abstracts: oral presentations

CELL BIO virtual 2020
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Wednesday, December 9, 2020

Keith Porter Lecture

A1
Biomolecular Condensates and their Implications for Physiology and Disease
A. A. Hyman; Max Planck Institute of Molecular Biology & Genetics, Dresden, GERMANY.

Cells organize many of their biochemical reactions by formation and dissolution of non-membrane-bound compartments. Recent experiments show that a common mechanism for such biochemical organization is phase separation of unstructured proteins to form liquid-like compartments, which can subsequently harden to form compartments with new material properties such as gels and glasses. These compartments can be described by principles elucidated from condensed-matter physics and are therefore termed biomolecular condensates. I will discuss potential roles of phase separation in organization of cellular biochemistry and the role of aberrant phase separation in disease. I will also describe how these discoveries were facilitated by the establishment of the Max Planck Institutes for Cell Biology and Genetics and the Physics of Complex systems, in Dresden.

EMBO Gold Medal

A2
Metabolic Rewiring in Cancer Progression
S. Fendt; VIB-KU Leuven Center for Cancer Biology, Leuven, BELGIUM.

Metabolic rewiring is a hallmark of cancer proliferation and metastasis formation. We provide evidence that plasticity of lipid metabolism and metabolic regulation through pyruvate metabolism enables cancer cells to evadedrug treatment and to seed in distant organs. In particular, we find that multiple cancers can repurpose the metabolic enzyme FADS2 to produce the unusual fatty acid sapienate. This alternative lipid desaturation pathway, which is controlled by the mTOR-SREBP signaling axis, allows cancer cells to proliferate in the presence of a classical desaturation inhibitor. Moreover, we find that cancer cells disseminating to the lung rely on microenvironmental pyruvate to remodel the extracellular matrix resulting in elevated metastasis formation. Specifically, pyruvate-fueled α-ketoglutarate metabolically regulates the activity of P4HA leading to collagen hydroxylation and a permissive metastatic niche. In conclusion, we discovered that metabolic plasticity and regulation contributes to cancer progression and may be targeted in the future in cancer therapy.

A3
How Evolution Hijacked Chemistry and Evolved a Dynamic and Robust Metabolism
M. Ralser; Institute of Biochemistry, Charité - Universitätsmedizin Berlin, Berlin, GERMANY.

Life runs on many thousands of different biochemical reactions, known collectively as cell metabolism. Metabolic reactions are vital for keeping cells and organisms growing and alive, and problems with cellular metabolism are implicated in ageing and diseases such as cancer, diabetes and brain disorders. Rather than thinking about cell metabolism as a collection of individual reactions, we are working to
understand metabolism as a dynamic, interconnected network of processes that adapts in response to changes and stresses in the environment, and that evolves and functions as an entity. But how does a complex biochemical network originate in evolution, and how does it stay dynamic in modern organisms, so that these can adjust it constantly to the ever changing environments? In this lecture, I’ll summarize our efforts to understand the nature of the earliest biochemical processes that did shape the emergence of life more than 4 billion years ago. Further, I’ll share our efforts to use mass spectrometry and simple traceable mode organisms, yeast in particular, for conducting hundreds to thousands of analytical measurements, allowing us to reveal basic design principles of metabolism. These help to understand how complex metabolic processes are controlled, and how they are reconfigured in response to environmental changes at a very high speed. In particular, I’ll present our findings that show that microbial cells uptake metabolites not only for growth but also to become more tolerant against toxic compounds, and that they collaborate and specialize in metabolism to generate single cell diversity to react robustly in stress situations.

Thursday, December 10, 2020

EB Wilson Medal Presentation and Address

A4
Enhancing Our Understanding of Eukaryotic Membrane-bound Organelles
J. Lippincott-Schwartz; HHMI Janelia Research Campus, Ashburn, VA.

All life on earth is comprised of either prokaryotic or eukaryotic cells. Eukaryotic cells, unlike prokaryotes, have numerous internal membrane-bounded organelles, including the nucleus, Golgi, endoplasmic reticulum, mitochondria, and lysosome. These organelles compartmentalize biological activities, such as uptake of molecules from outside the cell, the synthesis, processing and transport of proteins, energy production, and housing of genetic material. This capacity allowed eukaryotic cells to expand the complexity of their biochemical reactions and spatial organization, ultimately permitting them to specialize and work together, producing complex organisms such as ourselves. While we know the list of eukaryotic organelles and much of their functions, we still know very little regarding how organelles sort and distribute their components, how they vary in their function and distributions across different cell types, and how they form functional linkages with each other under different physiological conditions to maintain homeostasis. I will discuss the conceptual and technological advances being made on these topics by biologists, physicists, engineers, chemists, and computer scientists, who are using enhanced light and electron microscopy techniques to see more details of intracellular processes. Among the technologies I will cover are those that: increase spatiotemporal resolution dramatically, permitting simultaneous multispectral imaging of multicellular organelles and components; enable high-speed single molecule imaging to directly observe tethering and release dynamics of putative tethers in individual contact sites between organelles; and employ whole cell milling through Focused Ion Beam Electron Microscopy (FIB-SEM) to reconstruct all organelles in the entire cell volume at 4 voxel resolution. Together, these new tools are enabling researchers to construct an “organelle interactome” for describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of organelles and their trafficking pathways, and how disruptions of their normal functions due to genetic mutations may contribute to important diseases.
E.E. Just Award and Lecture

A5

A Biopsychosocial Approach to Addressing Health

L. A. Jones; Professor Emeritus, U.T.M.D. Anderson Cancer Center, Houston, TX.

For more than two decades, I have said that health alone will never solve the issue of health inequities. It never has and never will. Just look at how long we have attempted to address this issue. Until we climb out of our silos, we will never make the progress needed. So, what is a Biopsychosocial (holistic) Approach to addressing both health and health inequities? In 1977, George Engel eloquently stated that "To provide a basis for understanding the determinants of disease and arriving at rational treatments and patterns of health care, a medical model must also take into account the patient, the social context in which he lives and the complementary system devised by society to deal with the disruptive effects of illness, that is, the physician role and the health care system. This requires a Biopsychosocial model." In 1999, we changed Dr. Engel’s statement to reflect a more multidisciplinary approach to address health inequities, a medical model that must take into account the complex nature of health inequities that involves more than problems with access. It takes into account community, the social context in which they live, and the complementary system devised by society to deal with the disruptive effects of illness on their lives. That is a problem that can’t just be solved at the bench or in the clinic or by analyzing national datasets. With the impact of COVID-19, the Facilitated Assistance, Referral & Outreach is the perfect example of a Biopsychosocial Approach to address this issue. It does not negate scientific discoveries but provides that bridge for successful implementation.
SYMPOSIA

Tuesday, December 8, 2020

Cells in Distress and Disease

S1

Jamming-unjamming Transitions in Cancer Progression

P. Friedl¹,²; ¹Radboud University Medical Center, Nijmegen, NETHERLANDS, ²UT MD Anderson Cancer Center, Houston, TX.

Single-cell or collective invasion results from coordination of cell shape, deformability and actin dynamics relative to the tissue environment. Invading cancer cells exhibit particular ability to switch between collective and single-cell dissemination, controlled by cadherin-mediated cell-cell junctions, and microenvironmental signals. Using spatially defined organotypic culture, intravital microscopy of mammary tumours in mice and in silico modelling, we identify cell density regulation by three-dimensional tissue boundaries to physically control collective movement irrespective of the composition and stability of cell-cell junctions. Deregulation of adherens junctions by downregulation of E-cadherin and p120-catenin resulted in a transition from coordinated to uncoordinated collective movement along extracellular boundaries, whereas single-cell escape depended on locally free tissue space. Similar transition was identified by hypoxia and HGF stimulation, suggesting epithelial fluidization as an escape strategy from metabolically perturbed niches. These results indicate that cadherins and extracellular matrix confinement cooperate to determine unjamming transitions and stepwise epithelial fluidization towards, ultimately, cell individualization.

S2

How the Tubercle Bacillus Co-opts Host Pathways to Cause Cell Distress and Death

L. Ramakrishnan; University of Cambridge, Cambridge, UNITED KINGDOM.

Tuberculosis (TB), the most deadly infectious killer, is caused by the obligate intracellular pathogen Mycobacterium tuberculosis. This bacterium first takes up residence in host macrophages, which it can tweak in myriad ways to render into growth-permissive niches. Finally, to transmit to a new host, the bacterium must kill the cell and achieve extracellular residence. Hence, M. tuberculosis-induced macrophage necrosis is a critical pathogenic event that causes mortality and morbidity to the infected individual while sustaining the evolutionary survival of the pathogen and the global disease burden. We study TB pathogenesis in zebrafish infected with its natural pathogen Mycobacterium marinum, a close relative of M. tuberculosis. The optically transparent and genetically and pharmacologically tractable zebrafish is an ideal model host for in vivo cell biology. I will present one example of how mycobacteria accelerate macrophage necrosis by co-opting TNF, a key host protective cytokine. TNF induces in mycobacterium-infected macrophages increased mitochondrial reactive oxygen species that kill the cell through an elaborate signaling pathway that connects between components in the lysosome, cytosol and ER before returning to the mitochondrion to cause necrosis. I will discuss the metabolic perturbations underpinning this circuitry and its immediate therapeutic implications for TB treatment.
**Dynamic Intracellular Organization**

**S3**

**Microtubule Network Mechano-sensation**  
*M. Thery; French Alternative Energies and Atomic Energy Commission (CEA), Paris, FRANCE.*

The stochastic switching between microtubule growth and shrinkage is a fascinating and unique process in the regulation of the cytoskeleton. To understand it, almost all attention has been focused on the microtubule ends. However, recent experiments on reconstituted microtubule in vitro have revealed that tubulin dimers can also be exchanged in protofilaments along the microtubule shaft, thus repairing the microtubule and protecting it from disassembly. In this presentation we will discuss the role of microtubule self-repair in cells and provide novel evidence that it confers unexpected mechano-sensitive properties to the microtubule network. We applied controlled deformations to living cells and found that compressive forces can stabilise microtubules. This process allows the microtubule network to sense and adapt its architecture to the force field cells are submitted to, a process that has long believed to be the apanage of the actin network.

**S4**

**Fluid forces: Mechanics of Intracellular Phase Separation**  
*C. P. Brangwynne; Chemical and Biological Engineering, Princeton University/HHMI, Princeton, NJ.*

In this talk I will discuss our work to understand and engineer intracellular phase transitions, particularly liquid-like condensates which form through biomolecular phase separation, and play an important role in organizing the contents of living cells. We are interested in the role of mechanical forces on the dynamics of phase separation. Within the nucleus, mechanical constraints manifest through viscoelasticity of the densely packed chromatin, which impacts the formation, mobility, and shape of condensates such as the nucleolus. Biomolecular condensates can, in turn, generate forces that dynamically restructure their surroundings. We show that surface tension of model condensates can be harnessed to probe the local cellular viscoelasticity, and to generate spatiotemporally-controlled, piconewton-scale forces. Thus, while intracellular mechanics is typically viewed through the lens of solid-like biopolymer scaffolds, the presence of liquid condensate inclusions can have a major impact on the mechanical organization of the cell.

**Wednesday, December 9, 2020**

**Cell Shape, Cell Division, Migration and Death**

**S5**

**E. Coli Meets World: How The Environment Shapes A Bacterial Cell**  
*E. A. Mueller, P. A. Levin; Department of Biology, Washington University in St. Louis, St. Louis, MO.*

Like other single celled organisms, bacteria are uniquely sensitive to changes in their physical and chemical environment. With only the minimal protection offered by their cell envelope, fluctuations in nutrient availability, pH, osmolarity, and temperature have an immediate impact on diverse aspects of
cell physiology as bacteria struggle to adapt to the new condition. On a more extended time scale, nutrient availability is one of the major determinants of bacterial cell morphology. Escherichia coli cells are three times larger when cultured at steady state in nutrient rich conditions than in nutrient poor ones, due to the actions of nutrient-dependent division inhibitors, accelerated lipid synthesis and a concomitant increase in plasma membrane capacity, and other yet to be identified factors. Little is known, however, about the effect of other environmental conditions on bacterial cell morphology. Focusing on one environmental variable, I will discuss how modest changes in pH—too small to affect growth rate—alter the activity of the essential proteins that construct E. coli’s peptidoglycan cell wall, increase resistance to clinically relevant antibiotics, and reduce cell length by as much as 20%. Together this work identifies pH as a significant environmental determinant of bacterial physiology and morphogenesis whose impact is mediated primarily through changes in the cell envelope.

S6
Maintenance and Remodeling of Epithelial Cell-Cell Junctions during Cell Shape Changes
A. L. Miller; University of Michigan, Ann Arbor, MI.

Epithelial cell-cell junctions adhere cells to one another and maintain a selectively permeable barrier between cells. Cell-scale forces (e.g. cytokinesis) and tissue-scale forces (e.g. morphogenesis) drive changes in cell shape that challenge cell-cell junctions. These junctions must maintain adhesion and barrier function during cell shape change events to ensure that epithelial tissue function is not severely disrupted. In my talk, I will describe our recent efforts to gain a better understanding of how epithelial cell-cell junctions are maintained and remodeled in response to physiological forces that challenge adhesion and barrier function. Using quantitative live imaging of developing Xenopus laevis embryos as a vertebrate model organism, we have shown that local breaches in barrier function often occur as junctions elongate. These barrier leaks are rapidly repaired by transient, localized Rho activation, which we termed “Rho flares”. We discovered that Rho flares restore barrier function by driving concentration of tight junction proteins through actin polymerization and ROCK-mediated localized contraction of the cell boundary. Additionally, we recently found that a transient increase in cytoplasmic calcium accompanies Rho flares and is needed for efficient repair of tight junction breaches. We propose that Rho flares are part of a rapid tight junction repair pathway that reinforces epithelial barrier function, thus minimizing leakiness and allowing epithelial cells the flexibility to change shape during dynamic processes like cytokinesis and tissue morphogenesis.

Growth, Pattern and Form

S7
Sensing Fluid Flow by Immotile Cilia for Left-Right Patterning
H. Hamada; RIKEN, Center for Developmental Biology, Kobe, JAPAN.

Directional fluid flow generated by motile cilia plays an essential role in left-right (L-R) symmetry breaking in the fish, frog and mouse while bird and reptile break L-R symmetry without motile cilia/fluid flow. In the mouse embryo, rotational movement of motile cilia at the node generates leftward fluid flow, which is sensed by immotile cilia located at the periphery of the node. However, it remains unknown how the fluid flow is sensed. I will discuss 1) involvement of ciliary Ca2+ in flow sensing, 2) whether immotile sense mechanical stimuli or not, and 3) how the fluid flow leads to degradation of Cerl2/Dand5 mRNA, the readout of flow-induced signal.
**S8**

**Using the Cell Biology of Embryogenesis to Inform Tissue Regeneration and Repair in the Heart**

K. Red-Horse; Stanford University, Stanford, CA.

Using the cell biology of embryogenesis to inform tissue regeneration and repair in the heart. Blood vessels experience variations in the mechanical forces of blood flow, which provide signals that shape the vascular network. In the context of coronary artery development in the heart, we found that mechanical signals trigger pre-artery cells to migrate against the direction of blood flow, providing a cell source for arterial growth without the need for proliferation. We found that the transcription factor Dach1 facilitates endothelial cell polarization, alignment, and migration against the direction of blood flow through its induction of the chemokine Cxcl12. Accordingly, Dach1-deficient mice have small coronary arteries, and Dach1-overexpressing mice have more arteries. Finally, Dach1-overexpression in adult heart endothelial cells protected mice from experimental myocardial infarction (i.e. heart attacks), resulting in increased survival and decreased tissue fibrosis. Thus, understanding the cell biology of endothelial interactions with blood flow during artery development can teach us how to enhance recovery following cardiac injury.

**Information Processing**

**S9**

**Dynamic Changes in tRNA Modifications and Abundance during T-cell Activation**

T. Pippe, R. Rak, M. Polonsky, I. Eizenberg, O. Dahan, N. Friedman; Weizmann Institute of Science, Rehovot, ISRAEL.

The tRNA pool determines the efficiency, throughput, and accuracy of translation. Previous studies have identified dynamic changes in the tRNA supply and mRNA demand during cancerous proliferation. Yet, dynamic changes may occur also during physiologically normal proliferation, and these are less characterized. We examined the tRNA and mRNA pools of T-cells during their vigorous proliferation and differentiation upon triggering of the T cell antigen receptor. We observe a global signature of switch in demand for codon at the early proliferation phase of the response, accompanied by corresponding changes in tRNA expression levels. In the later phase, upon differentiation of the T cells, the response of the tRNA pool is relaxed back to basal level, potentially restraining excessive proliferation. Sequencing of tRNAs allowed us to also evaluate their diverse base-modifications. We found that two types of tRNA modifications, Wybutosine and ms2t6A, are reduced dramatically during T-cell activation. These modifications occur in the anti-codon loops of two tRNAs that decode “slippery codons”, that are prone to ribosomal frameshifting. Attenuation of these frameshift-protective modifications is expected to increase proteome-wide frameshifting during T-cell proliferation. Indeed, human cell lines deleted of a Wybutosine writer showed increased ribosomal frameshifting, as detected with a reporter that consists of a critical frameshifting site taken from the HIV gag-pol slippery codon motif. These results may explain HIV’s specificity to proliferating T-Cells since it requires ribosomal frameshift exactly on this codon for infection. The changes in tRNA expression and modifications uncover a new layer of translation regulation during T-cell proliferation and exposes a potential trade-off between cellular growth and translation fidelity.
Getting in Touch with Mechanical Pain

D. M. Bautista; University of California, Berkeley, Berkeley, CA.

Nociception, the conversion of a noxious stimulus into an electrical signal to elicit a protective behavioral response, is a highly conserved nervous system function. While studies have elucidated the mechanisms by which noxious temperature or chemicals are transduced, our understanding of how the mammalian nervous system detects and transduces noxious mechanical stimuli remain poorly understood. In this talk I will discuss data demonstrating that the bioactive signaling lipid, sphingosine 1-phosphate (S1P) and S1P Receptor 3 (S1PR3) signaling in neurons plays a key role in noxious mechanotransduction under normal conditions, and also contributes to pain, itch and inflammation under disease conditions.

Thursday, December 10, 2020

Cellular Identity

Principles of Cellular Compartmentalization

A. Spang; University of Basel, Basel, SWITZERLAND.

Eukaryotic cells contain membrane-bound organelles to separate distinct cellular functions. Nevertheless, the organelles need to communicate with each other and the extracellular space. Specialized domains on organelles and the plasma membrane support communication through at least three different ways: 1) transport vesicles that are formed at a donor compartment -exit site- and fuse with and are consumed at a specific target compartment -arrival site- 2) transport containers/organelles that briefly fuse with each other, yet maintain their overall identity; a mechanism referred to as ‘kiss-and-run’ or 3) membrane contact sites at which two compartments come into very close contact that allows exchange of lipids and ions between organelles or organelles and the plasma membrane. Key regulators of these specialized compartments are small GTPases of the Arf and Rab families. In their activated, GTP-bound state, they recruit effector molecules, which locally change the membrane and protein environment and thus give raise to specialized compartments. These small GTPases are regulated themselves through the action of GEFs and GAPs allowing for the tight temporal and spatial control of GTPase activity and hence membrane compartmentalization. Compartmentalization is not restricted to membranes but takes also place in the nucleus and the cytoplasm, where membrane-less condensates are formed to fulfill dedicated functions. For example, under stress, processing bodies and stress granules form in the cytoplasm and degrade or store RNA. When stress subsides, they can be turned over, and some RNA can even be returned to translation. I will discuss our current understanding of intracellular compartmentalization, rules that govern these processes and where we are headed.
A Numbers Game Goes Awry: How Aneuploidy Affects Cell Behavior and Identity

R. Li\textsuperscript{1,2}; \textsuperscript{1}Department of Cell Biology, Johns Hopkins University, Baltimore, MD, \textsuperscript{2}Mechanobiology Institute, National University of Singapore, Singapore, SINGAPORE.

Multiple layers of fidelity and checkpoint mechanisms maintain euploid chromosome copy numbers through cell cycles and organismal generations. Stress, mutations, aging and perhaps inflammation can elevate the error rate of chromosome transmission, resulting in aneuploidy. Aneuploid cells with different chromosome numbers, or karyotypes, exhibit diverse phenotypes as a result of gene expression changes where mRNA and protein levels tend to scale with gene copy numbers. Secondary effects on downstream target genes not directly affected by copy number or on the chromatin state can cause more dramatic phenotypic shifts such as myosin-independent cell division or altered sexual identity. These strong, and heritable, effects of aneuploidy can help explain the role of chromosome instability and the consequent karyotype heterogeneity in rapid stress adaptation in yeast, drug resistance of pathogenic fungi, and progression in cancer. Interestingly, hidden beneath the phenotypic diversity due to gene-specific changes, aneuploidy causes a set of common deficiencies, ranging from endocytic defects to metabolic dysregulation. These common phenotypes result from a hypo-osmotic-like state characterized by an increase in turgor pressure and cell swelling. These biophysical changes can be explained by using a simple osmotic model in which partially assembled complex or unpartnered monomers due to proteome imbalance contribute excess solutes and hence increase intracellular osmolarity. These findings shed light on the potential evolutionary driving force for the maintenance of euploidy and motivate inquiries into how aneuploidy alters cell identity and drives pathogenic processes including cancer progression and drug resistance.

How Different Cells Interact: Sex, War, Competition, Symbiosis and Parasitism

Mycobacterium Tuberculosis and Macrophages: A Tug of War

M. G. Gutierrez; The Francis Crick Institute, London, UNITED KINGDOM.

To cause disease and disseminate to other hosts, \textit{M. tuberculosis} needs to replicate within human cells. Work in the last decades have shed light into some aspects of tuberculosis pathogenesis, however, we still do not understand how \textit{M. tuberculosis} manages to survive within eukaryotic cells and why some cells are able to eradicate this lethal pathogen. This surprising gap in knowledge is in part due to the lack of appropriate imaging technologies that have precluded comprehensive understanding of the fundamental biology that underpins \textit{M. tuberculosis}-host cell interactions. Our research focuses on the fundamental molecular and cellular mechanisms that regulate the interactions between \textit{M. tuberculosis} and host cells. We aim to dissect the host cell factors that contribute to \textit{M. tuberculosis} control as well as the \textit{M. tuberculosis} factors that this pathogen uses to hijack host cells. To this end, we use a variety of cutting-edge imaging approaches and model systems. In this seminar, I will present some recent data from our group regarding the environments where \textit{M. tuberculosis} survives in human cells and the barriers that these environments represent for therapy.
S14

**Epic-genetic Battles and Other Tales of Innate Immune Memory**

*M. Mhlanga*1,2,3, 1Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, NETHERLANDS, 2Radboud University Medical Center, Nijmegen, NETHERLANDS, 3Epigenomics & Single Cell Biophysics Group, Department of Cell Biology, Radboud University, Nijmegen, NETHERLANDS.

The finetuned regulation of gene expression programs is fundamental to all multicellular organisms. Our lab has sought to understand the intersecting roles of chromatin architecture, epigenetics and RNA in achieving this regulation. Recently we have demonstrated at a mechanistic level how each of these work together to orchestrate innate immune memory or what is commonly termed “trained” immunity. Importantly, these studies have revealed how deeply linked this regulation is linked to plethora of external and environmental influences and most likely is subordinate to metabolic states. Innate immune memory can be “written” and “erased” with many positive regulators of innate immune memory having been identified. However, negative regulators of innate immune memory and their gene expression programs remain elusive. I will describe work from our laboratory demonstrating how innate immune memories are “written epigenetically” and more recent work identifying negative regulators of trained immunity. I will also describe how such memories are heritable and transmissible with implications for not only innate immune memory but potentially for other genetic circuits.

Friday, December 11, 2020

**Collective Cell Behavior**

S15

**Sources of Regenerative Capacity in Animals**

*A. Sanchez-Alvarado*; Howard Hughes Medical Institute, Stowers Institute for Medical Research, Kansas City, MO.

Under normal physiological conditions, the functions of many organs depend on the continuous destruction and renewal of their cells. Equally remarkable is the fact that the adult tissues and organs of many organisms can be fully restored after amputation. In fact, metazoans have evolved a series of renewal and repair mechanisms to respond to both trauma and normal wear and tear. Such mechanisms are under tight regulatory control such that the form and function of tissues, organs, and systems can be maintained throughout life. As important as repair and restoration are to the survival of multicellular organisms, we know little about how these processes are effectuated and regulated at the cellular and molecular levels. Here, I will discuss how the study of two research organisms, the planarian *Schmidtea mediterranea* and the African killifish *Nothobranchius furzeri* is beginning to shed light on the way adult animals regulate tissue homeostasis and the replacement of body parts lost to injury.
Adult stem cells support tissue homeostasis and repair throughout the life of an individual. Numerous changes occur with age that result in altered stem cell behavior and reduced tissue maintenance and regeneration. Changes can be cell autonomous including changes in cell cycle progression, decreased bio-energetic efficiency, increased DNA damage, and epigenetic alterations. In addition, poorly understood changes to the local and systemic environments occur that result in decreased stem cell activity or alterations in commitment or differentiation potential. In *Drosophila melanogaster*, advanced age leads to changes in the intestine, including an increase in intestinal stem cell (ISC) proliferation, an increase in bacterial load, activation of inflammatory pathways, increases in ROS levels, and loss of intestinal barrier function. However, the relationship between these phenotypes remains unclear.

Experiments in our lab are focused on teasing apart the links between age-related changes in the behavior of *Drosophila* ISCs and loss of the intestinal barrier, as well as understanding the molecular mechanisms contributing to changes in barrier function over time. We've found that aging results in the mis-localization of occluding junction proteins in absorptive enterocytes (ECs) of the posterior midgut. Acute loss of proteins that localize to either the bi-cellular or tri-cellular junction between differentiated cells results in premature emergence of intestinal aging phenotypes, including increased ISC proliferation and initiation of stress signaling in intestines from young flies. Therefore, compromised occluding junction function is sufficient to alter ISC behavior in a non-autonomous manner. These studies have also revealed additional roles for cell adhesion proteins in the intestine, including potentiation of self-renewal signaling in ISCs. As the function of these proteins are highly conserved in mammalian systems, our work will likely have implications for the treatment of age-onset changes to intestinal function that contribute to disease, including colon cancer.

The Genome

S17

The Role of Spatial Proximity in Genome Regulation

W. Bickmore; MRC Human Genetics Unit, University of Edinburgh, Edinburgh, UNITED KINGDOM.

Imaging and genomic technologies have opened up large-scale interrogation of 3D genome organisation in the cell nucleus. This has revealed multiple layers of organisation and at different scales, including distal interactions of genome compartments, topologically associating domains (TADs) and specific cis interactions between either active or repressive elements. Progress is being made toward understanding the molecular mechanisms that mediate these layers of organisation. However, understanding the functional significance of 3D organisation across genomic scales lags behind. To what extent does 3D organisation drive genome regulation, or are some aspects of 3D organisation simply an emergent property of genome and protein functions? In this talk I will describe our efforts to determine the functional significance of the 3D genome - from the action of polycomb complexes in bridging distal interactions between repressed loci, to the role of TADs and enhancer-promoter proximity in enhancer-driven gene activation.
Loop Extrusion with Barriers as a Genomic Communication System
L. Mirny; Massachusetts Institute of Technology, Cambridge, MA.

There is now strong support for the process of cohesin-mediated loop extrusion delimited by CTCF barriers as a major mechanism of interphase chromosome organization in vertebrates. However, the role of loop extrusion in mediating functional interactions is still controversial. Here we explore how loop extrusion with barriers may influence regulatory communication. Our models and analysis of Hi-C and Micro-C data expands the current paradigm by demonstrating CTCF play a dual role as both insulators and facilitators of contacts. We propose that the system of extrusion with barriers enables a “dock-and-scan” strategy to mediate targeted functional interactions between specific elements. Taken together, our results replace the picture of TADs as isolated regulatory neighborhoods, with a landscape of oriented genomic communication lines established by extrusion barriers of varying strength.
Wednesday, December 2, 2020

Education Minisymposium: Teaching Excellence Amidst Uncertainty

EM1

The social context of STEM education
B. M. Dewsbury; University of Rhode Island, Kingston, RI.

The success of students in STEM classrooms can be enhanced through inclusive mindsets and consequently practices by practitioners in the classroom. For inclusive practices to be authentic and effective, practitioners must be cognizant of how broader social factors potentially impact the students’ ability to be in an ideal learning space. Students struggling with affordability, identity contingencies or other symptoms of social disenfranchisement may arrive at first-year STEM courses with unearned barriers. In this talk I discuss a study where we explored the relationship between pre-enrollment socioeconomic factors on student success in introductory biology courses at a large, public research institution. In response we enacted several strategies to ensure that students felt a strong sense of belonging and become academically successful. I discuss the psychological implications of these social factors and suggest ways in which practitioners can mitigate their effects. The enactment of inclusive practices involved the reduction of course content in favor of providing students opportunities to cultivate their own agency and power. We show evidence here that these approaches enable the students to not only be successful in the introductory biology course, but in subsequent Biology courses.

EM2

Faculty conceptions of diversity in higher education
N. A. Suarez¹, S. Wang¹, S. Brydges², S. M. Lo³; ¹Mathematics and Science Education, University of California San Diego, La Jolla, CA, ²Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, ³Cell and Developmental Biology, University of California San Diego, La Jolla, CA.

Institutions have increasingly made the commitment to diversify higher education, and faculty play integral roles in creating an inclusive culture and environment. Our study asks: How do faculty conceptualize diversity in higher education, and how do these conceptions influence curriculum and instruction? Using phenomenography as the theoretical framework, we examined the qualitatively different ways in which faculty experience and understand diversity. These ways of understanding are organized into an outcome space with specific aspects that describe the phenomenon of diversity and variations within each aspect that distinguish the individual experiences. Data were collected through semi-structured interviews with 32 faculty from two-year and four-year minority-serving institutions. Transcripts were analyzed using grounded theory, and data were coded by two independent researchers to ensure reliability. Qualitative codes were constructed to capture how each participant described diversity. Five aspects were identified from our data: student features, legitimized membership, intelligence mindset, faculty role, and learning environment. Variations among experiences were organized into an outcome space with three distinct conceptions of diversity. In Conception I (which we termed essentialist), faculty attend to demographic features of students and view students with a fixed mindset of intelligence and as outsiders to higher education. This is aligned with equal treatments of all students and a curriculum approach that considers diversity as an impediment to learning.
Conception II (functionalist), faculty attend to different student viewpoints and consider students with a deficit mindset and as guests who transiently pass through higher education institutions. This is aligned with accommodations for student needs and a curriculum approach that supports struggling students. Conception III (existentialist) includes and expands on Conception II by attending to how lived experiences intersect with demographic features and viewpoints to shape the kinds of learners that individual students become in the classroom. Implicit power dynamics are considered, and students are viewed as rightfully present in higher education regardless of their backgrounds. Specific curriculum approaches are intentionally implemented to foster productive conversations around different student characteristics and to center social justice issues, and diversity enriches learning in the classroom. Overall, our results indicate that faculty acknowledge different student features and have varying understanding for what diversity means and why it is important in higher education, and some conceptions of diversity do not necessarily suggest an inclusive culture.

EM3

Examining the Intersection of Equity, Trauma-Informed Pedagogy, & STEM Education

M. Imad; Pima Community College, Tucson, AZ.

In order to be able to help students, we need to first be able to recognize trauma in ourselves. This session will address the impact of the ongoing pandemic and the long-ignored calls for racial justice on our sense of self and overall well-being. What lessons can we learn from neuroscience to help us better negotiate the pain and anxiety in ourselves and our students? How can we leverage the healing power of the community to help us move forward and help ourselves and our students continue to learn and thrive? How might we transform STEM education from content-centered to human-centered where we are invited by scholars like bell hooks to "make the classroom a place that is life-sustaining and mind-expanding, a place of liberating mutuality where teacher and student together work in partnership."

EM4

Student buy-in toward and utilization of in-person and online formative assessments

B. A. Couch, K. R. Brazeal, L. A. Wheeler; University of Nebraska-Lincoln, Lincoln, NE.

Active learning in the form of formative assessment (FA) represents an important way to improve student learning and persistence in STEM courses. While the use of FAs (e.g., Just-in-Time Teaching, Peer Instruction) has increased in recent years, it has also been accompanied by challenges such as students resisting them or using them in ways that may undermine learning. Student buy-in and utilization thus represent critical factors that potentially limit FA adoption and efficacy. We have conducted mixed methods investigations to understand how instructor-based activity characteristics influence student perceptions and behaviors related to FA activities. I will present findings from open-ended interviews highlighting how students perceive specific activity characteristics (e.g., content, grading policy) to affect their FA engagement. Furthermore, I will show results from the closed-ended Formative Assessment Buy-in and Utilization Survey (FABUS) demonstrating quantitative connections between student buy-in, utilization, and performance. Finally, I will share suggestions about how FABUS can be used by instructors to monitor and improve their FA implementation to help students succeed.
Empirical insights into the negative mentoring experiences of life science doctoral students

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Effective mentoring has been linked to several positive outcomes for graduate students in STEM fields, including greater scholarly productivity, academic performance, and well-being. Yet, mentoring, like any other relationship, can also have negative elements. There has been little research aimed at understanding the problematic experiences graduate students can have with their research mentors, despite the growing body of research suggesting that negative mentoring experiences occur in the workplace and undergraduate research settings and can have detrimental effects. This is particularly concerning given the alarmingly high rates of attrition from STEM graduate programs and increasing concerns over the mental and physical well-being of graduate students. Here we report the results of our exploratory, qualitative study to define and characterize the negative mentoring that graduate students experience during their graduate research. We conducted interviews with life science doctoral students (n=40) who represented diverse institutions, socio-demographics, and program timepoints, and who rated their mentoring experience as negative or less favorable. We analyzed their qualitative accounts using standard content analysis procedures, drawing from research on negative mentoring in workplace and undergraduate research settings. Doctoral students in our study attributed their negative mentoring experiences to multiple levels of the science research ecosystem, ranging from problematic mentor characteristics to poor relationship quality with mentors to research group, organizational and disciplinary issues in science that influence mentor behaviors. Collectively, doctoral students perceived that these experiences had detrimental effects on their personal and professional development, including their research self-efficacy, career interests, and physical and mental well-being. Some of our findings resemble the negative mentoring experiences reported by mentees in workplace and undergraduate research settings, but other results indicate that graduate students experience distinct forms of negative mentoring that are unique to their stage of development and the context of academic research. This work begins to provide an evidence base for responding to recent calls from the National Academies to improve STEM graduate education and mentorship practices. Our findings will be used to develop a quantitative measure to determine the prevalence and impact of negative mentoring experiences in graduate education and ultimately to test interventions to prevent these experiences and mitigate their effects.

The Genomics Education Partnership: Genuine undergraduate research experiences for online courses

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Since 2006, faculty members participating in the Genomics Education Partnership (GEP) have incorporated authentic genomics research experiences into the undergraduate curriculum, introducing thousands of students to eukaryotic gene structure, comparative genomics, and the evolution of Drosophila. The GEP is a consortium of over 150 diverse colleges and universities that provides Course-based Undergraduate Research Experiences (CUREs) for thousands of students from a variety of backgrounds. The GEP provides a centralized support system for students and faculty to engage in
research in bioinformatics and genomics. The COVID-19 pandemic has forced many colleges and universities to provide remote instruction, presenting a challenge for faculty teaching biology laboratory courses. The GEP is uniquely positioned to address this challenge by providing research experiences in genomics for faculty and students within a completely online setting. Additionally, the GEP is providing remote, virtual teaching assistants for GEP students. Preliminary results from our faculty report suggest that the majority of GEP faculty teaching during the Spring 2020 semester find the GEP curriculum and infrastructure is well-suited for online instruction. The GEP assessment results from a pre/post quiz and survey administered in the Spring 2020 semester are similar to the results from previous semesters where most students were instructed face-to-face. However, even though only an internet connection and a computer are needed to complete a GEP research project, some students and faculty have limited access to reliable internet connections and computers. Moving forward, as we work to increase diversity in biology, inequities in access to technology will need to be addressed.

Monday, December 7, 2020

Cell Biology of the Nervous system in Development and Disease

M1

Synaptic active zone assembly requires scaffold molecule phase separation

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In neuronal synapses, the active zone is the site of release of vesicles containing neurotransmitters. While the composition of active zones is well characterized, how active zone proteins assemble together and recruit synaptic release machinery is not clear. Here, we show that two widely conserved active zone components, C. elegans SYD-2/Liprin-α and ELKS, undergo a liquid-liquid phase separation that is critical to assemble the active zone. We first find developing active zones are dynamic and recover quickly after photobleaching, consistent with a possible phase separation. In contrast, mature active zones become highly static with nearly zero cytoplasmic exchange. We find that SYD-2/Liprin-α and ELKS, two core active zone scaffolding proteins, are capable of liquid-liquid phase separation in vitro and in vivo, and rely upon multiple disordered motifs for this activity. By selectively mutating these motifs, we are able to specifically ablate phase separation activity without affecting the scaffolding functions of the proteins. We endogenously introduced the SYD-2 and ELKS phase separation-blocking mutations in vivo with CRISPR/Cas9, and find they localize normally, but lack fluid dynamics even in developing synapses. Consequently, we find additional active zone components fail to assemble properly and synaptic vesicles fail to cluster, causing synaptic transmission and behavioral defects. Critically, when SYD-2’s phase motif is replaced with that of an unrelated phase separating protein, FUS, assembly of the active zone is rescued, confirming the central importance of phase separation activity. Finally, we reconstituted SYD-2 and ELKS phase-separated scaffolds in vitro, and find they are able to incorporate additional active zone components UNC-10/RIM and GIT. We find this incorporation depends on SYD-2 and ELKS fluid dynamics; SYD-2 and ELKS-1 condensates that have matured and solidified, or condensates of mutants with decreased dynamics, are unable to evenly incorporate UNC-10 and GIT. Our data show that presynaptic active zones are assembled through a phase separation of core scaffold
molecules. This phase separation is critical for the accumulation and incorporation of active zone components at a nascent synapse before maturation into a stable structure.

M2
Zebrafish NADPH oxidase 2 regulates retinal ganglion cell guidance downstream of slit2/robo2
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Reactive oxygen species (ROS) have recently been established as a signaling molecule in many cellular events, contributing to cell homeostasis and physiology. In the central nervous system (CNS), ROS are associated with development, neuronal progenitor maintenance, establishment of neuronal polarity, and maintaining proper actin dynamics in the growth cone. An important source of cellular ROS is a special enzyme family called NADPH oxidases (Nox), which generate superoxide and hydrogen peroxide (H₂O₂). We previously showed that zebrafish nox2−/− mutants exhibit axon guidance defects throughout the CNS, including mis-targeted retinal ganglion cell (RGC) axons in the optic tectum (OT). Therefore, we speculated whether Nox2 could act downstream of guidance cue receptors to modulate axonal pathfinding. To investigate whether Nox2 could act downstream of slit2/Robo2, we took in vitro and in vivo approaches with cultured RGCs and double heterozygous mutants, respectively. We found that slit2‐mediated growth cone collapse was lost in mutant RGC growth cones, suggesting a cell‐autonomous relationship between Nox2 activity and axonal growth. Furthermore, H₂O₂‐specific biosensor imaging showed that slit2 increased intracellular H₂O₂ levels in wildtype, but not in nox2−/− or astray−/− (Robo2‐deficient) growth cones. To address whether slit2/Robo2 and Nox2 are in the same signaling pathway controlling RGC pathfinding, we performed genetic interaction experiments in vivo. We found that partial loss of Nox2 and Robo2 individually did not affect RGC innervation of the OT; however, when combined, partial loss of both genes affected retinotectal innervation in vivo. Taken together, our results provide evidence that Nox2 acts downstream of slit2/Robo2 mediating growth and guidance of developing zebrafish RGC neurons.

M3
Astrocyte signaling restricts motor dendrite dynamicity to a critical period of plasticity
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Critical periods - brief intervals where neural circuits can be modified by sensory input - are necessary for proper neural circuit assembly. Extended critical periods are associated with neurodevelopmental disorders, including schizophrenia and autism; however, the mechanisms that ensure timely critical period closure remain unknown. Here, we define the extent of a critical period in the developing Drosophila motor circuit, and identify astrocytes as essential for proper critical period termination. During the critical period, decreased activity produces larger motor dendrites with fewer inhibitory inputs; conversely, increased motor neuron activity produces smaller motor dendrites with fewer excitatory inputs. Importantly, activity has little effect on dendrite morphology after critical period closure. Astrocytes invade the neuropil just prior to critical period closure, and astrocyte ablation prolongs the critical period. Finally, we use a genetic screen to identify astrocyte-motor neuron signaling pathways that close the critical period, including Neuroligin-Neurexin signaling. Reduced signaling destabilizes dendritic microtubules, increases dendrite dynamicity, and impairs locomotor behavior,
underscoring the importance of critical period closure. Previous work defines astroglia as regulators of plasticity at individual synapses; here, we show that astrocytes also regulate large-scale structural plasticity to motor dendrites, and thus, circuit architecture to ensure proper locomotor behavior.

M4

**Genomic instability drives the neurodevelopmental and neuroinflammation defects due to RibonucleaseH2 loss in mice**

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Aicardi-Goutières syndrome (AGS) is a monogenic type I interferonopathy that is characterized by severe brain dysfunction, cerebellar atrophy, upregulation of type I interferon signaling and neuroinflammation. Mutations in various genes that function in nucleic acid metabolism, including Rnaseh2 are linked to AGS. Ribonuclease H2 (RNaseH2) is a genome surveillance factor that protects DNA by removing RNA-DNA hybrids and ribonucleotides incorporated into the DNA during replication. The central nervous system is severely affected in AGS, although it is unclear why Rnaseh2 mutations result in neurological symptoms. Moreover, a key question is if persistent upregulation of type I interferon signaling or DNA damage drive the AGS pathology. Here we investigate how brain-specific deletion of RNaseH2 affects the nervous system in mice. Contrary to previous findings, we show that loss of RNaseH2 in the mouse brain profoundly affects early neurogenesis resulting in cerebellar atrophy, activation of interferon-responsive genes and neuroinflammation. The neuroinflammation is not coincident with ribonucleotide mediated DNA damage, but occurs after defective neurodevelopment. Furthermore, the cerebellar atrophy due to RNaseH2b deletion is rescued by p53 inactivation suggesting that DNA damage signaling, rather than neuroinflammation, is responsible for neuronal loss. Importantly, p53 activation in RNaseH2-deleted brains is not solely dependent upon ATM kinase activity as deletion of Atm did not rescue the cerebellar phenotype. In fact, coincident deletion of Atm and Rnaseh2 resulted in a worsened cerebellar phenotype dependent upon activation of non-homologous end-joining (NHEJ) leading to mice developing ataxia. The loss of ATM also exacerbates neuroinflammation. Thus, ATM suppresses the genomic damage and neuroinflammation after RNaseH2 inactivation by inhibiting toxic NHEJ. Collectively, our data suggest that it is DNA damage-dependent signaling rather than type I interferon upregulation that underlies the neurodegeneration in AGS, thereby providing critical insight into understanding how RNaseH2 and ATM prevent neurologic disease.

M5

**Identification of extrinsic cues promoting target-selective axon regeneration**

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Regenerating axons in the peripheral nervous system must extend over long distances to reconnect with their original synaptic targets for functional recovery. However, re-establishing a complex trajectory that includes navigating multiple choice points and then selecting the appropriate target long after this circuitry was established during development, represents a unique challenge. To visualize regenerating axons as they navigate stepwise choice points, we established the larval zebrafish pectoral fin, equivalent to tetrapod forelimbs, as a vertebrate model system in which to study this process. Each pectoral fin is innervated by four motor nerves containing dozens of axons that branch to stereotypically-innervate specific regions of two muscle layers. Using a laser, we transect the fin motor
nerves and monitor axon regeneration in real time. We have characterized the stepwise choices faced by regenerating axons including sorting at the plexus to select the correct muscle layer, selectively fasciculating with the appropriate axonal partners to grow into the fin, and then defasciculating to reinnervate their original individual muscle fibers. By labeling single axons, we observe robust, specific, and functional regeneration of motor axons back to their original domains within 48 hours post injury, indicating the existence of local cues within the fin to guide selective reinnervation. To identify extrinsic injury-dependent guidance cues in the pectoral fin, we have employed an RNAseq approach. We present results from this RNAseq analysis from denervated fins at timepoints that precede important axon guidance decisions including choosing a muscle layer, sorting at the plexus, and specific target selection. We predict that changes in gene expression may reflect regional cues important for axon growth and guidance and are testing mutants in genes that are upregulated after injury to determine their functional role in axon guidance. Funding: NINDS NRSA F32NS103219

M6

Genetic Disruption of the WASH Complex Drives Endo-lysosomal Dysfunction and Cognitive-Movement Impairments in Mice and Humans

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Emerging evidence suggests that mediators of protein trafficking may be critical to cognitive and motor disorders. Long-range projections, such as the corticospinal motor tract, are particularly vulnerable to disrupted trafficking because they require protein delivery to their distal processes, which measure tens to hundreds of centimeters in length. The endosome-associated WASH complex has been strongly implicated in protein trafficking in non-neuronal cells by facilitating the formation and scission of cargo-laden vesicles for transport, but its trafficking effects have not been explored within the nervous system. Human mutations in WASH complex components and associated endosomal sorting complexes, result in movement and cognitive disorders such as Parkinson’s disease, hereditary spastic paraplegia, and intellectual disability, suggesting that WASH complex dysfunction could be causative in the development of these disorders. However, how these mutations manifest in neurologic dysfunction remains unknown. We first identify the neuronal WASH complex proteome, revealing a network of neuronal endosomal proteins it associates with in vivo. Then, to uncover how dysfunction of endosomal WASH leads to disease, we generate a mouse model of a human WASH complex mutation. Using a spatial proteomics approach coupled with a systems-level analysis of protein covariation networks, we find that this mutation destabilizes the WASH complex and significantly perturbs endosomal and lysosomal pathways in mouse brain. Cellular and histological assays confirm that this mutation has a significant impact on neuronal endo-lysosomal trafficking in vitro and in vivo, with evidence of neurodegenerative pathology. Behavioral analyses reveal that disruption of the WASH complex not only impacts cognition, but also causes significant progressive motor deficits in mice. Remarkably, a retrospective analysis of patients harboring this mutation confirms motor deficits in humans. Taken together, this work is the first to examine how the WASH complex functions within neurons in vivo. Our results also establish that
impaired WASH complex-dependent trafficking drives pathophysiology relevant to movement disorders in humans.

**Cell Cycle and Proliferative/Non-proliferative Signaling**

**M7**

*Network-imaging of cellular transitions between proliferative and Non-proliferative states*

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Cellular transitions between proliferative and Non-proliferative states are essential for single cell survival and the development of organisms. Non-proliferative cellular states, such as quiescence, dormancy and terminal differentiation, are ubiquitous, however, it is still unclear how cells integrate stress or developmental stimuli to enter such states for long periods of time. This occurs, in part, because stimuli that suppress proliferation often trigger several signaling pathways, which are hard to simultaneously track in single cells. Here we used budding yeast to show how a combination of machine learning and spectral imaging allows to simultaneously track at least six different pathways in single cells. Using this approach, we imaged the main components of the network controlling the proliferation-quiescence transition in 30,203 cells undergoing quiescence entry in response to starvation. We found that quiescence entry is defined by (1) the rewiring of the cell cycle machinery by coherent and incoherent feed-forward loops in stress-activated pathways and (2) the action of hysteretic stress responses based on transcriptional repressors such as Stb3 and Xbp1. Our results show that the imaging of protein networks provides a comprehensive understanding of cellular transitions into and out of proliferation.

**M8**

*Contact Inhibition in Mother Cells Shifts an Activator-Inhibitor Balance to Direct Newborn Cells to Quiescence*

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Contact inhibition of cell proliferation regulates tissue size and prevents uncontrolled cell expansion. When cell density increases, contact inhibition can force proliferating cells into quiescence. Here we show that the memory of local cell density experienced by mother cells controls an activator-inhibitor balance in newborn cells that decides between quiescence and proliferation. We find that increasing local cell density in mother cells suppresses ERK activity and tightly controls cell fate by reducing the ratio between the levels of cyclin-dependent kinase (CDK) activator cyclin D1 and inhibitor p27 in newborn cells. Markedly, contact inhibition and mitogen signals compete with each other to control cell fate simply by shifting the cyclin D1/p27 ratio in opposite directions. Together, our study identifies an activator-inhibitor balance that integrates contact inhibition and mitogen signals in mother cells to control, at the population level, the percentage of daughter cells that keep proliferating.
M9
Single-cell measurements of cyclin-dependent kinase activity in *C. elegans*

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*C. elegans* is well-suited to dissect the genetic networks regulating cell proliferation, differentiation, and morphogenesis. One new approach in *C. elegans* that will facilitate our interrogation of these cellular behaviors is the use of kinase translocation sensors. These sensors are genetically engineered fluorescent kinase substrates that change their subcellular location upon phosphorylation. The first translocation-based sensor of kinase activity in *C. elegans* allowed for the quantitative and qualitative assessment of extracellular signal-regulated (ERK) activity *in vivo*. More recently, we and others have developed a cyclin-dependent kinase (CDK) sensor to analyze CDK activity live in several post-embryonic lineages, including the sex myoblasts, uterine cells, vulval precursor cells, and seam cells. These *C. elegans* lineages have served as paradigms for understanding mechanisms that underlie migration, cell fate specification, tubulogenesis, and asymmetric cell divisions, respectively. Here, we describe a *C. elegans* sensor of CDK-4 activity *in vivo*. Though the current CDK sensor reads out CDK-2, and possibly CDK-1, activity, the ability to follow CDK-4 activity in single cells is critical to studying the start of cell cycle entry. We specifically interrogate an invasive uterine cell, the anchor cell (AC). During larval development, the AC is programmed in a cell cycle regulated manner to invade the underlying vulval epithelial tissue to facilitate future egg-laying. We genetically and pharmacologically perturb the AC in order to drive cell cycle entry and thereby block cellular invasion, visualizing the G1/S transition with the CDK-4 sensor. This work suggests that CDK4/6 inhibition may inadvertently select for metastatic cellular behavior, with broader implications for cancer treatment and drug development.

M10
An expanded topology of the human cell cycle

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Our current understanding of the topology of the human cell cycle has been assembled piecemeal over decades of careful biochemical and genetic experiments. This accumulated knowledge has culminated into a comprehensive description of the molecular events that drive a typical human cell through DNA replication and division. Recent evidence suggests, however, that cells do not always take the same molecular path through the cell cycle and that heterogeneity in cell cycle trajectories exist even within an isogenic population. Furthermore, we know comparatively little about the paths cells take once they exit the proliferative phases of the cell cycle and enter a state of arrest. To obtain a more complete atlas of cell cycle states and the molecular trajectories that connect them, we performed iterative indirect immunofluorescence imaging (4i) of 47 cell cycle and signaling effectors in human epithelial cells in the absence and presence of various cell cycle stresses. We then used manifold learning approaches to reconstruct a consensus cell cycle topology from these high dimensional, single cell signatures of cell cycle state. Using this data-driven approach and without prior knowledge, we obtained a structural representation of the human cell cycle that: (1) reveals multiple proliferative (G1/S/G2/M) and arrest (G0) trajectories, (2) incorporates and displays the dynamics of cell cycle and signaling effectors along these trajectories, and (3) identifies the molecular architectures that govern exit into multiple cell cycle
arrest states with distinct molecular signatures. This expanded cell cycle topology provides a more comprehensive understanding of the structure and connectivity of the cell cycle and the mechanistic determinants that guide cells through this fate space.

M11
The bistable, biphasic regulation of PP2A-B55 accounts for the dynamics of mitotic substrate phosphorylation
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The entry into and the exit from mitosis are accompanied by dramatic changes in the phosphorylation state of the cellular proteome. During mitotic entry the cyclin-dependent kinase 1 (Cdk1) phosphorylates hundreds proteins at thousands of sites thereby bringing about the mitotic state. During mitotic exit, rapid dephosphorylation of these substrates promotes the transition into interphase. These mitotic transitions are decisive, all-or-none events which are governed by an intricate network of positive and double-negative feedback loops regulating the activity of Cdk1 and its counteracting phosphatases PP1 and PP2A-B55. Using Xenopus laevis egg extracts we have biochemically dissected the contributions of these different regulatory motifs to the overall dynamics of mitotic progression. We show that in extracto the Cdk1-counteracting phosphatase PP2A-B55 but not PP1 functions as a bistable switch, even when the bistability of Cdk1 activation is suppressed. In addition, Cdk1 regulates PP2A-B55 in a biphasic manner; low concentrations of Cdk1 activate PP2A-B55 and high concentrations inactivate it. As a consequence of this incoherent feedforward regulation, PP2A-B55 activity rises concurrently with Cdk1 activity during interphase and suppresses substrate phosphorylation. PP2A-B55 activity is then sharply downregulated at the onset of mitosis. During mitotic exit Cdk1 activity initially falls with no obvious change in substrate phosphorylation; dephosphorylation then commences once PP2A-B55 spikes in activity. These new insights suggest that the regulation of the phosphatase, more than the kinase, defines the timing and dynamics of mitotic transitions - a theme which might also apply to other cell cycle transitions as I will discuss.

M12
Regulation of the oxidative stress response via dynamic regulation of p53 and MAPK activity
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Reactive oxygen species (ROS) naturally occur within cells as a byproduct of normal metabolic processes and can lead to DNA damage, growth arrest, and cell death. Paradoxically, many cancers exhibit elevated levels of ROS and oxidative stress that favor tumor progression and hinder chemotherapeutic treatment. We sought to understand how individual cells determine cell fate in response to oxidative stress. Prior single cell studies of p53 have observed that temporal dynamics can specify distinct stress responses and dictate cell fate. Using a fluorescently tagged p53 fusion protein, we measured single cell expression dynamics of p53 by time lapse microscopy in response oxidative stress induced by hydrogen peroxide (H₂O₂) and compared this to the well-characterized DNA double strand break (DSBs) response induced by neocarzinostatin (NCS). Interestingly, we observed that single cell p53 dynamics were
quantitatively similar between NCS or H2O2 treatment. However, treatment with H2O2 significantly increased cell death as compared to NCS suggesting alternative pathways cooperate with p53 to encode stress specificity. To explore alternative signaling networks, we paired our fluorescent p53 with biosensors for the mitogen activated protein kinases (MAPK) ERK, JNK, and p38. We found that these kinases were differentially activated in response to NCS or H2O2 and regulated p53 expression in a kinase and stressor specific manner. Single cell analysis of surviving and dying cells revealed that patterns of JNK activity correlated with induction of cell death and pharmacological inhibition of JNK diminished cell death. RT-PCR analysis of p53 target genes also revealed differences in gene expression patterns between NCS and H2O2 treatment suggesting that p53 and MAPK-regulated transcription factors form a network of coherent feedforward loops to coordinate expression of pro-survival and pro-apoptotic transcripts. These findings suggest that by integrating temporally distinct signals from p53 and MAPKs, cells can increase the specificity of stress responses through regulation of key cell fate regulators. Additionally, we may be able to enhance the induction of cell death within tumors by manipulating the dynamics of these pathways in conjunction with specific chemotherapies.

Cytoskeleton and Cell Complexity

M13
FLN-1/Filamin is required to organize the cytoskeleton, organelles, and promote cortical stability in a contractile tissue
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Actomyosin networks are organized in space, direction, size, and connectivity to produce coordinated contractions across cells. We use the C. elegans spermatheca, a tube composed of contractile myoepithelial cells, to study how actomyosin structures are organized. FLN-1/filamin is required for the formation and stabilization of a regular array of parallel, contractile, actomyosin fibers in this tissue. Loss of fln-1 results in the detachment of actin fibers from the basal surface, which then accumulate along the cell junctions or are captured at the nucleus by the linker of nucleoskeleton and cytoskeleton complex (LINC) complex, where they form large foci. Loss of filamin results in surprising disruption to cell integrity, with apparent protrusion of fibers into neighboring cells. Nuclear positioning and morphology, distribution of the endoplasmic reticulum and the mitochondrial network are also disrupted. These results demonstrate that filamin is required to prevent large actin bundle formation and detachment, to prevent excess nuclear localization of actin and myosin, to stabilize cell membranes, and to ensure correct positioning of organelles.

M14
Dynamics of clathrin-mediated endocytosis and actin in the native tissue context
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Clathrin-mediated endocytosis (CME) is an essential eukaryotic process, which has been extensively studied using cultured cells. These studies have shown that the assembly dynamics of endocytic events and the required proteins vary considerably within cells and between cell types. For example, the life time of individual endocytic events and the requirement for actin polymerization have been reported to vary significantly. Cultured cells, however, lack the tissue context, which influences their mechanical properties and developmental state. It remains unclear how the variability and heterogeneity of
endocytosis in cultured cells relates to endocytosis in cells within their natural environment. To understand how CME functions in the native tissue context, we studied it in *Drosophila melanogaster*, and used CRISPR to create fly lines with endogenously tagged endocytic proteins. We then used fluorescence microscopy to image CME in pupae, which are non-motile and have all major organs developed. Due to the exquisite contrast of fluorescent knock-ins and optimized imaging, we were able to image individual endocytic events with a similarly high spatiotemporal resolution as in cell culture. We focused on the epithelium at the surface of the pupal notum, where specialized mechanosensory bristles are formed along a highly stereotypic developmental trajectory. We used these cells as model system to image CME throughout cell differentiation.

We found that endocytic dynamics are highly regular in bristle cells, contrasting many reports from cultured cells, and change as bristles develop. From imaging the clathrin adaptor protein AP-2, we found that coat assembly and disassembly phases remain stereotypic, but a variable delay phase in between changes during cell growth, which we hypothesize is linked to changing requirements for actin polymerization in CME. Bristles have a highly organized actin cytoskeleton composed of long and thick actin bundles at the plasma membrane, and local and dynamic actin assemblies between the bundles. To study how these actin structures are involved in endocytosis throughout bristle development, we simultaneously imaged AP-2 and the actin binding protein cortactin. We found that endocytosis is corralled between the bundles, and associated with highly dynamic actin networks. In summary, we have found evidence that CME is reprogrammed during development, likely in response to changing cellular function and architecture.

**M15**

+TIP-dependent Tuning of Microtubule Mechanical Flexibility Protects Cells Navigating Confined Environments.

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Cells in living organisms must be able to navigate highly crowded 3D environments. Moving through confined spaces presents significant mechanical challenges for cells and requires adaptive protection mechanisms. Microtubules play key roles in migration, but their role in 3D confined environments is poorly understood. Here we report a dynamic microtubule cortico-nuclear cage that is necessary to preserve the integrity of cells when they migrate in confinement. This cage is mechanically tuned by lattice repair mechanisms governed by the +TIP CLASP. Loss of the +TIP CLASP results in an increase in mechanical breakage of microtubules due to both a decrease in tubulin acetylation and a loss of polymer stability. This results in the catastrophic rupture of cells in confined environments due to a loss of cortical integrity driven by aberrant spatio-temporal activation of RhoA. We propose that microtubules regulate the cell cortex to preserve cell integrity. This requires localized mechanochemical tuning mechanisms to locally heal and reinforce the lattice to spatiotemporally coordinate contractile forces at the cell cortex required for cell shape changes and movement. Our study reveals a mechano-protective role for microtubules and +TIPS that is essential for cells navigating 3D confined environments.
A Non-Centrosomal Microtubule-Organizing Center with Unique Architecture and Gamma-Tubulin-Independent Microtubule Assembly Mechanisms in Adipose Cells

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Non-centrosomal microtubule-organizing centers (ncMTOCs) are a prevalent feature of differentiated cell types where centrosomes are frequently inactivated or degenerated. ncMTOCs are diverse in their sites of subcellular assembly, yet little is known of their structures, regulation, and what functions they serve in the specialized cell types where they are found. Here we show a unique molecular architecture for the ncMTOC in fat body cells, Drosophila analog of adipocytes and liver, that have critical secretory functions that are controlled by the ncMTOC. Remarkably, this ncMTOC does not require the prevalent microtubule nucleator gamma-tubulin for MT assembly. We compare and contrast this ncMTOC with another very different one that is assembled on the surface of mitochondria in spermatids that does require gamma-tubulin and is regulated by a variant of Centrosomin. We show that a prominent ncMTOC is assembled at the nuclear surface in fat body cells. This ncMTOC has several novel features and mechanisms of MT assembly that distinguish it from centrosomes and other MTOCs. Firstly, this MTOC is remarkable in its complete lack of dependence on gamma-tubulin, the broadly-employed MT nucleator at centrosomes and ncMTOCs. Secondly, we show that the Nesprin Msp300 establishes the MTOC on the nuclear surface together with the spectraplakin Shot. This complex recruits the MT minus-end regulators Patronin (CAMSAP homolog) and Ninein, which function redundantly to establish MT nucleation at the nuclear surface. Thirdly, Patronin + Ninein recruit the MT polymerase Msps (XMAP215 homolog) to generate radial MTs. We further show that Patronin and Msps associate. Overall, this MT regulatory axis involving Patronin + Ninein to stabilize or anchor MT minus ends while recruiting the MT polymerase Msps to assemble the radial MT array, anchored at the nucleus by Msp300 + Shot, and functioning independently from γ-tubulin, represents a novel paradigm for MTOC structure and function. We present a model for how Patronin and Msps cooperate in MT nucleation at the ncMTOC. Surprisingly little is known about the cell biological or physiological functions that diverse ncMTOCs serve in the variety of cell types. We show that the fat body perinuclear ncMTOC controls membrane trafficking, impacting plasma membrane homeostasis. The MT arrays radiating from the perinuclear ncMTOC coordinate dynein motor-dependent endocytic trafficking to maintain proper plasma membrane growth. The uncontrolled plasma membrane overgrowth that results from disruption of the ncMTOC causes extracellular entrapment of large secreted complexes like collagen within the convoluted membrane folds, a phenomenon comparable to liver fibrosis.

Kinesin-4 Motor Teams Effectively Navigate Dendritic MT Arrays Through Track Switching and Regulation of MT Dynamics

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Microtubules constrain and direct active transport mediated by the dynein and kinesin motors that move cargos through the cell. The arrangement of microtubules into an organized cellular network allows for efficient targeted transport. However, it is not clear how directed transport is established in environments such as the dendrites of neurons, where the microtubule cytoskeleton is organized with mixed polarity. KIF21B is a robust plus-end directed kinesin-4 motor involved in long range cargo transport in axons and dendrites. We queried the ability of KIF21B to navigate mixed polarity microtubules in dendrites in live and extracted neurons, and on engineered microtubule bundles reconstituted in vitro. Using optogenetics to recruit KIF21B to cargos in live neurons, we found that KIF21B can effectively navigate the mixed polarity dendritic cytoskeleton, driving bidirectional movement with a net retrograde bias. On stabilized cytoskeletal arrays generated from extracted neurons, purified KIF21B motors exhibit pronounced bidirectional switching between oppositely oriented MT tracks. As KIF21B is both a microtubule motor and an effector of microtubule dynamics, we explored the underlying mechanisms by reconstituting KIF21B motility on dynamic, bipolar microtubule arrays. In addition to altering assembly dynamics at low nanomolar concentrations, we observed that teams of KIF21B motors use their C-terminal microtubule-binding domains to effectively switch between adjacent tracks. Together these results suggest a mechanism where KIF21B directionality in the cell is regulated by the formation of cargo-bound teams that effectively switch between oppositely oriented MTs to mediate transport over long distances.

**Cytoplasm properties limit cytoskeleton dynamics**

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The cytoplasm is a very crowded environment harboring most of the metabolic reactions necessary for cellular function. Its biophysical properties are influenced by cellular metabolism. Indeed, changes in cytoplasm mechanical properties have been linked to cellular differentiation and changes in cytoplasm concentration to cell death. However, the reverse relationship has not been extensively studied. So, here we tried to address the effects of the cytoplasm biophysical properties on various dynamic processes happening inside cells from protein diffusion to cytoskeleton dynamics. Our results demonstrate the sensitivity of cellular dynamics, specifically of the cytoskeleton dynamics, to cytoplasm concentration. In fission yeast, changing cytoplasmic density reversibly affects all the dynamic processes we looked at from protein diffusion to complex dynamic processes such as actin and microtubule dynamics. We found that microtubule dynamic is inversely correlated to cytoplasmic density. Increasing cytoplasm density slowed down microtubule growth and shrinkage rates in a dose-dependent. This effect of cytoplasm density on cytoskeleton dynamics did not seem to depend on stress pathway. We extended our observation to other eukaryotes, mammalian, and plant cells, and confirmed the effect of cytoplasm density on microtubule dynamics suggesting that we are probing an inherent property of the cytoplasm. We discovered that the effect of cytoplasmic density on the cytoskeleton can be alleviated and that a dilution of the cytoplasm leads to an increase in microtubule dynamics even if tubulin concentration decreases. We set out to explore the effect of the environment biophysical properties on microtubule dynamics in vitro by growing microtubule in buffers of various viscosity and obtained supporting evidence. Our results highlight how important the biophysical properties of the cytoplasm...
are to cellular metabolism in eukaryotes and suggest that the viscosity of the cytoplasm limits how fast processes such a cytoskeleton dynamic can happen in cells. This study reveals the interplay between biology and physics happening in the cytoplasm. Our assay is a first step in understanding how the biophysical properties of the cellular environment affect the biological processes it encloses.

**Genes in Diseases**

**M19**

*Caging the nucleus: Lamins and LINC complex proteins facilitate microtubular organization around myonuclei*

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Centrosomes serve as microtubule organizing centers (MTOCs) in many types of eukaryotic interphase cells. An exception is differentiated striated muscle cells, where the nucleus serves as the MTOC. This unique microtubular arrangement is generated when myoblasts fuse to form myotubes. During this developmental process, the microtubule nucleating activity switches from a centrosomal to a nuclear based mechanism. This switching process and the components involved in organizing microtubules around the nucleus are not well understood. Our studies on Drosophila nuclear envelope proteins reveal that lamins, intermediate filament proteins that line the inner nuclear membrane, and components of the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex are necessary for the formation of nuclear MTOCs in Drosophila muscles. We have generated Drosophila models of skeletal muscle laminopathies, a collection of diseases caused by mutations in genes encoding nuclear envelope proteins. These models recapitulate many aspects of human muscle disease, such as Emery-Dreifuss muscular dystrophy. Studies using these models revealed that muscles expressing specific mutant lamins show a loss of nuclear MTOC formation. Normally, microtubules are arranged in a cage-like structure around the nucleus; however, the mutant lamins cause a loss of the microtubular cage and the microtubules are arranged in arrays parallel to the longitudinal axis of the muscle. To investigate reasons for the loss of the nuclear MTOCs, we examined the localization of components of the LINC complex, made of proteins that span the inner and outer nuclear membrane and connect in the perinuclear space. Immunostaining with antibodies to a Drosophila orthologue of human nesprin, Klarsicht (Klar), showed abnormal Klar localization in muscles expressing mutant lamins. Consistent with these data, RNAi knock-down of Klar also caused a loss of nuclear MTOCs, as evidenced by immunostaining. Taken together, these data suggest that lamins organize Klar to build microtubular cages around myonuclei, a structure that protects the nucleus from mechanical stress experienced by muscles. We hypothesize that the loss of the microtubular cage might explain why nuclei from laminopathy patients and disease models show nuclear envelope rupture and increased DNA damage.

**M20**

*Chemical intervention of influenza virus mRNA nuclear export*

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Influenza A viruses are human pathogens with limited therapeutic options. Therefore, it is crucial to devise strategies for the identification of new classes of antiviral medications. The influenza A virus genome is constituted of 8 RNA segments. Two of these viral RNAs are transcribed into mRNAs that are alternatively spliced. The M1 mRNA encodes the M1 protein but is also alternatively spliced to yield the M2 mRNA during infection. M1 to M2 mRNA splicing occurs at nuclear speckles, and M1 and M2 mRNAs are exported to the cytoplasm for translation. M1 and M2 proteins are critical for viral trafficking, assembly, and budding. Here we show that gene knockout of the cellular protein NS1-BP, a constituent of the M mRNA speckle-export pathway and a binding partner of the virulence factor NS1 protein, inhibits M mRNA nuclear export without altering bulk cellular mRNA export, providing an avenue to preferentially target influenza virus. We performed a high-content, image-based chemical screen using single-molecule RNA-FISH to label viral M mRNAs followed by multistep quantitative approaches to assess cellular mRNA and cell toxicity. We identified inhibitors of viral mRNA biogenesis and nuclear export that exhibited no significant activity towards bulk cellular mRNA at non-cytotoxic concentrations. Among the hits is a small molecule that preferentially inhibits nuclear export of a subset of viral and cellular mRNAs without altering bulk cellular mRNA export. These findings underscore specific nuclear export requirements for viral mRNAs and phenocopy down-regulation of the mRNA export factor UAP56. This RNA export inhibitor impaired replication of diverse influenza A virus strains at non-toxic concentrations. Thus, this screening strategy yielded compounds that alone or in combination may serve as leads to new ways of treating influenza virus infection and are novel tools for studying viral RNA trafficking in the nucleus.

M21
Structure of the C9orf72 complex haploinsufficient in ALS/FTD
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Mutation of C9ORF72 is the most prevalent defect in amyotrophic lateral sclerosis (ALS) and frontal temporal degeneration (FTD). Together with hexanucleotide repeat expansion, haploinsufficiency of C9ORF72 contributes to neuronal dysfunction. We determined the structure of the C9orf72-SMCR8-WDR41 complex by cryo-EM. C9orf72 and SMCR8 are both longin-DENN domain proteins, while WDR41 is a beta-propeller protein that binds to SMCR8 such that the whole structure resembles an eye slip hook. Contacts between WDR41 and SMCR8 spray drive lysosomal localization in amino acid starvation. The structure suggested that C9orf72-SMCR8 was a small GTPase activating protein (GAP). We found that C9orf72-SMCR8-WDR41 is a GAP for Arf family small GTPases. These data rationalize the function of C9orf72 both in normal physiology and in ALS/FTD.

M22
An intrinsically disordered, glycine-rich protein governs mycobacterial actin-based motility
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Examining how microbial pathogens manipulate the eukaryotic actin cytoskeleton during infection has yielded significant insight into the regulation of actin dynamics. Some intracellular pathogens co-opt the host actin polymerization machinery at the microbial surface to drive movement within and between host cells in a phenomenon known as actin-based motility. Mycobacterium marinum, a model organism to study the significant human pathogen Mycobacterium tuberculosis, stimulates actin-based motility...
within host cells by recruiting and stimulating the host proteins WASP and/or N-WASP to nucleate actin filaments through the Arp2/3 complex. However, the bacterial protein governing this phenomenon has remained elusive. Here, we report the identification of the *M. marinum* actin-based motility factor, which we designate mycobacterial intracellular rockets A (MirA). During infection, *mirA* mutant bacteria are unable to recruit WASP/N-WASP to stimulate actin polymerization at their surface, and are unable to spread from cell-to-cell. MirA co-localizes with N-WASP at sites of actin polymerization near or at the bacterial pole. Using an affinity purification/mass spectrometry approach, we show that MirA interacts with N-WASP along with N-WASP binding proteins CDC42 and the WIP family. Curiously, when expressed in eukaryotic cells, MirA also localizes to and stimulates actin polymerization at the surface of host lipid droplet organelles. This propels lipid droplet organelles through the cytoplasm and raises the possibility that MirA may interact with host lipid droplets during infection for an unknown purpose. MirA is a member of the enigmatic PGRS protein family, the largest family of substrates translocated into host cells during mycobacterial infection. PGRS proteins are remarkably glycine-rich, and little is known of their functions due to their lack of homology to other proteins and sequence similarity with each other. Thus, MirA is radically dissimilar to known WASP/N-WASP binding partners and likely uses a distinctive mechanism for activating WASP/N-WASP. Further, dissecting the MirA-WASP interaction will glean new insights into actin regulation as well as reveal how the peculiar mycobacterial PGRS proteins are able to intercept host cell biological pathways.

**M23**

**Defining Mechanisms Underlying Virus Regulation of Mitochondrial Bioenergetics During Infection**

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Alterations of mitochondrial functions and cellular metabolism are hallmarks of nearly all viral infections. As obligate intracellular parasites, viruses rely on mitochondria for the production of biosynthetic precursors and energy necessary for generating new viral particles. The prevalent pathogen human cytomegalovirus (HCMV) alters both mitochondrial structure and metabolism during its replication. However, how HCMV induces oxidative phosphorylation remains unknown. Employing a multidisciplinary approach integrating virology, microscopy, and mass spectrometry-based proteomics, we discover that a previously uncharacterized viral protein, pUL13 targets the mitochondria and increases oxidative phosphorylation during infection. We establish that pUL13 is required for productive HCMV replication. We further demonstrate pUL13 is sufficient to increase cellular respiration, not requiring the presence of other viral proteins. By characterizing the temporal pUL13 functional interaction networks during infection, we discover and validate that pUL13 targets the MICOS complex, a critical regulator of mitochondrial architecture and electron transport chain (ETC) function. Our findings address the outstanding question of how HCMV modulates mitochondria to increase bioenergetic output and expands the knowledge of the intricate connection between mitochondrial architecture and ETC function.

**M24**

**ZeroCostDL4Mic: an open platform to simplify, access and use Deep-Learning in Microscopy**

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Deep Learning (DL) methods are increasingly recognised as powerful analysis tools for microscopy and can carry out many tasks like image segmentation, classification, object detection, denoising. Additionally, their potential to outperform conventional image processing pipelines is now well established. But, despite the enthusiasm and innovations fuelled by DL technology, the need to access powerful and compatible resources and the complexity in setting up the necessary computational tools all lead to an accessibility barrier that most biology-focused laboratories find difficult to cross. Here, we present ZeroCostDL4Mic, an entry-level teaching and deployment DL platform which considerably simplifies access and use of DL for microscopy. This is achieved by exploiting computational resources provided by Google Colab: a free, cloud-based service accessible through a web browser. ZeroCostDL4Mic allows researchers with little or no coding expertise to use some of the most powerful DL networks available today, e.g. U-net and Stardist (segmentation), CARE and Noise2Void (denoising), fnet (artificial labelling), Deep-STORM (super-resolution microscopy), YOLOv2 (object detection) and pix2pix and CycleGAN (image-to-image translation). Importantly the platform allows the user to perform every step of the process necessary to DL: training of the models, quality control of the network output with quantitative and image-based assessments as well as batch analysis on new data once the performance of the model is validated. We also implemented two DL-specific features to the pipeline: (1) Data augmentation allowing the user to artificially increase the diversity of the training dataset and therefore improve generalization performance, (2) Transfer learning allowing the user to exploit the availability of pre-trained models and improve the training of their own models. We demonstrate the application of the platform to study various biological processes including cell migration (tracking and automatic detection of cell shapes), mitochondria dynamics (denoising live-cell 3D microscopy), nanoscale cytoskeleton assembly (super-resolution). We show that the implementation of the networks within the platform can be combined either together or with conventional image analysis to extract meaningful information about the imaged samples. In conclusion, we show that our platform helps democratising the access to DL to a wider range of researchers from the biomedical community and can be used as a stepping stone for laboratories to establish new capabilities. -------- von Chamier, L. Laine R.F. et al. ZeroCostDL4Mic: an open platform to simplify access and use of Deep-Learning in Microscopy. http://biorxiv.org/lookup/doi/10.1101/2020.03.20.000133 (2020)

Mechanobiology of Collective Cell Behaviors

M25

Local, heterogeneous regions of active RhoA drive asymmetric junction contraction

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Convergent extension events during morphogenesis rely on the tight spatiotemporal control over cell-cell junction lengths. RhoA-mediated contractile actomyosin forces, acting at adherens junctions, alter junction lengths as a cyclic ratchet. During such ratcheted contractions, tricellular vertices exhibit sliding behavior, where a motile vertex contracts into a less motile vertex to facilitate asymmetric junction shortening. Failure to facilitate these dynamic, asymmetric junction behaviors results in convergent extension failures and embryonic lethality. While this phenomenon is well documented, the molecular and cellular origin of this asymmetry is unknown. To uncover the mechanism of asymmetric junction
contraction, we use a bottom-up approach with the TULIP optogenetic system to exogenously target a RhoA activator, or RhoGEF, to drive local RhoA activation at cell-cell junctions in model Caco-2 epithelia. Upon uniform junctional RhoGEF localization, we find that this contraction culminates in a surprising and consistent contractile asymmetry. Using the predictive power of computational modeling, we examine the parameters that could specify these asymmetries. Our simulations predict that vertex asymmetry arises from the relative tensions at the tricellular vertices. However, when we experimentally measure vertex stiffness, we find no correlation, suggesting that asymmetry is independent of tissue mechanics. We find that this asymmetry is similarly independent of differential neighbor coupling and basal substrate tethering. By examining the local mechanochemical signaling acting at the junctions, we find that contractile asymmetries arise from stochastic regions of stable, persistent RhoA flares which are skewed to the less motile vertex. Consistent with this, activating half to a third of the junction drives asymmetric contraction where the localization of activation correlates with the less motile vertex. Additionally, at immobile vertices, E-cadherin is recruited to locally stiffen the vertex to slow its movement. Stable RhoA flares partition in regions of low interfacial E-cadherin and high plasma membrane, which also separate into anti-correlated microdomains along the junction. These membrane domains concentrate PIP2 and Anillin to create stochastic regions of stable RhoA that persist upon junction contraction. Here, Anillin increases RhoA’s residence time at the membrane, essentially protecting RhoA from GTP hydrolysis so that these sites can drive contractile asymmetry. Upon RhoA localization, RhoA recruits E-cadherin to immobilize and stiffen that vertex movement. Altogether these data decipher how these functional, contractile units are assembled and coordinated to respond to contractile forces during morphogenesis.

M26
The nature of mitotic forces in epithelial monolayers
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Epithelial cells undergo vast morphological changes during division as, a spread parent cell transitions to a rounded morphology, the mitotic cell elongates and constricts at the center from metaphase to cytokinesis, and finally as the daughter cells reintegrate into the epithelium. These changes occur despite dividing cells being confined by neighboring cells, indicating the need for extracellular force generation. While the forces generated during mitotic rounding are now well understood, the nature of the forces generated after mitotic rounding remain unknown. Here we studied MDCK and MCF10A epithelial monolayers, and measured changes in cell-cell stress during division. We identified two distinct stages of force generation that follow mitotic rounding: (1) protrusive forces along the mitotic axis that drive mitotic elongation, and (2) more uniform outward forces associated with post-mitotic re-scaping. Next, we sought to determine the origin of forces generated during mitotic elongation. Neighboring cells could in principle generate these forces. However, neighboring cells were randomly positioned with respect to the dividing cell’s axis, did not display any consistent movements or adhesion remodeling, and perturbation of cadherin-based adhesions did not diminish observed mitotic forces.
Elongation of the dividing cell, however, was accompanied by deformation of adjacent cells exclusively along the mitotic axis, suggesting that mitotic elongation is driven by forces originating from the dividing cell. Thus, we examined the mechanisms of protrusive force generation within the dividing cell. Volume during division was nearly conserved, and computational modeling showed that with near volume conservation, contraction of the cytokinetic ring should enhance cell elongation. Consistent with this, pharmacological inhibition of cytokinetic ring contraction at metaphase reduced cell elongation and chromosome separation, indicating that extracellular forces powering mitotic elongation originate primarily from cytokinesis, and are important not only for elongation, but also to properly separate genetic material. Deformation of adjacent cells during mitotic elongation was observed in epithelia from *Drosophila*, *C. elegans*, mouse, and *Xenopus* model organisms as well. Our results show that cells dividing within epithelial monolayers generate distinct forces in three mitotic stages: rounding, elongation, and spreading, and that force generation during mitotic elongation is a fundamental aspect of cell division within epithelia.

**M27**

**Trans-epithelial Fluid Pumping Performance of Renal Epithelial Cells and Mechanics of Cystic Expansion**

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Using a novel microfluidic platform to recapitulate fluid absorption activity of kidney cells, we report that renal epithelial cells can actively generate hydraulic pressure gradients across the epithelium. The fluidic flux declines with increasing hydraulic pressure until a stall pressure, at which the fluidic flux vanishes—in a manner similar to mechanical fluidic pumps. The developed pressure gradient translates to a force of 50-100 nanoNewtons per cell. For normal human kidney cells, the fluidic flux is from apical to basal, and the pressure is higher on the basal side. For human polycystic kidney disease (PKD) cells, the fluidic flux is reversed from basal to apical with a significantly higher stall pressure. For mouse cells, complete deletion of PKD-2 gene decreased the apical-to-basal fluid flux but did not reverse it. Molecular studies and proteomic analysis reveal that renal epithelial cells are highly sensitive to hydraulic pressure gradients, developing different expression profiles and spatial arrangements of ion exchangers and the cytoskeleton in different pressure conditions. These results, together with data from osmotic and pharmacological perturbations of fluidic pumping, implicate mechanical force and hydraulic pressure as important variables during morphological changes in epithelial tubules, and provide further insights into pathophysiological mechanisms underlying the development of high luminal pressure within renal cysts.

**M28**

**Calcium signaling contributes to epithelial tight junction remodeling during cell shape changes**

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Cell shape changes challenge epithelial cell-cell adhesion and barrier function. In order for an epithelium to serve as an effective barrier, tight junctions must be remodeled during events that cause cell shape changes, such as morphogenetic movements and cytokinesis. Using live imaging in the epithelium of gastrula-stage *Xenopus laevis* embryos, we have shown that junction elongation correlates with local loss of tight junction proteins, resulting in a leaky barrier. These leaks are rapidly repaired by localized, transient activation of RhoA – termed “Rho flares” (Stephenson *et al*., 2019) – which promote actomyosin-mediated reinforcement of tight junction proteins. However, the mechanism underlying local activation of Rho flares is unknown. Here, we show that a transient increase in intracellular calcium precedes activation of Rho flares and is localized to the site of Rho flares. Further, using drugs to deplete calcium inside the cell, we show that calcium is required for efficient repair of tight junction breaks in a Rho dependent manner. Because Rho flares are associated with junction elongation and a local apical plasma membrane deformation, we propose that mechanically-triggered calcium channels may be an important mechanism by which cells sense and respond to transient leaks in barrier function. Piezo1 is a mechanosensitive calcium channel (MSC) that senses the membrane tension caused by stretch and curvature of the membrane and allows calcium ions to pass through the membrane. We found that Piezo1 localizes to apical cell-cell junctions, and upon inhibiting Piezo1 (using the antagonist GsMTx4), the frequency of barrier breaches and the frequency of Rho flares increases significantly compared to controls. Further, we show that mechanosensitive calcium channel mediated calcium influx is required for efficient reinforcement of ZO-1 and sustained activation of Rho flares, suggesting that the increase in the frequency of barrier breaches is due to impaired repair of ZO-1 loss. We are currently testing the effect of Piezo1 knockdown on the dynamics of calcium influx during Rho flares and the effect on barrier function. Overall, this work will shed light on the basic molecular mechanisms that convert a mechanical cue into a local biochemical signal to regulate barrier function in the vertebrate epithelium.


M29

**Mechanical Priming Regulates Future Invasion of Epithelial Cells through Collagen Remodeling**

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Due to mechanical heterogeneities present in tissue microenvironments, epithelial cells encounter matrices of varying stiffness and fibrosis in their paths of collective invasion. Across a mechanical gradient, we recently showed stiff-primed cells migrate faster even after arriving in soft regions. However, it remains unknown whether past stiff-priming can alter collective cell invasion through 3D collagen matrices. To test this, we developed an *in vitro* model using polyacrylamide (PA) gels of defined stiffness, 0.08 kPa (soft) and 16 kPa (stiff), encased in 3D rat-tail collagen type 1 (COL). Mammary epithelial cells (MECs), MCF10A, were mechanically primed on PA gels for 5 days. Subsequently, cell invasion into COL was tracked for 3 days. We found that stiff-primed MECs invaded farther and faster into the surrounding 2.3mg/ml collagen (COL2.3) compared to the soft-primed ones. After leaving the priming surface, stiff-primed MECs first pulled on collagen fibers, then invaded, which was visualized via Second-Harmonic Generation (SHG) imaging and live movement of embedded beads. This collagen pulling was then quantified using particle image velocimetry. Upon inhibition of myosin activity, via
blebbistatin treatment, COL deformation relaxed, causing an outward motion and a net loss in invasion. We hypothesized that during this priming-dependent collagen pulling, cells might be densifying or crosslinking the collagen before invading through it. To test this possibility of collagen priming before cell invasion, we varied collagen density (1.5 and 3.1 mg/ml) and photo-crosslinked it using Riboflavin (COL2.3+RF). Across these conditions of varying collagen compositions, we saw that higher collagen density or crosslinking both aided invasion, presumably because these matrices better support the higher forces generated by stiff-primed cells. To test the role of cell-cell coordination in priming-dependent invasion, we depleted α-catenin using a lentiviral vector affecting cell-cell adhesions, which led to a loss in collagen deformation and invasion of stiff-primed cells. However, soft-primed cells with α-catenin knockdown increased collagen deformation and collective invasion. Taken together, our results show that past stiff-priming of MECs increases force generation, collagen remodeling, and the subsequent invasion into the surrounding 3D microenvironment. In contrast, soft-priming lowers cellular contractility, restricts collagen remodeling, and thus hampers future cell invasion. In both cases of mechanical priming, cellular force development and cell-cell coordination are needed for collective invasion.

M30

α-catenin regulates cell junction fluidity by cooperative mechanosensing

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Cell adhesions dynamically tune their mechanical properties during tissue development and homeostasis. Fluid connections required for cell mobility can switch to solid links to maintain the mechanical rigidity of epithelial layers. Changes in the composition and clustering of adhesion molecules have been proposed to modulate cell junction fluidity, but the underlying mechanisms are unclear. α-catenin has been shown to play a fundamental role in different adhesion sites. At adherens cell-cell junctions (AJ), α-catenin localizes in cadherin-catenin complexes, where it provides a mechanical link between β-catenin and the actin cytoskeleton. However, its function is controversial owing to the low affinity between actin and the α-β-catenin heterodimer. Outside AJ, α-catenin binds itself to form homodimers that connect the cell membrane to the actin cytoskeleton to promote adhesion and migration, but its mechanosensitive properties are inherently unknown. Here, using ultra-fast laser tweezers (Capitanio et al., Nature Methods, 2012) we show that a single mammalian α-catenin molecule displays very different force-bearing properties depending on whether it is associated to β-catenin or not. We found that a single α-β-catenin heterodimer slips along an actin filament in the direction of force, while a single α-catenin homodimer forms a strong asymmetric catch-bond with actin, in which the bond lifetime increases, and the protein unfolds with force. Importantly, assemblies of multiple α-β-catenin heterodimers show force-bearing and unfolding properties similar to the α-catenin homodimer. Our results indicate that, outside AJ, single α-catenin homodimers act as a mechanical link with the actin cytoskeleton that resists force efficiently. Nonetheless, inside AJ, α-catenin’s capability to hold cell-cell connections under physiological loads critically depends on the recruitment of multiple (5-10) complexes. Our data support a molecular model in which α-catenin clustering and intercellular tension engage a fluid-to-solid phase transition at the membrane-cytoskeleton interface.
Organizational Principles of Genome, RNA, and Synthetic Cells

M31
Coordination of RNA abundance with cell size
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Populations of cells often contain a highly variable number of transcripts for any given gene. This ‘heterogeneity in gene expression’ was originally regarded as noise arising from stochastic transcription events, however it has more recently been shown for many individual genes in human cells that transcript abundance at the single cell level is closely coordinated with the cellular phenotype. For example, one common contributor to transcript abundance variation is cell size, with larger cells containing more transcripts. The mechanistic basis of such ‘size-scaling’ and also more gene-specific heterogeneity of mRNA abundance at the single-cell level are largely unexplored. Previous work on individual genes in human cells indicates that size-scaling is achieved by increasing mRNA production rate with cell size, rather than decreasing degradation. Using RNA metabolic labeling with image-based readout, we now reveal that bulk messenger RNA and ribosomal RNA production rates are highly variable between single cells, yet are closely coordinated with cell size and also cell cycle stage. We have further extended this assay using highly-multiplexed (44-plex) immunofluorescence, which reveals that the abundance and activity of transcription machinery as well as nuclear speckles, global chromatin state and RNA export factors, are all heterogeneous in cell populations, yet are linked to differences in RNA production at the single cell level. In addition to this detailed characterization of unperturbed cells, we have also performed an image-based genome-wide siRNA screen to examine the relationship between transcription rates and cell size in the context of genetic perturbations. After accounting for cell size and cell-cycle stage, the screen revealed a large number of perturbations which result in up-(6%) or down-regulation (3%) of bulk transcript production rate. Systems-level analysis of these genes as well as characterization of the resulting single-cell phenotypes by single-molecule RNA FISH and highly-multiplexed immunofluorescence reveals links between RNA production and degradation in RNA concentration homeostasis.

M32
Fat Droplets Have High Interfacial Tension and are Sufficiently Rigid to Deform and Rupture the Nucleus

Lipid droplets (LD), considered to be intracellular emulsions surrounded by a phospholipid monolayer, are present in fat tissue but have also been identified in other body tissues during disease. Specifically, build-up of lipid droplets has been linked to pancreatic cancer and fatty liver disease. We have previously shown that LD presence in cells undergoing adipogenesis may impose indentions on the nucleus similar to those generated by stiff probes. Our previous studies also showed that high nuclear curvature imposed by pores, stiff probes, or small micronuclei correlates with nuclear rupture, mislocalization of multiple DNA repair factors, and subsequent DNA damage. However, it remains unclear if the bending of nuclear lamin filaments resulting from lipid droplet imposition is sufficient to cause nuclear envelope rupture. Micropipette aspiration experiments indicate a high interfacial tension for physiological fat cells in tissue and for lipid droplet extraction out of the cell. FRAP experiments found no difference in mobility fractions of molecules within the fat droplets or for recovery half times
over the 24°C to 37°C temperature range. Lipid droplet induction into cultured U2OS cells using oleic acid produced predominantly small sized (< 3 µm) droplets that impose high curvature deformations on the nucleus regardless of fixation time over the course of seven days. Droplets in both 2D imaging and 3D migration assays were found to indent the nuclear lamina and are associated with increased mislocalization of the nuclear DNA repair factor Ku80. Finally, LD-containing cells transfected with the cytosolic DNA sensor cGAS demonstrated accumulation of cGAS at indentation sites, indicative of LD-caused nuclear envelope rupture. Understanding how LD physically interact with the nucleus and if they can play a role in DNA damage in tissues where their presence is not common may provide insights into their contribution to cancer initiation and progression.

M33

HP1α is a chromatin crosslinker that dictates nuclear and mitotic chromosome mechanics, morphology, and function

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Chromatin, which consists of DNA and associated proteins, provides not only the genetic information of the cell but also acts as a major mechanical component of the nucleus. Previous studies have shown that increasing histone methylation, a mark often associated with compact heterochromatin, increases nuclear and mitotic chromosome stiffness. Constitutive heterochromatin also includes HP1α (CBX5), a protein unexplored as a mechanical component but that is implicated in heterochromatin function through compaction and/or as a crosslinker. We used a novel HP1α Auxin-inducible Degron (AID) cell line generated by the 4D Nucleome consortium to degrade HP1α in 4 hours and determine its role in nuclear and mitotic chromosome mechanics. Single nucleus isolation and micromanipulation force measurements reveal that HP1α is an essential mechanical component to chromatin-based short extension nuclear force response, while not contributing to lamin A-based strain stiffening at longer extensions. As with other perturbations that decrease chromatin-based nuclear rigidity, degradation of HP1α results in abnormal nuclear morphology, which is a hallmark of human disease. Nuclear mechanics and morphology are partially rescued in HP1α-depleted cells by increasing methylation of histones, suggesting that HP1α and histone methylation levels contribute independently. Rescue experiments reveal that HP1α mechanistically functions as a dimer/crosslinker, as the dimerization mutant HP1αI165E failed to rescue nuclear shape, contrary to HP1αWT. Simulations of nuclear mechanics recapitulate HP1α depletion as loss of chromatin-chromatin crosslinks, but not lamin-chromatin crosslinks. Furthermore, mechanical extension experiments on single mitotic chromosomes reveal that HP1α has a similar mechanical contribution to mitotic chromosomes that aids mitotic alignment and faithful segregation. Thus, HP1α is the first chromatin crosslinker protein shown to provide mechanical support to chromatin and aids key cellular functions.

M34

Modulation of single cell volume and mass growth by intrinsic and extrinsic factors

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Cells that make up the same tissue or organ tend to have a stereotypic physical size, while variability in cell size is a marker of abnormal cell growth. A well-defined cell size with low variance is crucial for the intimate coordination between cell growth and division, which drives physiological functions like cell proliferation, differentiation, and embryonic development. But, how size homeostasis is achieved and what size parameter is measured is still an open question for mammalian cells. In this work, we combine two independent methods for single live cell size measurement: quantitative phase microscopy to measure cell dry mass and fluorescence exclusion, to measure cell volume, on a number of cell types over a complete cell cycle. Specifically, we aim to: 1) Understand fluctuations in mass and volume growth during cell-cycle and, 2) Study mechanisms that couple mass and volume thus modulating cell density. We show that cells grow linearly and steadily in mass without many plateaus, unlike volume, over the complete cell cycle. Additionally, mass added across each cell cycle is independent of the initial mass of the cell, corresponding to the same adder-like behaviour observed for volume (Cadart et al Nat Com 2018). We also show that the density of cells is not constant, and instead the cells are born denser as mass increase starts immediately at mitotic exit whereas volume increase is delayed in new born cells (Zlotek-Zlotkiewicz, JCB 2015). Our initial results show that density starts to decrease several hours into the cell cycle and is proportional to mean mass growth rate rather than volume. Density perturbations are also seen during cell spreading on adhesive substrates. We aim to understand the factors which modulate the density and mass/volume coupling which might also correspond to dynamic cell shape changes due to migration/spreading in adhesive cultured cells. This work aims at a fundamental understanding of how cells grow and regulate their size. It will also elucidate functional consequences of modifications in mechanisms of mass/volume coupling on cellular functions. References: 1. Cadart, C., Monnier, S., Grilli, J., Sáez, P. J., Srivastava, N., Attia, R., ⋯ Piel, M. (2018). Size control in mammalian cells involves modulation of both growth rate and cell cycle duration. Nature Communications, 9(1), 3275. https://doi.org/10.1038/s41467-018-05393-0 2. Zlotek-Zlotkiewicz, E., Monnier, S., Cappello, G., Le Berre, M., & Piel, M. (2015). Optical volume and mass measurements show that mammalian cells swell during mitosis. The Journal of Cell Biology, 211(4), 765-774. https://doi.org/10.1083/jcb.201505056

M35
Size-dependent increase in RNA Polymerase II initiation rates mediates gene expression scaling with cell size
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Cell size varies during the cell cycle and in response to external conditions. This necessitates a tight coordination, or “scaling”, of cellular components quantities with cell volume in order to maintain biomolecules concentrations and cell density. In fission yeast, mRNAs quantities, global transcription rates and RNA polymerase II (RNAPII) occupancy of most genes have been found to scale with cell size, revealing that transcription and cell volume are coordinated globally. Here, we used a combination of single-molecule in situ hybridisation (smFISH) and mathematical modelling in single cells to uncover the precise molecular mechanisms that control transcription scaling with cell size. We found that linear scaling of mRNA quantities occurs in single cells during a normal cell cycle. Transcription of both constitutive and periodic genes is a Poisson process with transcription rates scaling with cell size, and
without evidence for transcriptional off states. In diploid cells, transcription rates of individual gene units do not scale with cell size, instead both copies contribute partially to scaling. In multinucleated cells, transcription scaling is coordinated with nuclear volume and local cytoplasmic area but not overall cell size. In addition, we find that scaling is regulated at the level of initiation of RNAPII transcription and not elongation, pause-release or mRNA degradation and that RNAPII is a limiting factor. We show using real-time quantitative imaging that this is mediated by concentration independent recruitment of RNAPII onto chromatin. Integrating our observations in a mechanistic model of RNAPII mediated transcription, we propose that scaling of gene expression with cell size is the consequence of competition between genes for limiting RNAPII.

M36

Vascular Smooth Muscle Cell Phenotype is Regulated by IncRNA Heterogeneous Expression

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Our understanding of the 10^6 disease-causing single nucleotide polymorphisms (SNPs) in the genome is limited as the vast majority are located in non-coding regions. We previously found that SNPs in the non-coding locus with the strongest association with coronary artery disease (CAD), i.e. 9p21, affects vascular smooth muscle cells (VSMCs) contractility (Lo Sardo et al, Cell 2018); during CAD, VSMCs switch from a contractile to a “synthetic” or proliferative phenotype. Using induced pluripotent stem cells (iPSCs) from patients homozygous for risk (R) or non-risk (N) SNPs along with knock out cells (R KO), we differentiated iPSCs into VSMCs to study how variants altered phenotype. We found that between and within patients, there was significant heterogeneity in adhesion strength—even within a clone—when sorting cells using a microfluidic device. Adhesion heterogeneity correlated with differences in synthetic and contractile VSMCs as they were morphological, contractile, and proliferative differences between subpopulations of the same clonal population; however, adhesion heterogeneity was not consistent between clones as a greater percentage of cells detached for both R patients compared to R KO and N patients at the same shear stress, i.e. adhesion sorting into uniform populations was specific to each clone and cell autonomous. We further hypothesized that variable IncRNA expression within and between clones could drive heterogeneous expression resulting in variable penetrance of a synthetic phenotype. While all VSMCs stained positive for VMSC markers, RNA sequencing revealed VSMCs with the R genotype had reduced collagen binding integrin expression. Between and within patient clones, we universally found that the more weakly adherent fractions expressed more ANRIL, a IncRNA within 9p21, compared to the more strongly adherent fractions, suggesting that it plays a role in regulating VSMC adhesion and promotes a synthetic phenotype. RNA sequencing of these fractions between and within patient clones will also be used to further identify differentially expressed genes regulated by ANRIL in synthetic and contractile phenotypes. This work provides new perspectives on VSMC phenotype heterogeneity regulated by non-coding loci.
Quality Control and Intracellular Trafficking

M37
Mitochondria health and deterioration under nutrient stress are dependent on lipid trafficking pathways setup and controlled by vacuolar phase-separated microdomains
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Recent work in the budding yeast, *Saccharomyces cerevisiae*, has suggested that vacuole and mitochondrial activities are coordinated to permit survival under starvation and lifespan extension. Among the findings supporting this are that during nutrient stress sterol-enriched microdomains similar to those found in giant unilamellar vesicles arise on the vacuole’s surface, and the integrity of these domains directly affects lipid droplet (LD) uptake into the vacuole and long-term cell survival. Despite the existence of the phase-separated, vacuolar microdomains, it remains unclear whether the domains are necessary for the vacuole’s crucial role as regulator of mitochondria function, and if so, what functions the domains could serve. Here, we address these questions by showing that failure of the vacuole to phase partition its membranes during starvation leads to mitochondria dysfunction and reduced cell lifespan due to a disruption of lipid trafficking pathways necessary for mitochondrial growth and activity. We demonstrate that the phase-separated, vacuolar microdomains arise from autophagic delivery of sterol-enriched membranes from endosomes, a pathway triggered uniquely by acute glucose starvation. Once formed, the domains initiate a vacuole-derived lipid pipeline for delivering lipids into pathways for LD biogenesis and mitochondrial growth under starvation. The partitioned vacuole microdomains also permit more effective vacuole acidification, likely facilitating LD digestion and fatty acid release for mitochondrial use in energy production. We further find that the bioenergetic state of mitochondria can modulate the existence of phase-separated, vacuolar microdomains in a feedback mechanism, with impaired mitochondria causing the vacuole microdomains to disappear. These data indicate that mitochondria health and deterioration under nutrient stress are dependent on lipid trafficking pathways setup and controlled by vacuolar phase-separated microdomains.

M38
The Role of Calnexin in Regulating Proteostasis of RESET Substrates
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RESET is a protein quality control pathway that involves the clearance of select misfolded secretory pathway proteins out of the endoplasmic reticulum (ER) to the Golgi via vesicular transport [1]. RESET substrates include misfolded, disease associated mutants of prion protein and other GPI-anchored proteins [1], and select transmembrane proteins including amyloid precursor protein. RESET contrasts with ER associated degradation and autophagy pathways, which retain misfolded proteins for degradation at the ER. During RESET, misfolded proteins are released by the ER-resident chaperone, calnexin (CNX), and requisitely associate with p24 or Tmp21 for vesicular transport to the Golgi. The misfolded proteins subsequently transit the cell surface en route to lysosomes where they are destroyed. Thus, steady-state turnover of RESET substrates is concomitant with ER-export. Furthermore, physiological and non-physiological chemical ER stressors dramatically enhance ER-export and consequent lysosomal degradation. Here we address the questions (i) what are the mechanisms regulating constitutive ER-export of RESET substrates during steady-state conditions and (ii) how do
physiological ER-stress conditions enhance ER-export of RESET substrates for subsequent degradation? Our results suggest that the flux of RESET is regulated by competition of other unfolded/misfolded proteins for CNX-binding under steady-state conditions. Chemically blocking new expression of CNX substrates dramatically inhibits steady-state ER-export and degradation of RESET substrates. Conversely, an increase of competitor binding interactions with CNX during physiologically induced ER-stress conditions, including the upregulation of inflammatory glycoproteins, increases the flux of ER-export of RESET substrates. Critically, acutely triggering degradation of CNX by overexpressing E3 ligase Nixin/ZNRF4 causes the rapid release of RESET substrates out of the ER through the RESET pathway for downstream lysosomal degradation, which emphasizes CNX’s central role in regulating RESET. We present a new model that displacement from CNX by newly synthesized CNX substrates regulates the constitutive and stress-enhanced turnover of RESET substrates. These findings may offer insights into the connection between ER stress, inflammation, and the extracellular deposition of misfolded proteins as seen in prion and Alzheimer’s diseases. [1] Satpute-Krishnan et al. ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway. Cell. 2014 July 31

M39
**ZNF574 is a quality control factor monitoring ribosome biogenesis**


Each eukaryotic ribosome consists of 4 ribosomal RNAs (rRNAs) and ~80 ribosomal proteins. The synthesis, maturation and transport of individual ribosomal components and their assembly into ribosomal subunits requires the intervention of ~200 protein factors, and numerous small nucleolar RNAs. All of these components take part in hundreds of error-prone reactions. The complexity of the ribosome assembly pathway makes it possible for mutations and/or environmental stress to introduce mistakes with potential deleterious consequences for cell viability and human health. Indeed, mistakes during ribosome assembly have been linked to diseases, such as ribosomopathies and cancer. However, how cells detect and cope with defective biogenesis intermediates is not well understood. Here, we identified a novel quality control factor that monitors the assembly of the large subunit, and determined how this factor ensures continuous ribosome production. We used a mutant ribosomal protein to block biogenesis of a fraction of the maturing large subunits in mammalian cells. We discovered that these trapped biogenesis intermediates are rapidly detected and degraded. We used CRISPR-Cas9-based screening to identify factors necessary for the detection and clearance of these defective ribosomes. One of the top hits in our screen was a zinc-finger protein with unknown function, ZNF574. Depletion of ZNF574 leads to accumulation of defective 60S biogenesis intermediates, which sequester away biogenesis factors, causing global inhibition of ribosome production, cytotoxic stress, and ultimately cell death.

M40
**Distinct regions of Chlamydia trachomatis effector protein IncE interact with components of retromer and endosome vesicle fusion machinery**

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Chlamydia trachomatis is the leading bacterial cause of sexually transmitted infections. During infection this obligate intracellular pathogen resides in a membrane-bound compartment, the inclusion, which it modifies by secretion of a unique class of effector proteins, the inclusion membrane proteins (Incs). Incs are ideally poised at the host-pathogen interface to interact with host proteins. Given the challenges of performing conventional genetics with Chlamydia, we used affinity-purification mass spectrometry to systematically identify interaction partners of Incs. This analysis identified members of the retromer (sorting nexins (SNX) 5 and 6) as well as components of the vesicle fusion machinery (syntaxins (STX) 12 and 7) as predicted high affinity interaction partners with IncE. We have previously published that the C-terminal 24 amino acids of IncE are necessary and sufficient to bind to SNX5 and displace its native interaction partner, the Cation-Independent Mannose-6-Phosphate receptor. We now provide data that IncE engages with STX12 and STX7, components of the soluble NSF attachment protein receptor (SNARE) vesicle fusion machinery that are involved with early and late endosome fusion, respectively. Using a C. trachomatis strain that overexpresses IncE, we show that endogenous STX7 and STX12, along with STX7-associated SNAREs Vamp7/8, co-immunoprecipitate with IncE. Transfection studies reveal that amino acids 37-100 of IncE are necessary and sufficient to co-immunoprecipitate with endogenous STX7 or STX12. We further demonstrate that the SNARE and transmembrane domains of STX7 or STX12 are required for interaction with IncE. Live cell microscopy of C. trachomatis-infected cells reveals STX7- and STX12-containing vesicles adjacent to and fusing with the inclusion. Depletion of STX7 results in decreased inclusion formation and production of infectious progeny. Finally, we generated a C. trachomatis IncE null mutant strain and complemented the mutant strain with plasmids encoding informative IncE deletion constructs. We show that IncE is require for efficient fusion of inclusions. Whether the interaction of IncE with the retromer components and the vesicle fusion components is simultaneous, sequential, or mutually exclusive is under investigation. Together, these findings suggest that IncE is a master multi-tasker that has the capacity to both interfere with retromer function and to recruit a specific subset of vesicle to potentially promote fusion of nutrient-rich endosomal vesicles with the inclusion.

Controlling intracellular transport to tune cell-based (immuno)-therapies
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Recent years have brought a major change in the paradigm of tumor treatment. In addition to the collection of classical treatments (surgery, chemotherapy, radiation), the use of immunology-based approaches has opened new roads with promising results for cancer treatment. In particular, cell-based therapies based on the expression of a Chimeric Antigen Receptor (CAR) at the surface of T cells (CAR-T) that mimics T cell receptor activity have shown impressive effects to cure certain B cell malignancies. Two products, Kymriah and Yescarta, were approved by the U.S. Food and Drug Administration (FDA) and by European Medicines Agency (EMA) starting from 2018. Although very efficient, these treatments come with critical side effects, that can be life threatening. These includes cytokine release syndrome and neurotoxicities, that are correlated with an overactivation and expansion of the CAR-T cells. In addition, only limited success has been obtained against solid tumors or against non-immunogenic tumors for example. Because these cell-based therapies rely on efficient protein trafficking, we decided to exploit our knowledge in intracellular transport control to try to improve cell-based therapy and in particular CAR-T cell. More generally, we strongly believe that the power of cell biology can be
harnessed to develop novel tunable cell-based therapies. Several years ago, we have set-up a system (the Retention using selective hooks, or RUSH, system), which is now widely used, that allows to synchronize the transport of virtually any secretory protein cargos. Cargos bearing a Streptavidin-binding peptide (SBP) are retained in the endoplasmic reticulum (ER) using a stably located hooking protein fused to Streptavidin. Addition of biotin allows rapid release of the cargo and its export to its target compartment. We modified our system so that the transport and secretion of CAR-T proteins, or modulating factors like certain cytokines or chemokines, can be controlled by the simple addition of biotin in living cells, and in particular in T cells. With this system, that we call CELLTune, we could elicit interferon or IL2 signaling for example. We could also reprogram T cell using such a modulated CAR-T so that toxicity can be controlled by biotin. We are now bringing the system in vivo. Our first results indicate that we can control the secretion of cytokines from reprogrammed and grafted cells in vivo by injecting mice with biotin. We could also stimulate release by simple feeding. We are now carrying out experiments to validate the use of our tuned system (CELLTune) to control tumor eradication in vivo. Bringing such a tunable system in the clinic may be a game changer in many situations and we are now implementing a large set of regulatory factors in our system to broaden its use.

M42
Cell migration in the very fast lane
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Actin based cell migration is usually studied in metazoan cells that migrate at around 5-10 micrometers per minute. We investigate a protozoan pathogenic cell, the Plasmodium sporozoite, which initiates malaria infections after the bite of an infected mosquito, and migrates in vivo an order of magnitude faster (1). To achieve this high-speed, Plasmodium evolved unusually stable microtubules that determine a peculiar crescent shape (2) and avoids to stick to its substrates (3). Rapid actin dynamics are essential for migration as are a number of proteins unique to Plasmodium (4,5). Retrograde flow of surface receptors generates forces in the dozen pN range and parasites are highly sensitive to substrate elasticity, yet promiscuously bind a multitude of substrate ligands (6,7). We will present recent studies investigating Plasmodium motility and show how they led to a new concept for experimental vaccination against malaria. (1) Amino et al., Nat Med 2006, (2) Spreng et al., EMBO J 2019, (3) Munter et al., Cell Host Microbe 2009, (4) Douglas et al., PLoS Biol 2018, (5) Kehrer et al., PLoS Path 2016, (6) Quadt et al., ACS Nano 2016, (7) Klug et al., eLife 2020
Tuesday, December 8, 2020

Building the Microtubule Arrays of the Neuron

M43

Microtubule polymerisation is required for microtubule bundle organisation in axons and coordinated by GSK-3β

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Axons are the cable-like, up to meters long extensions of neurons which electrically wire our bodies; their backbones are formed by parallel bundles of microtubules (MTs). In ageing and certain neurodegenerative diseases, axons form pathological swellings where MT bundles disintegrate into criss-crossed curling MTs. Here we report that defects in MT polymerisation cause axonal bundle disorganisation and explain the underlying mechanisms. We found that Eb1, XMAP215 and Tau cooperate during MT polymerisation in axons of Drosophila and Xenopus neurons. Loss of either of them causes shorter axons, reduced size, number, speed and lifetime of Eb1 comets, and enhanced MT disorganisation. Mechanistically, we found that Eb1 and XMAP215 are co-dependent to localise at polymerising MT plus ends, and Tau maintains Eb1 at plus ends by competitively preventing it from being sequestered to MT shafts. Any loss of Eb1 at plus ends affects the Eb1-Shot-mediated guidance of polymerising MTs into parallel bundles (Voelzmann et al., 2017, Sem Cell Dev Biol 69, 40ff), thus causing disorganisation. Such combined action of various MT regulators has to be orchestrated. A candidate orchestrator of MT regulation is GSK-3β (glycogen synthase kinase 3β): it is known to phosphorylate a number of MT-binding proteins and has established links to neurodegenerative diseases and axon regeneration. I found that functional up- or down-regulation of GSK-3β causes MT disorganisation in the fly and rat neurons, and have identified Shot as an important GSK-3β target, providing a means to change MT behaviours in a time and location-specific manner. By using Drosophila neurons for these studies, it is possible to reach across a wide range of MT regulators and to explore their orchestration, thus enabling better systemic understanding that will eventually have important translational potential. Funded by the Leverhulme Trust and BBSRC

M44

Significance of Minus-end-out Microtubules in Dendrites

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Microtubules (MTs) are intrinsically polarized and their arrangement has been studied in neurons: Axons have uniform plus-end-out polarity, while dendrites have minus-end-out polarity or mixed polarity in invertebrates and vertebrates, respectively. We aim to understand what aspects of axon and dendrite identity are controlled by their different arrangements of MTs. We developed an in-vivo system in Drosophila neurons that eliminates minus-end-out MTs in a subset of dendrites. This mosaic system has let us study both normal dendrites (90% minus-end-out) and flipped neurites (100% plus-end-out) in a single cell. In this system, we assessed the effect of flipped MT orientation on a variety of neuronal
properties: when we studied morphology, we saw a reduced number of tertiary branches in flipped neurites but no change in length. This suggests MT polarity partially affects morphology. Cargo trafficking is mediated by molecular motors that use MT tracks. In this system, a decrease in localization of ribosomes (dendritic marker) and an increase in ANF-containing dense core vesicles, supports that MT polarity affects cargo transport. We tested whether flipped neurites responded like axons or dendrites to severing because they tend to react through distinct machinery - severing of dendrites, but not axons, activates fidgetin and causes increased MT dynamics. The flipped neurites failed to show this dendritic-specific MT-response, resembling axons. Axonal MTs are known to show less MT dynamicity. The average number of growing MTs in dendrites were more than twice the number of MTs in flipped neurites. This suggests that the MTs in flipped neurites are more stable, resembling axons. One of the hallmark features of an axon is the presence of a diffusion barrier at its base. Surprisingly, fluorescence recovery after photobleaching (FRAP) assays in this mosaic system showed the presence of an additional diffusion barrier near the base of the dendritic arbor consisting of both flipped and normal neurites. This result is in contrast to control neurons where diffusion barrier is present only in axons. Overall, flipped neurites tend to lose dendritic features. Based on these results and previous studies, we propose that MT polarity directly affects cargo distribution, response to injury, and partially affects morphology, MT dynamicity and the presence of diffusion barrier.

M45
**Activation of cytoplasmic dynein through microtubule crossbridging**
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Cytoplasmic dynein is the main microtubule minus-end-directed transporter of cellular cargo in animal cells. Cytoplasmic dynein also functions in the organisation and positioning of mitotic spindles and the formation of ordered microtubule arrays in neurons and muscle. Activation of the motor for cargo transport is thought to require formation of a complex with dynactin and a cargo adapter. Here we show that recombinant human dynein can crossbridge neighbouring microtubules and can be activated by this crossbridging to slide and polarity-sort microtubule bundles. While single molecules of human dynein are predominantly static or diffusive on single microtubules, they walk processively for 1.5 μm on average along the microtubule bundles they form. Speed and force output of dynein are doubled on bundles compared to single microtubules, indicating that the crossbridging dynein steps equivalently on two microtubules. Our data are consistent with a model of auto-activation through the physical separation of dynein motor domains when crossbridging two microtubules. This enables cytoplasmic dynein to function effectively as a microtubule organiser and transporter without needing to first form a complex with dynactin and a cargo adapter.

M46
**Doublecortin contributes to neuronal morphogenesis by stabilizing the growth cone microtubule network.**
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Doublecortin (DCX) is a microtubule-associated protein that is frequently mutated in lissencephaly-spectrum neurodevelopmental disorders in which immature neurons fail to migrate correctly through the developing cortex. However, the molecular role of DCX in early neuronal morphogenesis is not
understood. To better analyze how DCX controls microtubule function in developing cortical neurons we generated a human induced pluripotent stem cell (hiPSC) line in which DCX is tagged with eGFP at the endogenous locus by CRISPR/Cas9 genome editing. DCX-eGFP dynamics at physiological expression levels confirm our previous findings that DCX specifically decorates straight GDP-lattice microtubules (Ettinger et al., Curr. Biol. 26:1549-1555). Notably, DCX-eGFP never tracks growing MT ends in neurons and is absent from the EB1/3 domain at growing microtubule ends. In addition, DCX only binds to microtubules in the growth cone and is absent from the neurite microtubule bundle. Although this DCX gradient is mirrored by a reverse gradient of microtubule acetylation, acetylation does not alter the DCX-microtubule interaction. Instead experiments with pharmacological inhibitors indicate that DCX-microtubule binding is controlled by dephosphorylation in the growth cone. Taxanes reverse the microtubule geometry specificity of DCX. DCX dissociates from straight growth cone microtubules and instead relocalizes to curved microtubule segments within minutes. In neuronal growth cones, this taxane-induced DCX relocalization is accompanied by microtubule bending and retraction from the growth cone periphery leading us to hypothesize that DCX functions to stiffen and/or stabilize growth cone microtubules. To directly test this hypothesis, we next generated a DCX knockout hiPSC line. Compared with control neurons, DCX knockout growth cones display substantial structural differences of the microtubule network with increased curling of microtubules in the central growth cone domain and reduced penetration of microtubules into the growth cone periphery. Although these DCX knockout hiPSCs still differentiate, neurite extension is impaired. Thus, we propose that impaired growth cone dynamics during brain development underlie the neurodevelopmental phenotype of lissencephaly-associated DCX mutations.

M47

Understanding the role of microtubule-associated protein 9 (MAP9) in C. elegans ciliated neurons
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Microtubules are intracellular polymers involved in various essential processes ranging from cell division to maintaining cell structure. These functions rely on specific microtubule organizations that are in part created by microtubule associating proteins (MAPs). While we understand how many MAPs function to help build organizations such as the mitotic spindle or the parallel arrays in axons, the diversity of how MAPs affect other microtubule organizations is not well understood. One such organization is the microtubule doublets found in cilia. Cilia are unique microtubule based cellular projections important for sensing the environment and motility; perturbation in this organelle is the cause of a class of diseases known as ciliopathies. Despite the importance of cilia, little is understood about the unique doublet microtubules that are central to the structure, maintenance, and growth of cilia. This study seeks to understand the role of MAP9, an understudied ciliary MAP, in the recognition/building of microtubule doublets and modulation of other ciliary components. The C. elegans MAP9 family member (MAPH-9) is expressed in cilia, however, nothing is known about its function or role. We have endogenously tagged MAPH-9, and in Vivo find that it only localizes to cilia and specifically to the areas of the cilia where doublets are found. This is a novel localization for a MAP and may provide insight into the mechanism of building or recognition of doublet microtubules. We also find that knockout of MAPH-9 perturbs localization of dynein, a component important for cilia function. Parallel studies in Vitro show MAPH-9 causes singlet microtubules to adopt bundled conformations and confirms its binding to doublet microtubules. These in Vitro and in Vivo data together suggest a unique role for MAPH-9 to
directly and specifically bind microtubule doublets affecting the transport of motors essential for robust function of cilia.

M48

**ATAXIN-2 is an essential regulator of cytoskeletal dynamics in Drosophila neurons**

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Ataxin-2 (Atx2) is a highly conserved RNA binding protein. Atx2 undergoes polyglutamine expansion leading to amyotrophic lateral sclerosis (ALS) or spinocerebellar ataxia (SCA). However, the normal physiological functions of Atx2 remain unknown, likely because of functional redundancy between Atx2 and Atx2-like genes in mammals. Here we use the powerful genetics of *Drosophila*, that have only a single Atx2 gene, to show that Atx2 is essential for normal cytoskeletal dynamics and for neurodevelopment. We depleted Atx2 specifically in neurons and found severe impairments in cytoskeletal dynamics both in microtubule and actin networks. Microtubules became hyper-stabilized, as demonstrated by increased acetylation and resistance to microtubule depolymerising drugs, and kinesin-driven microtubule-microtubule sliding was inhibited. Similarly, we found F-actin was hyper-stabilized as shown by resistance to actin depolymerising drugs. Further, in neurons both *in vitro* and *in vivo* we found that transport of multiple cargoes was severely decreased. Beyond the defects in the cytoskeleton, we found neuron-specific depletion of Atx2 caused many morphological defects in the nervous system of third instar larvae. These include reduced brain size, impairments in optic lobe innervation and decreased dendritic arborization in sensory neurons. Defects in the nervous system of these larvae caused loss of the ability to crawl and were lethal at the pupal stage. Taken together, these data mark Atx2 as a master regulator of the cytoskeleton and denote Atx2 as an essential gene in neurodevelopment, as well as a neurodegenerative factor. These data will provide insight into therapeutic interventions for Atx2 polyglutamine disorders.

**Cancer Cell Signaling, Dissemination, and Therapy**

M49

**Histone H3.3 mutations that drive pediatric gliomagenesis suppress both epigenetic regulation and mitotic phosphorylation of the pericentromere**

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Diffuse midline gliomas are lethal brain tumors in children, with poor prognosis and limited treatment options providing only short-term benefits. The majority have a lysine-to-methionine substitution at residue 27 in histone H3 genes – predominantly in the H3.3 variant. Previous work has shown that K27M causes global reductions in H3Lys27 tri-methylation (H3K27Me3), comprehensive epigenetic reprogramming, and is a key driver in gliomagenesis. Here we show that the H3.3K27M mutation also inhibits H3.3 Ser31 phosphorylation, resulting in chromosome missegregation during mitosis, and generating chromosomally unstable daughter cells. Ser31 is 1 of 5 amino acid substitutions differentiating H3.3 from canonical H3.1. Mitotic phosphorylation of H3.3Ser31 is restricted to pericentromeric heterochromatin, where it plays a role in chromosome segregation. We show that the K27M mutation affects neighboring Ser31 phosphorylation and pericentromeric heterochromatin organization. We demonstrate that (i) H3.3K27M protein is defective for Ser31 phosphorylation in vitro;
(ii) K27M mutant cell lines are chromosomally unstable and have significantly decreased mitotic Ser31 phosphorylation; and (iii) CRISPR-reversion of H3.3K27M to Lys27 restores phospho-Ser31, Lys27 tri-methylation, and significantly DECREASES chromosome instability. Expression of H3.3K27M or non-phosphorylatable H3.3S31A mutants in human cells (normal diploid or glial tumor) results in chromosome missegregation; co-expression of phospho-mimetic H3.3K27M/S31E that restores phospho-Ser31 suppresses this. Chromosome missegregation in normal cells triggers a p53-dependent G1 cell cycle arrest blocking the proliferation of aneuploid daughters. However, p53WT cells expressing H3.3K27M or S31A failed to arrest following missegregation. Finally, in an RCAS-TVA mouse model of glioma, 100% of H3.3WT-P2A-PDGFB control mice survived 100 days, with only low grade tumors. However, mean survival of H3.3K27M-P2A-PDGFB or H3.3S31A-P2A-PDGFB mice was 81 and 68 days: 100% of H3.3S31A mice developed diffuse high-grade tumors with palisading necrosis. H3.3S31A is WT for Lys27 tri-methylation and thus, loss of Ser31 phosphorylation alone is oncogenic. Together our work demonstrates that the K27M mutation decreases mitotic fidelity by decreasing the amount of core histone H3.3 at the pericentromere that can be phosphorylated during mitosis. This loss of histone phosphorylation is sufficient to drive gliomagenesis in our mouse model, even in the absence of epigenetic defects. Our study demonstrates that oncogenic histone H3 mutations act via two distinct mechanisms: global re-programming of the epigenome and chromosome instability induced by loss of Ser31 phosphorylation at the pericentromere.

M50
Regulation of MLK3 and JNK during ovarian cancer cell cycle progression
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Mixed lineage kinase 3 (MLK3) is a serine/threonine MAP3K that promotes the activation of multiple mitogen-activated protein kinase pathways, and is required for invasion and proliferation of ovarian cancer cells. Often, ovarian cancer patients are diagnosed when ovarian cancer is in the late stages and treatment options are limited. Therefore, understanding the molecular mechanisms that cause aberrant division of ovarian cancer cells will give us insights to create novel cancer therapies. It has been demonstrated that inhibition of MLK activity causes G2/M arrest in HeLa cells; however, the regulation of MLK3 during ovarian cancer cell cycle progression, and specifically during mitosis, is not known. We hypothesize that a Cyclin-dependent kinase (CDK) regulates MLK3 during ovarian cancer cell division. To investigate this regulation, ovarian cancer cells were treated with nocodazole to arrest cells in mitosis, and MLK3 protein was analyzed by western blot. Interestingly, we found that MLK3 exhibited a mobility shift in the nocodazole-treated cells. To test whether this is a phosphorylation event, we performed a phosphatase treatment assay. We found that phosphatase treatment eliminated MLK3 mobility shift, which suggests that the shift is due to phosphorylation. To determine the protein kinase responsible for this phosphorylation during M phase, we tested several kinase inhibitors for their effects on MLK3 phosphorylation and found that inhibition of CDK1 prevented MLK3 phosphorylation. This suggests that CDK1 promotes phosphorylation of MLK3 in mitotic ovarian cancer cells. Furthermore, using a double thymidine block assay, we found that phosphorylation of MLK3 occurred exclusively during M phase. We also observed that c-Jun N-terminal Kinase (JNK), a downstream target of MLK3, was activated in G2 when CDK2 activity is increased and then inactivated at the beginning of mitosis concurrent with the increase in CDK1 and MLK3 phosphorylation. To test whether CDK1 and CDK2 can phosphorylate MLK3, we decided to perform an in vitro kinase assay. We discovered that CDK1 and CDK2 can phosphorylate
MLK3 in vitro; furthermore, CDK1 phosphorylates MLK3 in vitro on Ser\textsuperscript{548}, and CDK2 phosphorylates MLK3 in vitro on Ser\textsuperscript{770}. Taken together, we propose a model where phosphorylation of MLK3 by CDK1 and CDK2 during G2 and M is important for the regulation of JNK activity and proper G2/M transition in ovarian cancer cells. Future investigations will be focused on characterizing the mechanisms by which CDK1 and CDK2 regulate MLK3 and JNK during G2 and M, and the significance of this phosphorylation in mitotic progression.

M51
Lateral macrophage mitochondrial transfer functions as a signaling source in cancer cells
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Unconventional modes of cellular communication such as the sharing of cellular components can have large impacts in the context of homeostasis and disease. Previously, we discovered that macrophages transfer cytoplasm to melanoma cells in vivo, with 70% of cytoplasm-receiving melanoma cells disseminating from the primary transplantation site. These data suggest macrophages transfer a “package” of molecules that influence recipient cell behavior. However, central questions remain: 1) what is identity of the transferred molecules? and 2) how do these molecules influence cell behavior? Here, we report that cell contact-dependent mitochondrial transfer occurs from macrophages to cancer cells, and recipient cancer cells exhibit an increased proliferative index. By genetically labeling mitochondria in primary human macrophages and breast cancer cells, we found that donated mitochondria do not incorporate into the host mitochondrial network, but instead persist as a separate, intact population. Interestingly, with the use of live dyes and a genetically encoded biosensor, we found that transferred mitochondria have reduced membrane potential and accumulate high levels of reactive oxygen species (ROS). Furthermore, with the use of a mitochondrial localized antioxidant we have shown that we can inhibit proliferation in recipient cancer cells. Taken together, these data suggest that the transferred mitochondria are serving as a signaling source rather than functioning as an energy-producing organelle. In future studies, we will address sufficiency by introducing purified mitochondria into cancer cells via microinjection and focus on identifying the ROS-responsive signaling pathway(s) underlying the proliferative response. Collectively, these studies will help to elucidate how donated molecules can lead to functional changes in recipient cells and define a type of cellular communication that contributes to disease progression.

M52
Inactivation of the Hippo Tumor Suppressor Pathway Promotes Melanomagenesis
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Melanoma, the deadliest form of skin cancer, is driven in greater than 50% of cases by an activating mutation in \textit{BRAF}, an essential component of the MAPK signaling pathway. Surprisingly, despite its prevalence, expression of oncogenic \textit{BRAF\textsuperscript{V600E}} alone is insufficient to promote melanoma in vivo, and instead elicits the formation of moles. This mirrors human nevogenesis, as human nevi are often \textit{BRAF\textsuperscript{V600E}}-positive and rarely transform into melanoma, with less than .0005% estimated transformations per year. As such, moles represent a significant barrier to melanomagenesis; however,
all the mechanisms by which moles restrain \( \text{BRAF}^{V600E} \)-positive melanocyte transformation remains unknown. Recent studies have shown mutations within \( \text{RAS} \), observed in \(~20\%\) of melanoma cases, can promote the formation of multi-nucleated tetraploid cells which ultimately senesce, preventing their further proliferation and possible transformation. Interestingly, human nevi are often observed to contain multi-nucleated melanocytes of unknown origins. Utilizing a doxycycline-inducible \( \text{BRAF}^{V600E} \) system within immortalized melanocytes, we demonstrate expression of \( \text{BRAF}^{V600E} \), akin to oncogenic \( \text{RAS} \), robustly promotes the formation of multi-nucleated tetraploid cells, similar to those seen within human nevi, via increased mitotic slippage. We find that \( \text{BRAF}^{V600E} \)-positive tetraploid melanocytes exhibit decreased viability due to activation of a major tetraploid tumor suppressor, the Hippo pathway, and that inactivation of this pathway, via disruption of the main Hippo kinases \( \text{LATS}1/2 \), is sufficient to rescue all growth deficits in vitro. Further, we demonstrate that melanocyte-specific inactivation of Hippo signaling, via deletion of murine \( \text{Lats}1/2 \) alone or with \( \text{Braf}^{V600E} \) expression, robustly promotes the formation of Sox10-positive, hypopigmented, spindle cell neoplasms positively diagnosed as melanoma. Moreover, we find heterozygous loss of one allele each of \( \text{Lats}1 \) and \( \text{Lats}2 \), which is observed in \(~15\%\) of human melanoma cases, is also sufficient to promote tumorigenesis. All together, these data suggest the Hippo signaling pathway may represent a novel, previously unappreciated, tumor suppressor in melanoma. Defining how Hippo pathway inactivation promotes melanocyte transformation will be essential to determine if the Hippo pathway represents a new therapeutic target for melanoma treatment.

M53

**Notch signaling contributes to proliferation of glioma cells via the regulation of Hexokinase2**

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Notch signaling, which plays important roles in cell-cell communication, promotes cancer proliferation including glioma cells. However, some types of gliomas are reported to be resistant to Notch inhibitors (Gamma-Secretase Inhibitors, GSIs). We previously found that Notch signaling negatively regulates gene expression of Hexokinase2 (HK2) in glial cells of zebrafish embryos (Notch-HK2 inhibitory pathway). Among glycolytic enzymes, HK2 is known to be involved in cell proliferation. Therefore, we hypothesized that Notch-HK2 inhibitory pathway mediates GSIs resistance of gliomas. In this study, we explored some conditions and factors necessary for the activation of Notch-HK2 inhibitory pathway. We evaluated HK2 expression levels in U87 cells (Human glioma cell line) which were exposed to GSI under various nutrient conditions. As a result, GSI did not affect HK2 levels compared to DMSO controls when U87 were cultured in the normal medium of EMEM + 10% FBS. On the other hand, HK2 was increased by GSI treatment under the condition that glutamine and serum were removed from normal medium (GS-free condition). In addition, U87 cells were co-cultured with Notch ligand expressing cells in order to identify which ligand especially activates Notch-HK2 inhibitory pathway. Under the GS-free condition, Notch activation due to Delta-like1 (Dll1) and Jagged1 induced the decrease in HK2 whereas Dll4 ligand did not. Furthermore, to assess whether HK2 inhibition by Notch also occurs in the in vivo environment, we established a xenograft model of zebrafish in which U87 cells were transplanted in the brain. In comparison with the results of in vitro experiments, the increase of HK2 expression by GSI exposure was more significant in U87 transplanted embryos. We also examined the influence of Notch inhibition on the viability of gliomas using live-cell imaging method. Consistent with previous studies, the viability of U87 was similar between the control group and the GSI-exposed group in xenograft embryos. Taken
together, our findings suggested that glioma cells activate Notch-HK2 inhibitory pathway depending on the nutritional state and the type of Notch ligands, which may lead to low sensitivity to GSIs.

M54

**N-Myristoyltransferase-1 is necessary for lysosomal degradation and mTORC1 activation in cancer cells**

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N-Myristoyltransferase-1 (NMT1) catalyzes protein myristoylation, a lipid modification that is elevated in cancer cells. NMT1 sustains proliferation and/or survival of cancer cells through mechanisms that are not completely understood. We used genetic and pharmacological inhibition of NMT1 to further dissect the role of this enzyme in cancer, and found an essential role for NMT1 in promoting lysosomal metabolic functions. Lysosomes mediate enzymatic degradation of vesicle cargo, and also serve as functional platforms for mTORC1 activation. We show that NMT1 is required for both lysosomal functions in cancer cells. Inhibition of NMT1 impaired lysosomal degradation leading to autophagy flux blockade, and simultaneously caused the dissociation of mTOR from the surface of lysosomes leading to decreased mTORC1 activation. The regulation of lysosomal metabolic functions by NMT1 was largely mediated through the lysosomal adaptor and NMT1 target LAMTOR1. Accordingly, genetic targeting of LAMTOR1 recapitulated most of the lysosomal defects of targeting NMT1, including defective lysosomal degradation. Pharmacological inhibition of NMT1 reduced tumor growth, and tumors from treated animals had increased apoptosis and displayed markers of lysosomal dysfunction. Our findings suggest that compounds targeting NMT1 may have therapeutic benefit in cancer by preventing mTORC1 activation and simultaneously blocking lysosomal degradation, leading to cancer cell death.

**Cellular Regulation in Tissue Development and Regeneration**

M55

**BioID network propagation identifies the TWIST1-dependent chromatin regulatory module directing cranial neural crest migration and specification**

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Protein interactions are critical regulatory events underlying cellular behavior and precise cell fate choices. To identify the cranial NCC regulators cooperating around the master regulator TWIST1 and their hierarchical relationship, we generated the first in situ TWIST1 protein interactome. Leveraging proximity-dependent biotin identification (BioID) proteomic approach, ChIP-seq, and transcriptome analysis, we characterized a NCC chromatin regulatory module (NCC-CRM). The NCC-CRM, for the first time, directly links TWIST1, craniofacial regulator with several chromatin regulators previously involved in the development of neural crest (CHD7) and neuroectodermal lineages (CHD8), as well as with the SET domain-containing H3K36-specific methyltransferase, WHSC1. Combinatorial perturbation of core members of the NCC-CRM in stem cells and mouse embryos revealed stage-specific functionality of the
module. In early neuroectoderm differentiation, the NCC-CRM cooperatively establishes the promoter/enhancer repertoires for patterning genes that predilects NCC while repressed neural stem cell fates. Later in the mature NCCs, the NCC-CRM enhanced the ectomesenchyme potential and cell migration. We have revealed a mechanism of NCC migration and specification coordinated by TWIST1-interacting regulators, which may be implicated in neurocristopathies. Moreover, our finding suggests that the specification of NCCs is accomplished by cross-repression of domains-specific cell fate programs that regulate the dorsal-ventral patterning of neural progenitors in the neural tube. Finally, our work implemented a network-propagation strategy to fully harness the predictive power and integrate various types of tissue-specific datasets. The NCC-CRM derived from the network-propagation is a unifying model regrouping and explaining the molecular functional relationship of previously unrelated rare diseases factors affecting NCC development. With the advent of increasing numbers of protein-protein interaction datasets, this strategy has foreseeable future utilities to explore delicate developmental and disease mechanisms.

M56
The mitophagy receptor NIX drives DRP1-dependent mitochondrial fragmentation to promote epidermal differentiation

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Morphogenesis of the epidermis requires orderly differentiation of keratinocytes to continually regenerate a protective barrier at the body surface. During the final stages of their maturation within the uppermost layers of the stratified epithelium, keratinocytes undergo a unique terminal differentiation process called cornification in which they eliminate intracellular organelles to form compacted, tightly adherent cellular sheets. The mechanisms regulating organelle degradation during construction of the epidermis remain unknown, but could reveal novel strategies to modulate skin barrier function and restore tissue homeostasis in disease. To examine organelle dynamics in a live stratified tissue model, we coupled spinning-disk confocal microscopy with three-dimensional organotypic cultures of human epidermis grown from primary keratinocytes. We focused on the fate of mitochondria during morphogenesis of the epidermis, hypothesizing that mitochondrial breakdown would be an essential step in terminal keratinocyte differentiation. In the uppermost tissue layers, we found that keratinocytes initiate fragmentation, depolarization, and acidification of mitochondria. Interestingly, this programmed mitochondrial degradation in the epidermis coincided with up-regulated expression of the mitophagy receptor NIX, which depended on hypoxia-regulated signaling and reactive oxygen species. Depletion of NIX using CRISPR/Cas9 gene targeting in human keratinocytes inhibited mitochondrial clearing and impaired epidermal maturation. In contrast, ectopic expression of NIX accelerated epidermal differentiation and induced premature mitochondrial fragmentation. While mutation of the NIX LC3-interacting region did not impair its ability to fragment mitochondria, disruption of NIX dimerization ablated NIX-induced mitochondrial breakdown. Exogenous delivery of NIX to undifferentiated keratinocytes enhanced mitochondrial localization of FIS1, a protein receptor for the membrane fission GTPase DRP1. Direct inhibition of DRP1 prohibited NIX-induced mitochondrial fragmentation and impaired the development of epidermal cultures. Together, our data indicate that mitochondrial degradation is a crucial step in human keratinocyte differentiation and we define an
essential developmental pathway operating via the mitophagy receptor NIX and DRP1 to drive proper epidermal morphogenesis.

M57
Genetic chimerism causes regeneration defects in the planarian
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Many basal animals reproduce asexually through fragmentation followed by regeneration, often using multipotent stem cells that are capable of self-renewal and differentiation to rebuild any missing body parts. This process is susceptible to accumulation of somatic mutations and emergence of cheating cells, which may over-proliferate and propagate across generations, neglecting their duties in making somatic tissues. Despite this obvious risk, it remains unknown whether these animals have immunological mechanisms in place to sense the presence of genetically altered cells, and if so, whether the regeneration process is affected. To answer these questions, we created chimeric planarians by surgically stitching together tissue fragments of two distinct genotypes of Schmidtea mediterranea. We observed that, while the chimeras integrated anatomically across fusion sites, the fusion caused an aberrant activation of activin signaling along with its non-canonical downstream effectors such as p38. Consistent with the previous observations that p38 activation represents the innate immune response to bacterial and fungal infections in planarians, our chimeras also strongly suppressed viral infections. To test if genetic chimerism has a penalty on regeneration, we amputated the chimeras and found that, when cut in the posterior portion of the body, the tail piece failed to regenerate a new head. This regeneration defect can be rescued by knocking down activin or wnt signaling, which is known to function downstream of activin pathway in the planarian. Our work reveals that activin signaling regulates both allogeneic response and regeneration, coordinating the balance between the two programs.

M58
A Spatial Gradient of Cell Size Regulates Genome Activation and Vertebrate Early Development
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In early embryogenesis, embryos divide without growth. After the rapid reduction in cell volume, the embryos turn on nascent transcription in a process named zygotic genome activation (ZGA), in which development switches from maternal to zygotic control. ZGA is essential for subsequent gastrulation, but the pattern of ZGA and its implication on embryonic development is less studied. Through metabolically labeling of nascent transcripts, we have identified a spatial and temporal pattern of ZGA in Xenopus embryos that occurs initially in small cells at the animal pole and is delayed by two hours in cells of the vegetal pole. This spatiotemporal pattern tightly correlates with the gradient of cell sizes present in the blastula embryos, which is induced by the asymmetric cell division and the difference in division timings. To study the developmental importance of the evolutionally conserved cell size
gradient and the resulting ZGA pattern, we developed an embryo temperature controller to eliminate or even reverse those patterns. It works by slowing down the division rate of the cells in the animal pole and speeding up the division of the vegetal cells. When setting different temperatures in animal poles and vegetal poles, cell division rates of each regions respond to the temperature accordingly. By generating *Xenopus* blastula embryos with reversed cell size gradients, we found that the spatial pattern of ZGA is also reversed; genome activation occurs first in the vegetal pole and is drastically delayed in the animal pole. Intriguingly, the ‘reversed’ embryos still have a similar threshold of cell size for genome activation onset, regardless of which the temperature gradient they have experienced. Those ‘reversed’ embryos tend to arrest in gastrulation as they fail to close the blastopore before neurulation. This work suggests that regulated spatial patterns of ZGA are important for coordinated embryonic development, particularly in blastula embryos that contain gradients of cell sizes.

M59

**Mapping the complex paracrine response to hormones in the human breast at single-cell resolution**

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The human breast undergoes continuous remodeling in response to fluctuations in ovarian hormones across menstrual cycles. These dynamic changes also increase cancer susceptibility. Many of the biological effects of hormones in the breast are indirect—only a minority of cells express hormone receptors, and this subset of hormone-responsive (HR+) cells initiates a complex network of paracrine signals that act on other cell types in the breast. Moreover, there is a high degree of woman-to-woman heterogeneity in breast architecture, cellular composition, and hormone-responsiveness, but the sources of this variability and its relationship to differences in cancer susceptibility are not well understood. By leveraging this heterogeneity, we developed a computational framework to identify coordinated changes in cell signaling states across cell types in the premenopausal human breast. Using this approach, we identify a set of highly correlated gene expression programs representing the *in situ* hormone response in HR+ cells and downstream paracrine signaling in other cell types. We find that the paracrine signaling response in basal cells depends on both the per-cell transcriptional response of HR+ cells to hormones and the overall proportion of HR+ cells in the epithelium. Further, we show that person-to-person heterogeneity in hormone-responsiveness in the breast is directly linked to two factors known to reduce premenopausal breast cancer risk. Prior pregnancy reduces the per-cell hormone transcriptional response in HR+ cells, whereas obesity reduces the overall proportion of HR+ cells. Together these findings demonstrate how the inherent inter-sample heterogeneity in single cell data can be used to provide mechanistic insight into the regulatory networks that control hormone signaling and modify breast cancer susceptibility.

M60

**Development and function of circadian rhythms in human intestinal organoids**

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Circadian rhythms regulate diverse aspects of gastrointestinal physiology ranging from the composition of microbiota to motility. However, development of the intestinal circadian clock and detailed molecular mechanisms regulating circadian physiology of the intestine remain largely unknown. The lack of appropriate human model systems that enable organ- and/or disease-specific interrogation of clock functions is a major obstacle hindering advancements of translational applications using chronotherapy. In this report, we show that both pluripotent stem cell-derived human intestinal organoids engrafted into mice and patient-derived human intestinal enteroids (HIEs) possess robust circadian rhythms, and demonstrate circadian phase-dependent necrotic cell death responses to Clostridium difficile toxin B (TcdB). Intriguingly, mouse and human enteroids demonstrate anti-phasic necrotic cell death responses. RNA-Seq data show ~4% of genes are rhythmically expressed in HIEs. Remarkably, we observe anti-phasic gene expression of Rac1, a small GTPase directly inactivated by TcdB, between mouse and human enteroids. Importantly, the observed circadian time-dependent necrotic cell death response is abolished in both mouse enteroids and human intestinal organoids (HIOs) lacking robust circadian rhythms. Our findings uncover robust functions of circadian rhythms regulating critical clock-controlled genes (CCGs) in human enteroids governing organism-specific, circadian phase-dependent necrotic cell death responses. Our data highlight unique differences between mouse and human enteroids, and lay a foundation for human organ- and disease-specific investigation of clock functions using human organoids for translational applications.

**Cilia and MTOCs in Action**

**M61**

**A newly identified primary cilium mechanically controls meiotic chromosomal pairing and germ cell morphogenesis in zebrafish and mouse**

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In meiosis, chromosomal pairing is established in the nucleus, but also depends on cytoplasmic mechanical forces. In pairing, telomeres associate with perinuclear microtubules (MTs) via Sun/KASH proteins on the nuclear envelope (NE). These centrosome-based MTs facilitate telomere rotation on the NE, shuffling chromosomes for homology searches. Ultimately, telomeres are pulled towards the centrosome and cluster on the NE, with their chromosomes looping toward the other side. This configuration stabilizes pairing and is called the zygotene chromosomal bouquet. How bouquet forces are generated and regulated in the cytoplasm is poorly understood. Here, we identify a primary cilium in zebrafish, that connects to the bouquet machinery and is required for its formation. We detected tubulin cables that extended from oocyte centrosomes as cellular protrusions. We confirmed these cables are primary cilia by molecular markers such as tubulin acetylation and glutamylation and Arl13b, as well as by ultrastructure analysis. Zygote oocytes develop in a compact cellular organization called the germline cyst. Three-dimensional rendering of entire cysts in EM resolution by SBF-SEM and confocal data, showed that cilia emanate from the centrosomes of zygote oocytes, and tangle between them like scaffolds throughout the cyst. The cilia formed specifically concomitantly with bouquet formation, and live imaging of ovaries showed cilia movements that coordinated with bouquet chromosomal rotations. These findings unravel a cytoskeletal cable system, extending from the cilium through the centrosome and MTs, to Sun/KASH-bound telomeres on the NE, as potential machinery for chromosomal pairing and as a physical framework for the cyst. We performed functional analysis using
the ciliary mutants, *cep290, kif7*, and their combination. In developing mutant ovaries the cilia were truncated or lost, bouquet formation was delayed and halted, and germline cysts were disintegrated. This resulted in degenerated ovaries in adults and female sterility. The centrosome MTOC was functional, confirming that ciliary defects likely directly cause these effects. Thus, the zygotene cilium is required for chromosomal pairing and germline cyst morphogenesis, and subsequently for ovarian development and fertility. Furthermore, we found that the zygotene cilium is conserved in germline cysts in mouse fetal ovaries and in zebrafish spermatocytes. Our work uncovers a novel concept of a primary cilium as a newly identified player in meiosis that mechanically regulates chromosomal pairing and physically reinforces germ cell morphogenesis.

**M62**

**Dancing basal bodies: basal bodies bend in response to ciliary force**

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Motile cilia beat asymmetrically to generate directed fluid flow and cell motility. Basal bodies (BBs) nucleate and anchor motile cilia, acting as the physical linkage to transmit forces from beating cilia to the cell for fluid mobility. BBs are composed of nine triplet microtubules arranged by linking proteins to form a nm-scale cylinder. BBs are $1/10^{th}$ the length of cilia and lack ciliary dynein motors that generate the ciliary beat stroke. Additionally, in contrast to ciliary doublet microtubules, BBs are formed of triplet microtubules. Thus, relative to cilia, basal bodies have largely been considered to be rigid anchorage structures that are structurally unaffected by beating ciliary forces. However, specific BB mutants produce unstable BBs that exhibit ciliary force dependent BB structural defects. We asked whether BBs are normally dynamic structures that resist ciliary forces. Using electron microscopy imaging techniques (HPF-FS / EM tomography), we captured BBs and cilia during the ciliary beat stroke to visualize BBs relative to cilia position in their beat stroke. BBs undergo consistent physical deformation at defined triplet microtubules during the ciliary beat pattern. This suggests that indeed ciliary beating forces produce dynamic changes to BBs. In addition, BBs display movements relative to cilia position in the beat stroke. These data provide the first description of BB structural dynamics and BB movements relative to ciliary beating.

**M63**

**Arp2/3-dependent functions in ciliary assembly and beyond in Chlamydomonas**

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The microtubule-dependent cilium has long been studied in the green alga Chlamydomonas reinhardtii due to its conservation of structures and assembly mechanisms relative to the cilium found on nearly all human cells. Our previous work found roles for actin in formation and trafficking of post-Golgi vesicles to cilia, as well as in the synthesis of ciliary proteins and organization of the gating region at the base of cilia. We have additionally tried to tease apart actin dependent ciliary functions and the nature of the actin filaments responsible for ciliary assembly by studying the Arp2/3 complex and branched actin. By using an inhibitor of the Arp2/3 complex, CK-666, and genetic mutants of the ARPC4 subunit of the
seven-member Arp2/3 complex, we found that branched actin defective cells, similar to cells completely devoid of actin filaments, have defects in ciliary maintenance and dramatically impaired ciliary assembly. However, surprisingly, loss of Arp2/3 has a more dramatic phenotype for early incorporation of pre-existing ciliary proteins than for incorporation of newly synthesized proteins. Furthermore, we found that Arp2/3 interacts with both the conventional actin, IDA5, and the secondary, divergent actin found in Chlamydomonas, NAP1 to maintain cilium length and gating. Based on these data and others from our lab showing a role for Arp2/3 complex in membrane protein endocytosis, we hypothesize that branched actin plays a larger role in initial membrane remodeling for ciliary regrowth relative to post-Golgi trafficking of newly synthesized protein later in the assembly process.

M64

**MTOC function at the centrosome and the ciliary base is driven by specific PCM protein**

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During development, the centrosome acts as a microtubule organizing center (MTOC) in mitotic cells, forming radial arrays essential to separate cellular components between daughter cells. Microtubules are organized at the centrosome by pericentriolar material (PCM) complexes. After mitosis, during cell differentiation the fate of the centrosome is going to be diverse depending of cell fate. In differentiated epithelial cells, the centrosome is inactivated, losing its PCM and having the MTOC function redirected to other cellular components, in contrast, other ciliated cells have the centrosome and its centrioles repurposed into basal bodies to ciliary structures. We are using *C. elegans* as a model to characterize MTOC recruitment and regulation at the centrosome. In *C. elegans*, the PCM is organized around the centrioles in a partial concentric overlay of protein spheres, in which the two main scaffolding protein, SPD-2/CEP192 and SPD-5 which localize the microtubule nucleating complex γ-TuRC to the centrosome, partially overlap delimiting two main body - an inner sphere with both partner and an outer sphere with SPD-5 (Magescas et al 2019). Upon differentiation centrosome lose their PCM, leaving ‘naked’ centrioles, like in intestinal cells. Interestingly, analysis of SPD-5 and γ-TuRC proteins revealed that ciliated sensory neurons, SPD-5 and most MTOC proteins remains at the ciliary base while centriolar protein and SPD-2 are lost. Those complexes organize the MTOC function at the base of cilia and are critical for ciliogenesis, as depletion of SPD-5 produces aberrant cilia. Interestingly, contrary to the current model, similar loss of SPD-2 in cycling intestinal cells prior to differentiation doesn’t result in the loss of SPD-5 at the centrosome, nor it impairs centrosomal function. Based on our data we propose that the PCM is composed of different subcomplexes revolving around SPD-5 that are differently regulated, working in parallel to drive the MTOC function.

M65

**Co-movement of astral microtubules, organelles and F-actin suggests aster positioning by surface forces in frog eggs**

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How bulk cytoplasm generates forces to separate post-anaphase microtubule (MT) asters in *Xenopus laevis* and other large eggs remains unclear. To inform models of aster separation movement, we
reconstituted aster separation in *Xenopus* egg extracts. Co-imaging of MTs, endoplasmic reticulum (ER), mitochondria, acidic organelles and F-actin showed that all cytoplasmic components moved together as a continuum. Interacting asters formed dynamic Voronoi tessellations, and the artificial centrosomes nucleating MTs moved to more regular spacing over time. Inhibition experiments showed that both dynein and actomyosin contributed to separation movement. To determine where dynein generates forces on asters we tracked organelles and observed a burst of inward movement at the growing aster periphery, which mostly halted inside the aster. This observation suggests that dynein exerts pulling force on MTs primarily at the aster periphery, and not all along their length as proposed previously. To interpret the actomyosin component of aster movement, we developed an active gel model in which actomyosin generates active stress proportional to its density. Disassembly of actomyosin caused by local AURKB activity along edges between neighboring asters generates anisotropies in density that trigger directed, continuum movement. These observations call for new models of aster positioning based on surface forces and internal stresses.

M66

The post-transcriptional regulations of centrosomal *plp* mRNA in *Drosophila*

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Centrosomes, functioning as microtubule organizing centers, are composed of a proteinaceous matrix of pericentriolar material (PCM) that surrounds a pair of centrioles. *Drosophila* Pericentrin (Pcnt)-like protein (PLP) is a key component of the centrosome that serves as a scaffold for PCM assembly. The disruption of *plp* in *Drosophila* results in embryonic lethality, while the deregulation of Pcnt in humans is associated with MOPD II and Trisomy 21. We recently found *plp* mRNA localizes to *Drosophila* embryonic centrosomes. While RNA is known to associate with centrosomes in diverse cell types, the elements required for *plp* mRNA localization to centrosomes remains completely unknown. Additionally, how *plp* translation is regulated to accommodate rapid cell divisions during early embryogenesis is unclear. RNA localization coupled with translational control is a conserved mechanism that functions in diverse cellular processes. Control of mRNA localization and translation is mediated by RNA-binding proteins (RBPs). We find PLP protein expression is specifically promoted by an RNA-binding protein, Orb, during embryogenesis; moreover, *plp* mRNA interacts with Orb. Importantly, we find overexpression of full-length PLP can rescue cell division defects and embryonic lethality caused by *orb* depletion. We aim to uncover the mechanisms underlying embryonic *plp* mRNA localization and function and how Orb regulates *plp* translation.

Intracellular Signaling and Spatial Organization of Metabolism

M68

Spatial Organization of Hexokinase 1 via O-GlcNAcylation

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Neuron harness energy through a stepwise breakdown of more complex substances, such as glucose, into simpler ones via a plethora of highly interconnected metabolic reactions. Polarity, non-uniform nutrient access, and energy needs are fundamental features of many cell types. However, our understanding of the molecular mechanisms that spatially partition metabolic biochemistry within the
cytoplasm is cursory at best. Here, we investigate how metabolic enzymes work coherently and efficiently within the complex cellular cytoarchitecture of neurons. Glycolysis is the first step in the breakdown of glucose, which occurs in the cytosol of a cell and starts with the first-rate limiting enzyme Hexokinase (HK). In this study, we report a new molecular mechanism that regulates HK1 (brain Hexokinase) activity and mitochondrial localization via the metabolic sensor enzyme O-GlcNAc transferase (OGT). OGT catalyzes a reversible posttranslational modification by adding a GlcNAc sugar moiety to serine and threonine residues (O-GlcNAcylation). The catalytic activity of OGT is regulated by intracellular UDP-GlcNAc concentrations, which fluctuate proportionally in response to glucose flux through the hexosamine biosynthetic pathway. In this study, we show that HK1 is dynamically modified with O-GlcNAcylation at its regulatory domain. O-GlcNAcylation of HK1 is elevated when OGT activity is upregulated. We further characterize that O-GlcNAc modification increases mitochondrial HK1, and also enhances both glycolytic and mitochondrial ATP production rates. We demonstrate that HK1 is enriched explicitly on the stationary mitochondrial pool in neurons. Our findings reveal a key molecular pathway which couple glycolysis to mitochondrial function via OGT, and how spatial metabolic organization is used for energy homeostasis in neurons.

M67
Altered tryptophan metabolism in growth and proliferation
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Tumors display increased uptake and processing of nutrients to fulfill the demands of rapidly proliferating cancer cells. Seminal studies have shown that the proto-oncogene MYC promotes metabolic reprogramming by altering glutamine uptake and metabolism in cancer cells. How MYC regulates the metabolism of other amino acids in cancer cells is not fully understood. Recently we found that transformation by MYC leads to an increase in the uptake and processing of the essential amino acid tryptophan. Tryptophan is the least abundant amino acid in all proteins, and its metabolism can give rise to biologically active byproducts including serotonin and metabolites in the kynurenine pathway. MYC induces the expression of the tryptophan transporters SLC7A5 and SLC1A5 and the enzyme AFMID involved in the conversion of tryptophan into kynurenine. SLC7A5, SLC1A5, and AFMID are elevated in colon cancer cells and tissues, and kynurenine is significantly greater in tumor samples than in the respective adjacent normal tissue from patients with colon cancer. Compared with normal human colonic epithelial cells, colon cancer cells are more sensitive to the depletion of tryptophan or kynurenine. Blocking enzymes in the kynurenine pathway causes preferential death of established colon cancer cells and transformed colonic organoids. Kynurenine, but no other tryptophan metabolite, functions as an oncometabolite to drive proliferation of MYC-transformed cells, in part, as a ligand for the transcription factor aryl hydrocarbon receptor (AHR), which regulates genes involved in protein synthesis.

M69
Seipin traps triacylglycerols in the ER to facilitate their nanoscale phase separation
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Seipin is a disk-like oligomeric ER protein important for lipid droplet (LD) biogenesis. We recently reported that seipin facilitates triglyceride (TAG) delivery to the LD. It is not known how this seipin-
mediated TAG flux is achieved. Here we investigate this through biomolecular simulations bridged to in vivo experiments in cells. Our data indicate that seipin can trap TAGs in the ER bilayer via the luminal hydrophobic helices of the protomers delineating the inner opening of the seipin disk. This promotes the nanoscale phase separation of TAGs at a concentration that by itself is insufficient to induce TAG clustering in a lipid membrane. We identify a key residue in the α3 helix and show that mutating it compromises the ability of seipin complexes to sequester TAG in silico and to promote TAG transfer to LDs in cells. Whilst a mutant compromised in TAG sequestering interacts poorly with promethin, the binding of nascent wild-type seipin complexes to promethin is promoted by TAGs. Together, these results suggest that seipin traps TAGs via its luminal hydrophobic helices, serving as a catalyst for seeding the TAG aggregate from dissolved monomers inside the seipin ring, thereby generating a favorable promethin binding interface.

M70  
**Structural mechanism for amino acid‐dependent Rag GTPase nucleotide state switching by SLC38A9**  
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The mechanistic target of rapamycin complex 1 (mTORC1) couples cell growth to nutrient, energy and growth factor availability. mTORC1 is activated at the lysosomal membrane when amino acids are replete via the Rag guanosine triphosphatases (GTPases). Rags exist in two stable states, an inactive (RagA/B GDP:RagC/DGTP) and active (RagA/BGTP:RagC/D GDP) state, during low and high cellular amino acid levels. The lysosomal folliculin (FLCN) complex (LFC) consists of the inactive Rag dimer, the pentameric scaffold Ragulator, and the FLCN:FNIP (FLCN‐interacting protein) GTPase activating protein (GAP) complex, and prevents activation of the Rag dimer during amino acid starvation. How the LFC is released upon amino acid refeeding is an outstanding question. We used a combination of cryo electron microscopy (cryo‐EM), hydrogen‐deuterium exchange mass spectrometry (HDX‐MS) and in vitro GTPase and nucleotide exchange assays to show the cytoplasmic tail of the human lysosomal solute carrier family 38 member 9 (SLC38A9) destabilizes the LFC and thereby triggers GAP activity of FLCN:FNIP2 toward RagC. The cryo‐EM structures of Rags in complex with their lysosomal anchor complex Ragulator and the cytoplasmic tail of SLC38A9 in the pre and post GTP hydrolysis state of RagC show that SLC38A9 binds in the cleft between the two G domains of the Rag heterodimer. This binding site overlaps with FLCN:FNIP2 in its GAP‐inactive state observed in the LFC explaining the destabilizing effect of SLC38A9 toward the LFC which in turn activates FLCN:FNIP2 GAP activity. SLC38A9 dissociation would then enable GDP to GTP exchange on RagA, generating the active Rag dimer. Subsequent recruitment and activation of mTORC1 with respect to the MiT/TFE transcription factors would then lead to inactivation of MiT/TFE‐dependent transcription, completing the chain of events.

M71  
**Spatial and Temporal Regulation of Sensors of a Mitogen Activated Protein Kinase Pathway Contribute to a Pathway‐Specific Response**  
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MAPK pathways are evolutionarily conserved signaling modules that regulate cell differentiation and the response to stress. In most organisms, MAPK pathways are regulated by proteins that can function in multiple pathways. Despite sharing components, different MAPK pathways sense specific stimuli and generate a unique response. The budding yeast *Saccharomyces cerevisiae* contains multiple MAPK pathways that share components. One allows cells to undergo filamentous growth in response to nutrient availability (fMAPK), and the other allows cells to respond to changes in external osmolarity (HOG). The pathways are regulated by different mucin-type sensors that converge on a common protein module that contains the tetraspan protein, Sho1p; Rho GTPase, Cdc42p; and the MAPKKK, Ste11p. We previously showed that the activity of the fMAPK pathway is cell-cycle regulated and peaks at M/G1. By comparison, the HOG pathway did not show cell-cycle regulation. The different regulatory patterns of these MAPK pathways could be explained by differences in the levels of the signaling mucins that regulate these pathways. The abundance of the mucin Msb2p (fMAPK) increased at M/G1. By comparison, the levels of the mucin Hkr1p (HOG), did not change throughout the cell cycle. We also found that the tetraspan protein, Sho1p, which interacts with both mucins, showed a dynamic localization pattern throughout the cell cycle and localized to the mother-bud neck during M/G1. Using indirect immunofluorescence to measure active Kss1p, the MAP kinase of the fMAPK pathway, we found that Sho1p localization at the mother-bud neck correlated with increased fMAPK activity. The importance of Sho1p localization at the mother-bud neck for fMAPK function was further corroborated in a conditional septin mutant, *cdc12-6*, which had mislocalized Sho1p and low fMAPK activity compared to the wild type. Moreover, a version of Sho1p that was uniformly targeted to the plasma membrane through N-terminal myristoylation, prevented Sho1p from enriching at the mother-bud neck and showed a reduction in fMAPK pathway activity. Together, the study identifies two ways MAPK pathways exhibit selectivity at the sensor level: one by altering the levels of a mucin sensor, and the other by regulating the localization of its direct effector. Spatial and temporal regulation of MAPK pathways may direct their functions in specific contexts.

M72

**Spatial heterogeneities in cortical mechanics control mitotic spindle positioning**

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Proper orientation of the mitotic spindle is crucial for cell fate and tissue morphogenesis. In animal cells, a conserved complex of Gai, LGN and NuMA recruits the motor protein dynein, which exerts pulling forces on astral microtubules to position the spindle. Upstream of these cues, recent studies have highlighted a role for external mechanical forces and tissue tension in regulating spindle orientation, likely through their effect on cortical mechanics. However, whether and how the mitotic spindle responds to small spatial changes in its mechanical environment is unclear. To investigate this, here we use optogenetics to locally modulate cortical tension and show that the mitotic spindle rapidly responds to changes in cortical mechanical properties. Remarkably, localized activation of RhoA-GTP at the poles of mitotic MDCK epithelial cells leads to re-orientation of the spindle 45° away from the poles, resulting in a change in the division axis of these cells. In contrast, localized activation at the equator does not perturb spindle orientation. Spindle re-orientation only occurs when spatial heterogeneities in cortical tension appear in metaphase after which spindle position remains fixed throughout anaphase until cytokinesis. Inhibition and localisation experiments indicate that spindle reorientation depends on cortical actomyosin contractility with spindles rotating towards intermediate myosin levels. We further
demonstrate that cortical pulling forces generated by NuMA/dynein and polymerisation of astral microtubules are necessary for spindle re-orientation. Finally, both experimental and modelling studies show that in addition to cortical pulling forces, pushing forces exerted by astral microtubules at regions of increased tension are essential for spindle re-orientation. Thus, local differences in mechanical properties appear to control mitotic spindle orientation to position the division axis towards regions of lower cortical tension. Spindle re-orientation may represent a key mechanism to fine tune cell packing within tissues to minimize tissue tension stresses.

**Nuclear Dynamics Across Models Systems**

**M73**  
*Drosophila* Wash and the Wash regulatory complex function in nuclear envelope budding  
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Nuclear envelope (NE) budding is a phenomenon wherein large macromolecular complexes, which are too large to be exported through nuclear pores, are packaged and expelled through the nuclear membranes. This pathway shares many similarities with the nuclear egress of herpesviruses, suggesting NE budding may be an endogenous host pathway utilized by the virus. Although a general outline of the cellular events occurring during endogenous NE budding can be inferred from the proteins known to be involved and visualization of the process, very little is yet known about the molecular machinery and mechanisms underlying the physical aspects of NE bud formation. Using genetics, biochemistry, and super-resolution imaging, we identify Wash, the Wash regulatory complex (SHRC), capping protein, and Arp2/3 as novel molecular components involved in the physical aspects of NE bud formation in a *Drosophila* model system. Interestingly, depletion of WASH in salivary gland nuclei causes wrinkled nuclei and loss of nuclear buds, whereas knockdown of SHRC results only in the loss of nuclear buds. Using double immunofluorescent staining and point mutations we show that Wash affects NE budding in two ways: 1) indirectly through general nuclear lamina disruption via an SHRC-independent interaction with Lamin B leading to inefficient NE bud formation, and 2) directly by blocking NE bud formation along with its SHRC. We also show that Wash requires Arp2/3 and capping protein for NE bud formation, suggesting Wash’s ability to form new branched actin networks may be needed. By mass spec and native PAGE, we show that Wash acts as part of multiple, separable nuclear complexes to affect its diverse set of nuclear properties/events. We are currently investigating the specific function of the protein components of each of these complexes to further elucidate Wash’s mechanistic role in NE budding and other nuclear processes. NE budding is emerging as an important endogenous nuclear process, as well as sharing many similarities with herpesvirus nuclear egress mechanisms, opening up potential avenues for exploration in both normal and disease biology.

**M74**  
*Atg39 Drives Remodeling of Inner and Outer Nuclear Membranes to Selectively Capture Nuclear Cargo for Autophagy*  
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Selective autophagy uses cargo receptors to degrade superfluous or damaged subdomains of organelles. While we have a clear conceptual understanding of how cytoplasmic organelles are cleared by
autophagy, how nuclear contents can be selectively cleared across both the inner and our nuclear membranes by a primarily cytosolic machinery remains enigmatic. To address this question, we have taken advantage of a recently identified membrane-spanning nuclear cargo receptor in budding yeast, Atg39. Interestingly, overproduction of Atg39 is sufficient to drive the formation of nuclear blebs that form independently of other autophagy proteins. Consistent with the conclusion that these blebs may represent functional intermediates in nucleophagy, we observe a remarkable sequestration of some, but not other, inner nuclear membrane (INM) proteins within the structures. Further, these blebs lack nuclear pore complexes (NPCs) suggesting that this mechanism is distinct from recently established NPC-phagy pathways. An investigation of the ultrastructure of the nuclear blebs by correlative light electron microscopy and tomography showed that they are composed of an expanded outer nuclear membrane (ONM) containing intraluminal membrane-bound structures derived from the INM. Atg39 thus drives coordinated remodeling of both nuclear membranes. This nuclear envelope remodeling likely occurs from the outside-in as we show using a split-GFP reporter system that, even when overexpressed, Atg39 accumulates specifically at the ONM. Thus, Atg39 targeting to the ONM and the formation of nuclear blebs with INM-cargo likely occurs through a physical coupling of both nuclear membranes. Consistent with this idea, we demonstrate that the formation of the nuclear blebs as well as cargo capture depends on the presence of the Atg39 lumenal domain. More systematic structure-function analyses of the lumenal domain of Atg39 supports the presence of unique ONM targeting and membrane-remodeling regions, suggesting that there may be the ability to directly interact with the INM. This is currently being investigated within minimal in vitro membrane reconstitution platforms. Consistent with the conclusion that the lumenal domain of Atg39 is critical for the execution of nucleophagy under physiological conditions, there is no autophagy-dependent degradation of known Atg39 cargos upon deletion of the Atg39 lumenal domain under nitrogen starvation conditions. We suggest a model that incorporates a novel translumenal bridge complex that connects the ONM and INM as a key element of nucleophagy.

M75

_Traip_ suppresses chromosome bridges via a mitotic DNA repair mechanism to control brain size

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Microcephaly is a failure to achieve proper brain size and neuron number during development. Most microcephaly-linked genes function either at the mitotic spindle or in DNA damage repair (DDR). Microcephaly is thought to reflect a loss of proliferation and/or increased cell death. However, few microcephaly genes are well-studied in neurogenesis, and whether the two classes of mutants cause microcephaly via a common pathway is unknown; thus, we are systematically characterizing microcephaly genes in _Drosophila_. Here, we studied the microcephaly gene _Traip_, known to function in DDR, and found that a mitotic DDR function suppresses DNA bridges to control brain size. We show that _Traip_ mutant flies have brain structural defects, fewer neurons, and marked loss of neural stem cells (NSCs) via caspase-dependent cell death. _Traip_ mutant NSCs have increased DNA damage in interphase, consistent with a DDR function. However, we were surprised to also find polyploid _Traip_ mutant NSCs, suggesting mitotic failure. High resolution live fluorescence microscopy of _Traip_ mutant NSCs revealed frequent mitotic DNA bridges, providing a possible explanation for the observed polyploid cells via cytokinesis failure. We characterized endogenously tagged _Traip_ transgenes; while _Traip_ is nuclear in interphase, in mitosis it localizes dynamically on spindles, furrow, and midbody, thus ensuring that it would encounter any DNA bridges. A _Traip_ variant lacking the nuclear localization signal (ΔNLS) is
evicted from the nucleus and fails to suppress DNA damage during interphase; however, ΔNLS Traip localizes properly in mitosis and rescues Traip mutant brain phenotypes, showing that a mitotic Traip function is sufficient to suppress microcephaly. A possible link between DNA bridges and mitotic failure is the abscission checkpoint; inhibiting the abscission checkpoint suppresses Traip mutant brain phenotypes, suggesting this is a key downstream effector. Together, our work challenges current thinking about the relationships between DDR, mitosis and microcephaly by showing that, rather than merely repairing DNA damage during interphase, the primary function of Traip is to monitor for and resolve mitotic DNA bridges and thus rescue NSCs from mitotic failure. Now, using Traip as a model microcephaly gene, we are using whole brain imaging and 3D analysis to screen for suppressors to uncover more downstream pathways in microcephaly. To date, we have found roles for neuronal stress response, Toll signaling, and caspase-dependent cell death. We are now testing whether these pathways also mediate the phenotypes of other microcephaly genes, including both DDR and mitotic spindle genes, and targeting these pathways as potential therapeutic targets to minimize neuron loss in microcephaly.

M76
Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B
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Chromosome segregation is the essential biological processes in which chromosomes are partitioned into the two daughter cells during cell division. In eukaryotes, chromosome segregation is carried out by the spindle, which is predominately composed of spindle microtubules. Spindle microtubules, whose dynamics vary over time and at different locations, cooperatively drive chromosome segregation. Measurements of microtubule dynamics and spindle ultrastructure can provide insight into the behaviors of microtubules, helping elucidate the mechanism of chromosome segregation. Much work has focused on the dynamics and organization of kinetochore microtubules, that is, on the region between chromosomes and poles. In comparison, microtubules in the central-spindle region, between segregating chromosomes, have been less thoroughly characterized. In this work, we investigated the relationship between central-spindle microtubules and chromosomes during anaphase in human mitotic spindles and Caenorhabditis elegans mitotic and female meiotic spindles. We used the same laser ablation, optical microscopy, and electron microscopy techniques on all of these spindles to avoid the potential complications of interpretation that can arise when different techniques are applied to different systems. We found that these central-spindle microtubules slide apart at the same speed as chromosomes, even as chromosomes move toward spindle poles. In these systems, damaging central-spindle microtubules by laser ablation caused an immediate and complete cessation of chromosome motion, suggesting a strong coupling between central-spindle microtubules and chromosomes. Electron tomographic reconstruction revealed that the analyzed anaphase spindles all contain microtubules with both ends between segregating chromosomes. Our results provide new dynamical, functional, and ultrastructural characterizations of central-spindle microtubules during chromosome segregation in diverse spindles and suggest that central-spindle microtubules and chromosomes are strongly coupled in anaphase.
M77

Rewiring quality control in C.elegans meiosis using a new chemically-induced proximity system
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During meiosis, the two sets of chromosomes inherited from both parents interact with each other and eventually segregate into haploid gametes. During the extended meiotic prophase, homologous chromosomes must pair, culminating with the assembly of synaptonemal complex (SC) along their lengths (synapsis). Synapsis is essential for crossover formation between homologs, and thus for their accurate segregation. In C.elegans, defects in synapsis are monitored by a quality control program called the synapsis checkpoint, as one or more pairs of unsynapsed chromosomes lead to a cell cycle delay and can eventually trigger apoptosis. Previous work from our lab revealed that the synapsis checkpoint requires the presence of unsynapsed pairing centers (PCs), special regions on each chromosome that promote homolog pairing and synapsis. However, how cells detect defects in SC assembly remains unknown. In C.elegans, the Polo-like kinase PLK-2 shows dynamic subnuclear localization during meiotic prophase: it is first recruited to PCs during early prophase, and following synapsis it relocates to the SC. This suggests that the localization of PLK-2 might be part of the signal that triggers the synapsis checkpoint. To test this idea, we developed a new chemically-induced proximity (CIP) system by modifying the auxin-inducible degradation system we previously implemented in C. elegans. We engineered mutations into the F-box protein TIR1 that were found to prevent it from interacting with other ubiquitin ligase components. By fusing one protein to this TIR1 sequence and another to a “degron” peptide, we can achieve induced proximity between the two tagged proteins using the small molecule indole acetic acid (auxin). Using this system, we successfully targeted PLK-2 to specific chromosomal/nuclear structures. We found that ectopic targeting of PLK-2 to the PCs of the X-chromosomes following synapsis was sufficient to induce apoptosis. Importantly, such induced apoptosis was abrogated by mutation of PCH-2, an essential component of the synapsis checkpoint. Meanwhile, the kinase activity of PLK-2 is also important for the phenotype caused by its ectopic recruitment. By combining this CIP system with C.elegans mutants lacking key meiotic regulators, we are currently probing how PLK-2 coordinates with other meiotic kinases and nucleoskeletons during meiotic quality control and cell cycle progression. Thus, we have developed a new CIP system and used it to gain mechanistic insight into the signal that triggers the synapsis checkpoint. This CIP system is robust and simple to use and will enable a wide variety of new experiments in C. elegans and other model organisms.

M78

pre-rRNAs contribute to chromosome periphery protein recruitment and affect chromosome congression
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Background. The chromosome periphery is a ribonucleoprotein layer that coats the outer surface of chromosomes during mitosis. This layer is composed by nucleolar proteins, precursor ribosomal RNA (pre-rRNA), DNA damage repair proteins, microtubule-interacting proteins and apoptosis regulators. The relocalisation of the nucleolar proteins to the chromosome periphery starts with Ki-67 exit from the nucleolus during late prophase followed by the other nucleolar components later in mitosis (between
late prophase to anaphase). However, it is not well understood how this layer is organised or how these proteins are recruited to the chromosome periphery. We propose that pre-rRNAs participate in the recruitment of nucleolar components to the chromosome periphery after Ki-67 relocates. **Results.** In order to decrease pre-rRNAs, we treated HeLa CDK1AS cells with a selective RNA polymerase I (RNAPI) inhibitor for a brief period in late G2 prior to release into mitosis. RT-PCR analysis of 45S and c-Myc (an RNAP II transcribed gene) transcript levels demonstrated that our treatment was affecting only RNAPI. Localisation of chromosome periphery proteins Ki-67, nucleolin, B23 and fibrillarin by immunofluorescence revealed that pre-rRNA downregulation disrupted the localisation of nucleolin, B23 and fibrillarin on the chromosome periphery during mitosis. Ki-67 levels were decreased, but its periphery localisation was clearly maintained. Previous reports showed that nucleolin depletion affects chromosome congression in HeLa cells. Thus, we performed an analysis of mitotic phases in the inhibitor-treated cells. We found that RNAPI inhibition resulted in an accumulation of prometaphase cells (almost 2-fold higher than untreated cells). **Conclusion.** These experiments suggested that pre-rRNAs help to organise the chromosome periphery downstream of Ki-67 recruitment, likely by promoting the recruitment of other nucleolar proteins such as nucleolin, B23 and fibrillarin. This has functional consequences in mitosis, as reflected by defects in chromosome congression and disjunction in anaphase.

**Physical Principles of Cell Adhesion and Migration**

**M79**

**Synergistic Phase Separation of Two Pathways Promotes Integrin Clustering and Focal Adhesion Formation**

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Integrin-mediated focal adhesions are plasma membrane-associated compartments that serve as signaling hubs where cells sense biochemical and physical cues in the environment and control processes including cell migration, differentiation and death. Local clustering of integrins receptors, coupled with rapid recruitment and concentration of cytosolic proteins, is critical to formation of signaling-competent focal adhesions. However, the molecular mechanisms that control integrin clustering and focal adhesion formation are not well understood. Focal adhesions can contain hundreds of different molecules including many multivalent adaptor proteins. In other systems, interactions among multivalent molecules can lead to the formation of stable, condensed phases that are highly concentrated in the constituents and exhibit liquid-like material properties. Since focal adhesions exhibit many characteristics of compartments formed thru phase separation and are enriched with multivalent adaptor proteins, we hypothesized that phase separation contributes to focal adhesion formation and function. To test our hypothesis, we first determined whether purified focal adhesion proteins could undergo phase separation *in vitro*. Indeed, we found that both p130Cas (Cas) and focal adhesion kinase (FAK) promote formation of liquid-like droplets that concentrate beta1 integrin in a kindlin-dependant manner. Next, we developed a novel *in vitro* experimental system to reconstitute beta1 integrin clustering on supported lipid bilayers. We attached beta1 cytoplasmic tail to supported lipid bilayers and assessed clustering with TIRF microscopy. If kindlin was present, phase separation of either Cas or FAK was sufficient to induce the formation of ~1-micron diameter integrin clusters with liquid-like material properties and an integrin density similar to newly formed focal adhesions in cells. Mutations in Cas or FAK that prevent phase separation *in vitro* also reduced focal adhesion formation in fibroblasts, while
mutations that enhance phase separation increased focal adhesion formation. Finally, we find that the Cas and FAK pathways act synergistically to promote phase separation, integrin clustering and focal adhesion formation in vitro and in cells. We propose that Cas- and FAK- driven phase separation provides an intracellular trigger for integrin clustering and nascent focal adhesion formation.

M80
Contractile Cytoplasmic Fragments Exhibit ROCK-dependent Oscillatory Motion
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Cytoplasmic fragments are enucleate structures that extend from cell body and are capable of independent motion after cleaving from the plasma membrane. As cells migrate through the in vivo complex fibrous environment, fragments of varying size and shapes are shed spontaneously. These fragments are vital to intercellular communication based on the proteins and RNA transcripts enclosed within them. Larger fragments have been also been used as model systems to deduce the role of nucleus in guiding cellular polarity, migration, and contractility. Larger fragments’ potential as an important diagnostic and therapeutic tool is well recognized, e.g., their uptake in murine fibroblasts leads to the loss of tumorigenic phenotype. To-date, fragments have been studied on flat 2D substrates which only partially capture the in vivo complexity. To better understand the emergence patterns and migratory phenotypes of fragments in fibrous environments, we used non-electrospinning Spinneret-based Tunable Engineered Parameters (STEP) technique to generate various fiber architectures: crosshatch, hexagonal and parallel. Owing to fragments’ contractile nature, we used Nanonet Force Microscopy (NFM), to estimate forces on single fragment of varying shapes and sizes, ranging from tens to hundreds of μm². Fragments formed on fiber networks undergo frequent shape changes (Aspect ratio AR=1 at t=0 min to AR=6.5 at t=45 min and reduced to AR=1 at t=60 min) and in instances, oscillate for more than sixteen hours, suggesting that the absence of nucleus renders fragments to be highly dynamic as opposed to the case for intact cells. NFM force platform based upon parallel arrangement of fibers allowed us to quantify forces (~10-45nN) exerted by individual fragments. Forces were estimated by applying distributed loading across the fragment length matching the experimentally observed focal adhesion distributions. We speculated the contractile nature to be ROCK (Rho-associated protein kinase) driven which also drives cell shape and motility. Hence, we added the drug Y27632 to reduce ROCK-based contractility which led to reduced formation of smaller circular shaped fragments. Notably, the reduction in contractility completely eliminated the oscillatory motion observed in fragments generated in control cases and significantly lessened the instances of AR changes. In conclusion, for the first time to the best of our knowledge, we report precise force measurements of individual fragments as well as their migratory behavior on ECM-mimicking fiber architectures. Our findings show fragments to undergo ROCK driven oscillatory behavior not captured by the use of standard 2D substrates, thus reinforcing the need to study these behaviors in environments that contextually represent in vivo settings.

M81
The nuclear piston generates migration paths in confining microenvironments
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Single-cell migration in three-dimensional (3D) microenvironments has been studied extensively, and it is now known that cells in 3D microenvironments can migrate using multiple strategies such as mesenchymal, amoeboid, lobopodial, and osmotic engine based migration. However, most of these studies utilize microenvironments in which migration paths or channels were pre-existing, or in which cells use proteases to degrade a migration path. Furthermore, it is also known from these studies that the nucleus plays a complex role in cell migration. As the largest organelle that is often stiffer than the rest of the cell, deformation of the nucleus is the limiting factor for 3D cell migration through micron and nanometer-size pores in the extracellular matrix (ECM). Conversely, cells have been shown to use the nucleus to find the optimum path of migration or to use the nucleus as a piston to generate increased pressure at the leading edge of cells. Here, we reveal a mechanism by which the nucleus drives migration of mesenchymal stem cells (MSCs) in fully confining microenvironments with no pre-existing migration path. Using a theoretical framework, we show how the actomyosin-based movement of the nucleus expands an initially shallow protrusion and generates a migration path in a 3D microenvironment. With this model of the nuclear piston that incorporates the transportation of both fluid and ions, we show that the expansion of the protrusion is regulated by the competition between increased hydrostatic pressure in the protrusion, which would promote water efflux, and increased osmotic pressure in the protrusion, which would promote water influx. The model predicts that the activation of mechanosensitive ion channels, upon pressurizing the protrusion by the nuclear piston, allows an influx of ions into the protrusion which in turn increases osmotic pressure in the protrusion to oppose the efflux of fluid. Furthermore, the model predicts that in the absence of protrusion ion channels, cell protrusions cannot expand as the fluid influx is almost equal to the fluid efflux. The model predictions are experimentally tested and validated by studying the migration of MSCs in 3D confining matrices. Taken together, the model and the experimental results elucidate how the nuclear movement, the influx/efflux of ions, and intracellular fluid work in concert to control the protrusion volume through balancing the hydrostatic and osmotic differences across the membrane. Therefore, while previous studies have established confinement as largely dictated by the stiffness of the nucleus, with the stiff nucleus limiting migration of cells through small pores, our studies reveal the nucleus as the key element used to overcome confinement to generate a migration path in a 3D viscoelastic microenvironment.

M82

Actomyosin-Dependent Force Generation is Hijacked to Promote Tumor Progression via ECM Remodeling and Immunosuppression

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It has been observed that many types of cancer cells, including breast cancer, lung cancer, prostate cancer and melanoma, can generate higher forces via actomyosin contraction, compared to their normal counterparts. It is known that higher actomyosin forces generated by cancer cells power cancer cell migration to invade distant tissues, driving further cancer development towards metastasis. We recently found that in addition to increase cell motility, cancer cells exploit the elevated actomyosin force generation to remodel extracellular matrix (ECM) and to deplete important co-stimulatory receptors on antigen presenting cells (APCs), facilitating tumor progression. In an in vitro organoid system, we observed that in a force-dependent manner, breast cancer cells align ECM fibrils and thereby enhance the diffusion of exosomes containing cytokines activating stromal cells to exhibit cancer-associated fibroblast (CAF) phenotypes. Suppression of force generation by perturbing Rho signaling, or ECM remodeling abolishes the enhancement of exosome diffusion and the subsequent CAF induction.
Moreover, we also observed that after cell-cell contact was established between breast cancer cells and antigen presenting cells, CTLA-4, a key molecule in immunosuppression, expressed on the breast cancer cells bind to CD80 expressed on the APCs, and underwent trans-endocytosis to deplete CD80. CD80 is a costimulatory receptor promoting T cell activation. Force measurement and live cell imaging revealed that upon binding to CD80, forces generated by breast cancer cells and transmitted via CTLA-4 are sufficiently strong to displace CD80 from the surface of APCs to be internalized by breast cancer cells, significantly attenuating the capacity of APCs to activate T cells. In contrast, normal mammary epithelial cells do not generate sufficient forces to internalize CD80. Inhibiting force generation by small molecules in cancer cells increases T cell activating capacity of APCs. Taken together, our results suggest targeting actomyosin force generation specifically in cancer cells can effectively stall tumor progression, providing a new strategy to treat cancer types where cancer cells generate abnormally high forces.

M83
Matrix rigidity controls epithelial-mesenchymal plasticity and tumor metastasis via a mechanoresponsive EPHA2/lyn complex

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Mechanical cues from the extracellular matrix (ECM) regulate various cellular processes via distinct mechanotransduction pathways. Breast tumors are often detected due to their apparent “hardness” compared to normal tissues, and increasing matrix stiffness correlates with distant metastasis and poor survival in breast cancer patients. These observations raise the question of how mechanical forces generated by the rigid tumor ECM impact tumor progression and metastasis. In a hydrogel coupled with 3D Matrigel overlay culture system that recapitulates the range of physiological stiffness from normal mammary glands to breast tumors, mammary epithelial cells form normal ductal acini with stable adherens junctions and intact basement membrane mimicking normal mammary ducts in the compliant matrix stiffness whereas they present weaker junctions and invade through basement membrane in the rigid stiffness similar to breast tumors. These cell morphological changes in response to increasing mechanical forces resemble the Epithelial-Mesenchymal Transition (EMT) program. We previously reported that the transcription factor Twist1 is an essential mechano-mediator that promotes EMT and metastasis in response to ECM stiffness. High matrix stiffness promotes Twist1 nuclear localization through phosphorylation-dependent regulation of its interaction with cytoplasmic anchor G3BP2. Using kinase screening and protein affinity purification, we identified a novel mechanosensitive EPHA2/lyn protein complex regulating EMT and metastasis in response to increasing ECM stiffness during tumor progression. High ECM stiffness leads to ligand-independent phosphorylation of ephrin receptor EPHA2, which recruits and activates the LYN kinase. LYN phosphorylates the EMT transcription factor TWIST1 to release TWIST1 from its cytoplasmic anchor G3BP2 to enter the nucleus, thus triggering EMT and invasion. Genetic and pharmacological inhibition of this pathway prevents breast tumor invasion and metastasis in vivo. In human breast cancer samples, activation of this pathway correlates with collagen fibre alignment, a marker of increasing ECM stiffness. Our findings reveal an EPHA2/lyn/TWIST1 mechanotransduction pathway that responds to mechanical signals from the tumor microenvironment to drive EMT, invasion and metastasis.
SCHEEPDOG: challenges and new approaches to using electrotaxis as a tool to program directed cell migration
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Electrotaxis describes the directed migration of cells under DC electric fields. That such fields arise endogenously during morphogenesis, healing, and disease raises the exciting possibility of developing bioelectric interfaces to let us literally program aspects of these processes by dynamically steering cell migration. A key goal of our laboratory is to both better understand the cell biological underpinnings of electrotaxis and develop increasingly advanced ways of harnessing electrotaxis to solve biological and bioengineering problems. Recently, we developed a new method that we call SCHEEPDOG that represents the first computer programmable electrotaxis system and allows us to program cell migration in both the X- and Y- axes to control migration through arbitrary 2D maneuvers. We will present the updated version of SCHEEPDOG, emphasize its new capabilities, and specifically address how to easily implement it more broadly. Additionally, we will present new research findings where we used SCHEEPDOG both to explore how cell-cell adhesion can interfere with electrotactic control, and to attempt to accelerate in vitro wound healing in barrier/scratch assays. Having demonstrated clean control of migration in large ensembles of weakly junctioned primary skin mouse skin cells, we have now begun to test electrotaxis in more mature primary skin monolayers. Surprisingly, and distressingly, we found that these more strongly junctioned mature tissues actually retract at the leading edge during electrotaxis, suggesting a mechanical or signaling imbalance likely caused by the presence of strong cell-cell junctions. Here, we will present our findings on how we think E-cadherin junctions enable this retraction due to strong contractility coupling from the bulk of the tissue towards the leading edge, and strategies we have developed to circumvent this by demonstrating how we can temporarily ‘disassemble’ mature tissues to make them easier to program and then reassemble them after electrotaxis by targeting E-cadherin. We are also exploring multi-directional field stimulation, and have now developed convergent electrotaxis bioreactors that can program tissues to migrate towards a point or line source, and we are using this to explore accelerated closure of in vitro wounds or as a way to separate a population into subsets. We will show new data from this system and hope for feedback on ways to improve the design to make it (and SCHEEPDOG) more accessible and useful to the broader cell biological community, as well as how to apply it to more organotypic cultures.

Thursday, December 10, 2020

Building and Decorating Cell Skeleton

M85

Probing the molecular organization of cells and organelles using cryo-electron microscopy
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Rapid freezing of cells can provide outstanding structure preservation and time resolution of dynamic cellular processes. Electron tomography of rapidly frozen specimens (cryo-ET) is a powerful technique
for imaging biological structures in their native state and in an unperturbed cellular environment. We integrate high resolution imaging using either cryo-ET and sub-tomogram averaging or TYGRESS (Tomography-Guided 3D Reconstruction of Subcellular Structures), with comparative genetics, biochemical methods and EM-visible labeling to visualize the in situ 3D structure and functional organization of macromolecular complexes and organelles inside cells. Among different model systems, we use e.g. cilia to advance techniques and approaches for high-resolution imaging of complex cellular structures. Cilia, which are conserved and ubiquitous eukaryotic organelles composed of more than 600 different proteins, have important biological roles in motility and sensation, and defects in their assembly or function cause severe human diseases. Our cryo-ET studies have visualized the three-dimensional structures of intact wild-type and mutant cilia, dissected the organization of key macromolecular complexes in different functional states, and revealed the molecular mechanisms of how dynein motors step on microtubules, and how inhibition, not activation of dyneins regulates ciliary beating. We are also advancing and applying cryo-focused ion beam milling to generate sections (“lamella”) from biological samples that would otherwise be too thicker (> a few hundred nanometers) for cryo-ET. Using latter approach, we study cytoskeletal assemblies and cellular machines inside model organisms ranging from yeast to worms. Our findings provide detailed insights into the structural basis and ultimately the function of many cellular processes.

M86
Microtubule dynamics and force production examined with multi-scale computational modeling and electron cryotomography
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Microtubules (MTs) are essential tubulin polymers that stochastically alternate between assembly and disassembly, exploring the intracellular volume and filling it with an array of transport tracks. MT dynamics can generate mechanical forces to affect cell shape, reposition organelles and segregate duplicated chromosomes during mitosis. Recently, we have shown that MTs grow by addition of curved guanosine triphosphate (GTP) tubulins to the tips of curved tubulin protofilaments in vivo and in vitro, challenging previous views about tubulin assembly, and yet establishing some continuity between crystallographic and biochemical studies of tubulin’s atomic structure and the pathway for this protein’s polymerization. Building on this structural finding, we now report results from a broad computational and experimental effort to understand this pathway for MT assembly and its implications for the cell biology of MTs. We have constructed a comprehensive model of MT dynamics and force generation, using three approaches at different scales: (1) all-atom molecular dynamics simulations to investigate effects of GTP-hydrolysis on the structure and stiffness of tubulin PFs; (2) Brownian dynamics simulations to link MT tip structure during assembly/disassembly with force production, (3) and Monte-Carlo simulations to examine MT transitions between growth and shrinkage on a longer time scale. Our simulations suggest that thermal fluctuations drive very frequent straightening of curved tubulin PFs to
form lateral bonds and support MT elongation. High flexural rigidity of curved PFs does not limit MT growth rate, provided the lateral bonds are sufficiently strong. MT catastrophe is a complex process, which is affected by both GTP hydrolysis and configurations at the MT tip. GTP hydrolysis probably affects the flexibility of tubulin PFs, rendering tubulin inter-dimer interfaces more rigid. We identify the activation energy barrier in lateral tubulin-tubulin interactions as a key hidden parameter, controlling the magnitude of pulling forces that can be generated during MT disassembly. To test our model predictions, we have used cryoET to examine MT tip shapes under a range of conditions, including the presence of a polymerization catalyst and tubulin-binding drugs. These analyses elucidate potential mechanisms for the regulation of MT assembly by associated proteins and drugs. Finally, our simulations provide a mechanism for load-dependent acceleration of MT assembly, offering a role for flared MT tips in synchronizing MT growth and shortening rates during chromosome oscillations. Supported in part by RFBR grant # 20-34-70159 and NIH grant # GM33787.

M87

Regulation of kinesin and dynein motility by tau and MAP7

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Microtubule (MT)-associated proteins (MAPs) regulate intracellular transport by affecting motor-based transport. The mechanism of this regulation has been attributed to an overlap between motor and MAP binding sites. We tested this model by investigating how MAP7, tau, and DCX affect MT motors using single-molecule and cryo-electron imaging in vitro. We found that all three MAPs inhibit both kinesin-1 and cytoplasmic dynein, except MAP7 that regulates kinesin-1 motility in a biphasic fashion. MAP7 projection domain activates kinesin-1 motility by interacting with its stalk, whereas the MAP7 MT-binding domain (MTBD) inhibits kinesin by overlapping with its MT binding site. Remarkably, tau and DCX inhibit dynein without an overlap. Instead, positive charges on tau’s MTBD prevent MT binding of dynein by competing for negatively-charged residues on the MT surface. Our results exclude the steric overlap model and suggest that motors are required to make favorable interactions with a MAP to walk on MAP-coated MTs.

M88

Distinct FHF complexes link dynein to different intracellular cargos

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The proper organization of intracellular components is crucial for many fundamental biological processes. The motor proteins dynein and kinesin facilitate long-distance transport of cargos along microtubules. While ~15 kinesins transport cargo toward the plus end of microtubules, a single dynein, cytoplasmic dynein-1, is responsible for cargo transport toward the minus end of microtubules. How a single dynein is capable of transporting a wide variety of different cargos is an unresolved question. A protein complex known as the “FHF” (FTS, Hook, and FAM160) complex links dynein to cargo in mammalian and fungal systems, making it an ideal model for understanding intracellular transport across kingdoms. The FHF complex is implicated in linking dynein to a single motile cargo, early
endosomes, in the filamentous fungus *Aspergillus nidulans*. While *A. nidulans* has only one Hook, FTS, and FAM160 protein, human cells have three Hooks (Hook1, Hook2, and Hook3), four FAM160 proteins (FAM160A1, FAM160A2, FAM160B1, FAM160B2), and one FTS. We hypothesize that gene expansion and functional divergence of FHF complex proteins leads to combinatorial assembly of FHF complexes, facilitating dynein binding to multiple cargos in human cells. Using proximity-dependent biotinylation (BioID), live-cell imaging, and biochemistry, we found that (1) different Hooks preferentially interact with different FAM160 proteins to form distinct FHF complexes and (2) these FAM160 proteins associate with different motile cargos. We tagged each FAM160 protein with BioID to identify its protein ‘interactome’. Via these BioID datasets and co-immunoprecipitations, we found that FAM160A1/A2 only associate with Hook1 and Hook3, while FAM160B1/B2 associate with all three Hooks. Furthermore, while the FAM160A1/A2 interactomes contain many early endosome-associated proteins, the FAM160B1/B2 interactomes have many Golgi-associated proteins. We found that FAM160A2 colocalizes with Rab5-tagged early endosomes. Additionally, purified FAM160A2 and GTP-locked Rab5B directly interact, demonstrating that FAM160A2 specifically recruits dynein to early endosomes via Rab5. FAM160B1, however, colocalizes with the ER-to-Golgi cargo VSV-G in live cells, suggesting that FAM160B1 is involved in ER-to-Golgi transport. Our findings suggest that the combinatorial assembly of different FHF complexes governs dynein-cargo specificity.

M89

*Structure reveals the transformation of Arp2/3 complex from inactive to nucleation-competent state*

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Regulated nucleation of actin filaments allows cells to assemble functional actin cytoskeletal networks, for number of important cellular processes. Arp2/3 complex, a 225kD seven-subunit protein assembly, is a key actin filament nucleator that stimulates new actin filament assembly in response to cellular signals. The nucleation activity of Arp2/3 complex is activated by nucleation promoting factor (NPF) proteins that bind to the complex to trigger structural rearrangements. Though decades of structural, biochemical and biophysical experiments have shed some light into the process of actin nucleation by Arp2/3 complex, however, it is still unclear how the complex is activated by NPFs, and upon activation how they initiate the process of actin polymerization? This is primarily due to a lack of high-resolution structures of the activated Arp2/3 complex. Here we report a ~3.9 Å resolution cryo-electron microscopy structure of activated Arp2/3 complex bound to the NPF Dip1 and attached to the end of the nucleated actin filament. The structure reveals dramatic conformational changes that transforms the two actin-related proteins in Arp2/3 complex to mimic an actin filament dimer and seed filament nucleation. Dip1 triggers these changes by a clamp twisting mechanism, in which it forces two core clamp subunits in Arp2/3 complex to pivot around one another, shifting approximately half of the complex into a new activated position. By revealing how NPF stimulates the active state, the structure provides new insight into how NPFs regulate Arp2/3 complex across diverse processes like cellular motility, endocytosis, and DNA repair.
M90
Structure-function analysis of the MyTH4-FERM myosins reveals insights into the determinants of actin track selection in polarized epithelia
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MyTH4-FERM myosins have evolved to play a role in the creation and function of a variety of actin-based membrane protrusions that extend from cells. Here, we performed a comparative structure-function analysis of the MyTH4-FERM myosins, Myosin-7a, Myosin-7b, and Myosin-10, to gain insight into how these myosins select for their preferred actin networks. Using enterocytes that have spatially-separated actin tracks in the form of apical microvilli and basal filopodia, we show that actin track selection is principally guided by the mode of oligomerization of the myosin along with the identity of the motor domain, with little influence from the lever arm. Chimeric variants of Myosin-7a and Myosin-7b fused to a leucine zipper parallel dimerization sequence in place of their native tails both selected apical microvilli as their actin tracks, while a truncated Myosin-10 used its native antiparallel coiled-coil to traffic to the tips of filopodia. Swapping lever arms between the Class 7 and Class 10 myosins did not change actin track preference. Surprisingly, fusing the motor-neck region of Myosin-10 to oligomerization sequences derived from the Myosin-7a and Myosin-7b cargo proteins USH1G and ANKS4B, respectively, re-encoded the actin track usage of Myosin-10 to apical microvilli with significant efficiency.

Cell-Cell Interaction and Morphogenesis

M91
Cellular maelstroms and tissue jousting: adventures in millimeter-scale tissue growth and collisions
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Cells in tissues behave as large communities where myriad interactions amongst cells give rise to the properties of the tissue such as healing and growth. Such collective migration is especially common in epithelial tissues that form surfaces such as the skin, surface of the eye, and lining of the kidney or gut. The key to these behaviors is understanding that where a cell is within a group can determine how it behaves, so even cells of the same type can behave very differently depending on if they are at the edge or center of a tissue. These same collective behaviors also determine what happens when two or more tissues ‘meet’, leading to phenomena such as fusion and tissue compartmentalization. While much is currently known about the behaviors of relatively small tissues in culture (< 1 mm critical length), less is known about how much larger tissues develop, raising exciting questions about scaling and interactions. Here, we explore the role of size and shape in determining how individual tissues and groups of interacting tissues grow based on new data from live imaging of millimeter-scale tissues over 1-4 days. We start with a seemingly simple question—which grows proportionally faster: a small, or a large tissue? Here, we explore the similarities and differences in how small (~10 mm²) and large (~50 mm²) ‘free-range’ epithelia grow when unconfined and allowed to expand. We will specifically discuss cell migration speeds, density variations, and cell cycle coordination. A key finding is that such large tissues exhibit surprisingly sharp decoupling between the behavior of cells in the ‘bulk’ and cells at the ‘edge’. Notably, while all such large tissues develop identically behaved boundary zones expanding at equal rates that drive overall expansion rates, small tissues develop massive, nearly-whole tissue, vortices that persist for nearly 24 hrs. Our results ultimately highlight the importance the history of how a tissue
reaches a certain size in its behavior. Having characterized single large tissues, we then asked what happens when two or more of them meet. Here, we differentially labeled epithelia of different sizes and shapes and allowed them to grow over several days and interact with each other. Variables here included tissue size, shape, cell density, and cell type (co-cultures). We again found stereotyped behavior at collision interfaces that allowed us to make a very simple computational model that closely predicted experimental data for any number of tissues of arbitrary sizes and shapes, which we used to design especially intricate ‘tissue patchwork’ patterns. Especially surprising is the extrusion of tissue occurring at the singularity where three tissues meet. Together, these findings can help to explore questions of tissue boundary formation and composite tissue engineering.

M92

Lights, Camera, Action: Investigating the mechanism of Rho flare activation using optogenetics

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Lights, Camera, Action: Investigating the mechanism of Rho flare activation using optogenetics

Shahana A. Chumki¹, Rachel E. Stephenson², and Ann L. Miller¹; Cell and Molecular Biology Graduate Program¹, and Department of Molecular, Cellular and Developmental Biology², University of Michigan, Ann Arbor, MI. Epithelial tissues generate barriers that compartmentalize multicellular organisms, support homeostatic nutrient and water transport, and prevent pathogens from invading the tissue. Epithelial cell-cell junctions, including tight junctions (TJs), which seal the paracellular space between cells, and adherens junctions, which promote cell-cell adhesion, are connected to an apical bundle of F-actin and Myosin II. Cell shape changes driven by dynamic events including cell division, wound healing, and developmental morphogenesis generate tensile stress on junctions and challenge cell adhesion and barrier integrity. We discovered that the small GTPase RhoA is activated at junctions in short-lived, local accumulations, termed “Rho flares”, which repair local leaks in barrier function via an actomyosin-dependent contraction of the junction. However, the mechanism by which Rho flares are activated at cell-cell junctions remains elusive. Because Rho flares are preceded by junction elongation and a local loss of TJ proteins, we predict that junction elongation may act as a mechanical stimulus that initiates tight junction leaks and the Rho flare repair pathway. Given the stochastic nature of Rho flares in the developing epithelium, I have adapted the previously described TULIP optogenetic system, which locally activates contractility upon light stimulation, for use in live Xenopus laevis embryos. I have shown that global light stimulation elicits recruitment of a photo-recruitable GEF followed by RhoA activation at epithelial cell-cell junctions in Xenopus embryos. By using regional light stimulation, I can induce regional epithelial contractility, which leads to an increased frequency of Rho flares at cell-cell junctions located nearby the region of stimulation. Using this approach, I am analyzing whether junction elongation occurs upstream of local TJ leaks and investigating the mechanism of Rho flare activation by GEFs. Our work highlights the versatility of the TULIP optogenetic system to induce contractility on demand to uncover the molecular players in this rapid TJ repair pathway.

M93

SWI/SNF chromatin remodeling regulates cell cycle exit and promotes cellular invasion in the C. elegans Anchor Cell

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The success of many metazoan developmental programs relies on the ability of specialized cells to transgress basement membranes (BM). Cancer metastasis also relies on cellular invasion. Chromatin remodeling complexes, such as the SWI/SNF complex, coordinate animal development through broad regulation of gene transcription, ensuring normal cell cycle control and cellular differentiation in lineage-specific and temporally restricted manner. Mutations in subunits of chromatin remodeling complexes are associated with a variety of diseases, including cancer. Here we utilize the in vivo model of Caenorhabditis elegans anchor cell invasion (AC) to identify the suite of chromatin remodeling genes that are involved in the promotion of cellular invasiveness. From a large-scale microscopy-based RNAi screen targeting ~400 chromatin modifiers, we identify multiple members of the conserved SWI/SNF complex as new regulators of AC invasion. Through the use of a ratiometric cyclin-dependent kinase (CDK) cell cycle sensor and fluorescent reporters for pro-invasive genes, we demonstrate that the SWI/SNF ATP-dependent chromatin remodeling complex is a critical regulator of AC invasion, with pleiotropic effects at both the level G0/G1 cell cycle arrest and activation of the invasive machinery. Using genome editing and RNA interference, we also show that SWI/SNF contributes to AC invasion in a dose-dependent fashion, with lower levels of activity in the AC associating with aberrant cell cycle entry and greater loss of invasion. Thus, we demonstrate that SWI/SNF is required for AC invasion in vivo both to arrest cell cycle progression and activate pro-invasive genes, which may provide valuable single-cell mechanistic insight into how and why mutations in subunits of the complex are associated with many cancers.

M94

Cell fate coordinates mechano-osmotic forces in intestinal crypt morphogenesis

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Intestinal organoids derived from single cells undergo complex crypt-villus patterning and morphogenesis. However, the nature and coordination of the underlying forces remains poorly characterized. Through light-sheet microscopy and mechanical perturbations, we demonstrate that organoid crypt formation coincides with stark lumen volume reduction, which works synergistically with actomyosin-generated crypt apical and villus basal tension to drive morphogenesis. We analyse these mechanical features in a quantitative 3D biophysical model and detect a critical point in actomyosin tensions, above which crypt becomes robust to volume changes. Finally, via single-cell RNA sequencing and pharmacological perturbations, we show that enterocyte-specific expressed sodium/glucose cotransporter modulates lumen volume reduction via promoting cell swelling. Altogether, our study reveals how cell fate-specific changes in osmotic and actomyosin forces coordinate robust organoid morphogenesis.

M95

Reconstituting Epithelial Branching Morphogenesis by Engineering Cell Adhesion

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During embryogenesis, many organs undergo branching morphogenesis to form a tree-like hierarchical structure. Stratified epithelia, such as in embryonic pancreas and salivary gland, branch by repeated clefting, where indentations at the surface extend inwards to split one epithelial bud into several.
Clefting requires growth factor signaling and involves extensive dynamics of epithelial cells and the extracellular matrix. However, it remains unclear how the numerous epithelial buds of each organ arise from the interplay of cell and matrix dynamics. Here, we used two-photon microscopy to perform long-term volumetric live imaging at high spatiotemporal resolution using transgenic mouse salivary glands expressing a green nuclear marker together with a red epithelial reporter or a red membrane marker. This imaging strategy has enabled us to follow individual cells within virtually the entire gland for several rounds of clefting. Through quantitative image analysis, we found that the surface epithelial cells form an integral layer with the basement membrane, which together expands uniformly and folds inward to drive clefting that leads to new bud formation. Interestingly, cell divisions of surface epithelial cells always produce one or two interior daughter cells, all of which eventually return to the surface to make a delayed contribution to the surface expansion. Based on the low and high E-cadherin expression levels of surface and interior epithelial cells, respectively, we propose that the robust surface return of surface-derived interior daughter cells is driven by differential cell-cell adhesion. At the surface, strong cell-matrix adhesions displace weak cell-cell adhesions of low-E-cadherin cells to expand the surface, which folds to cause branching. This model was supported by both numerical analysis of relative proliferation rates of surface vs. interior cells and the finding of accelerated branching upon basement membrane recovery from collagenase-mediated disruption. Importantly, we successfully reconstituted this mode of branching by experimentally reducing E-cadherin expression and inducing basement membrane formation in 3D spheroid cultures of engineered cells that normally do not branch. Furthermore, we showed that the reconstituted branching requires integrin-mediated cell-matrix adhesions, and can be promoted by elevating the strength of cell-matrix adhesion. Our results demonstrate a fundamental self-organization mechanism driven by a combination of strong cell-matrix adhesion and weak cell-cell adhesion that can explain how stratified epithelia undergo branching morphogenesis.

**M96**

*Extracellular-forces shaped by tissue stiffness drives inner ear morphogenesis*

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Tissue morphogenesis is powered by contractile actomyosin networks in most current models, while the extracellular matrix (ECM) may shape these forces through differential stiffness. Here we use the semicircular canals (SCCs) of the zebrafish inner ear as a model for epithelial morphogenesis. SCCs form from invaginations in the epithelium (buds) that extend across the otic vesicle, where they fuse to form the hub of each canal. We find that behaviors such as proliferation, intercalation and buckling, are not required for morphogenesis. Instead, SCC formation requires deposition of the extracellular matrix (ECM) component hyaluronan, which is patterned by expression of the biosynthetic enzymes *ugdh* and *has3*. Charged hyaluronan polymers below the epithelium swell due to osmosis, thereby creating pressure that pushes the overlying epithelium into extending buds. Notably, this ECM pressure is isotropic and thus not responsible for longitudinal bud extension. Instead, anisotropic bud extension is mediated by actomyosin rich- membrane protrusions we term cytocinches. Cytocinch distribution is biased in the circumferential axis and necessary for bud extension in the orthogonal axis. Overall, our results present a novel framework where ECM-derived forces power morphogenetic movements, while cytocinches provide differential stiffness to direct tissue movement.
Chromosome Structure and Segregation

M97
Evolution of the chromosome proteome during synchronous mitotic entry
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Interphase chromatin is organised in part by cohesin complexes and also by interactions with the nuclear membrane and nuclear pores to allow regulated expression of the genome. This elaborate organisation is disassembled as cells enter mitosis and form individualised mitotic chromosomes to facilitate segregation of the duplicated genome. The transition from interphase chromatin to mitotic chromosomes is triggered by activation of several kinases, including CDK1 at the beginning of mitosis. Indeed, CDK1 activity is absolutely required to trigger the transition from G2 into prophase. Here, we investigated how chromatin is re-organised during the first steps of mitotic chromosome formation. We exploited a CDK1α chemical-genetic system to trigger synchronous mitotic entry in chicken DT40 cells. Using the Chromatin Enrichment for Proteomics (ChEP) method, chromosomal proteins were cross-linked to DNA by formaldehyde and the chromatin fractions subsequently isolated under denaturing conditions from interphase and mitotic cells. The composition of proteins that were close enough to DNA to be crosslinked to it was then determined by mass spectrometry. We analysed the changes in abundance of proteins associated with DNA at 5 minute intervals over a period from G2 until after nuclear envelope break down (NEBD - i.e. early prometaphase). Hierarchical clustering revealed 38 different kinetic patterns among the 2547 proteins. Cluster size varied from 2 to 904. 1282 proteins (49.5 %) in four clusters went down (the proteins moved away from DNA) while 524 proteins (20.2%) in 11 clusters went up (the proteins moved onto the DNA). The remaining 756 proteins (29.2%) showed small (or no) deviations in either direction. The largest cluster, consisting of proteins that left the DNA at about the time of NEBD, was enriched for proteins involved in chromatin remodelling, histone modification, DNA repair and the DNA damage response. The first proteins to leave chromatin upon entry into mitosis were ribosomal RNA processing factors and mRNA splicing proteins together with some nuclear membrane proteins. Interestingly, RNA Pol I stayed longer on chromatin, accounting for the accumulation of unprocessed pre-rRNA that ends up coating the chromosome periphery later in mitosis. Two clusters containing 366 members account for all proteins known to associate with the chromosome periphery later in mitosis. These data constitute an important resource that provides a number of surprising insights into the behaviour of well-known complexes, including the outer kinetochore and the mitotic checkpoint complex.

M98
Linker histone regulates mitotic compaction through condensin I and TopoIIα
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DNA loop extrusion by condensin and decatenation by DNA topoisomerase II (Topo II) drive mitotic chromosome compaction and individualization, but it is unclear how these enzymes act together on chromatin. Here, we demonstrate that the linker histone H1.8 suppresses chromatin levels of both
condensins and Topo II through competitive inhibition. Chromosome morphology and Hi-C analyses suggest that H1.8-mediated suppression of condensin I shortens mitotic chromosome lengths by decreasing DNA loop numbers while increasing loop sizes. We also show that Topo II activity, which is enhanced by condensin I, is suppressed by H1.8 during mitosis, and that this suppression of condensin and Topo II by H1.8 limits chromosome individualization. Accordingly, depletion of H1.8 compromises spindle integrity with hyper-individualized, elongated chromosomes. While linker histones locally compact DNA by clustering nucleosomes, our study reveals the mechanism by which H1.8 controls megabase-scale DNA folding and topological organization through repressing condensin I and TopoII activities.

M99

Epigenetic and Genetic Mechanisms of Centromere Inheritance
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Accurate chromosome segregation during cell division is essential for the survival of all living organisms. Errors in chromosome segregation cause aneuploidy that leads to developmental disorders and cancer. Chromosome segregation requires the attachment of microtubules of the mitotic spindle to each chromosome at a unique locus called the centromere. In vertebrates, a specialized histone variant known as Centromere Protein A or CENP-A replaces histone H3 in centromeric nucleosomes within chromatin. CENP-A nucleosomes are sufficient to specify the kinetochore assembly site. During DNA replication, CENP-A nucleosomes are equally distributed to daughter chromosomes resulting in the dilution of CENP-A in chromatin. During G1-phase, a CENP-A-specific histone chaperone called HJURP delivers new CENP-A near existing CENP-A nucleosomes for assembly. Thus, the position of CENP-A in chromatin epigenetically determines where new CENP-A nucleosomes will form. At centromeres, CENP-A nucleosomes are interspersed with H3 nucleosomes on homogenous repetitive arrays of DNA sequences. The role of the centromeric DNA sequence in regulating the deposition and maintenance of CENP-A nucleosomes is poorly understood but known to be important for centromere formation on human artificial chromosomes. Precisely manipulating CENP-A concentration or repetitive centromeric DNA sequences in vivo is challenging. Therefore, we have developed a cell-free assay using Xenopus laevis egg extracts to quantify the deposition of epitope tagged CENP-A nucleosomes in chromatin substrates. Performing CENP-A ChiP-sequencing and k-mer based analyses of the repetitive sequences, we have identified novel centromeric DNA sequences in the X. laevis genome. Using the egg extract system, we have discovered that HJURP drives CENP-A deposition more efficiently on CENP-A chromatin assembled on frog centromere repeat DNA than on non-specific DNA. This finding indicates that centromeric DNA sequence actively influences CENP-A assembly. We are currently investigating the mechanisms through which centromeric DNA sequences promote new CENP-A assembly. These investigations will provide insights into genetically encoded mechanisms for regulating centromere maintenance and chromosome segregation.

M100

Organization and segregation of a highly segmented bacterial genome.
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Faithful inheritance of a species’ complete genome during cell division is a requirement of cellular life. Despite broad variations in genome size and segmentation level among bacteria, archaea, and eukaryotes, all have evolved mechanisms that ensure the full genome is passed on. *Borrelia burgdorferi*, the agent of Lyme disease and a spirochete bacterium, poses unusual challenges. Typical strains have their genomes composed of one linear chromosome and about 20 linear and circular plasmids, making these the most highly segmented genomes of all known bacteria. Intrigued by how a bacterium could accommodate such a highly segmented, eukaryote-like genome, we studied *B. burgdorferi*’s genome organization and segregation. For all the genome pieces that we visualized, we found that they are present as multiple copies per cell. The level of polyploidy changed depending on the growth stage of the culture, with fewer copies found in stationary phase. This was accompanied by decondensation of the genome, with possible implications for gene expression. We also found that the chromosomes were regularly spaced at near-equal intervals within the cell, a feature that favors faithful inheritance. Finally, we found that uniform subcellular chromosome distribution is controlled by a novel segregation system that involves the interplay between a modified parABS system, and a novel genus-specific chromosome partitioning gene.

**M101**

**Liquid-Liquid Phase Separation Drives Compartmentalization and Protection of Telomeres**

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Chromatin, a nucleic acid and protein-based polymer, is dynamically organized into spatially and functionally distinct compartments. Given chromatin’s polymeric nature, the 3D architecture of the genome may be compartmentalized by liquid-liquid phase separation (LLPS). Telomeres are a particularly interesting genomic compartment comprised of kilobases of repetitive DNA and a six-protein complex named shelterin that work together as a unit to protect the single-stranded ends of chromosomes from aberrant recognition by DNA damage repair proteins. Here, we tested the hypothesis that telomere organization is driven by LLPS. However, because telomeres rarely encounter one another in living cells due to their constrained subdiffusive motion, it is difficult to test in vivo whether two telomeres can coalesce, a key aspect of the liquid condensate model. To overcome this challenge, we use a novel optogenetic approach that brings two telomeres into close proximity and find that telomeres readily coalesce into and remain as a single liquid-like droplet. We further characterize the biophysical phase behavior of telomeres, which exhibit signatures of multicomponent condensates, wherein both homotypic and heterotypic interactions between the shelterin proteins and the scaffolding telomeric DNA build the compartment. These findings are consistent with in vitro experiments, which reveal that shelterin complex proteins readily phase separate together with telomeric DNA. This work not only develops a better understanding of the biophysical mechanism of the end-protection mechanism, but it also elucidates general principles underlying the structure and composition of nuclear compartments.
Regulation of sumoylation is key to condensate formation and telomere clustering in ALT cancer

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Telomerase-free cancer cells rely on an alternative lengthening of telomeres (ALT) pathway that employs homology-directed DNA repair to maintain telomere length for immortality. One hallmark of ALT cancer is the re-localization of promyelocytic leukemia (PML) nuclear bodies to telomeres to form APBs (ALT-associated PML bodies) and clustering of telomeres in APBs to provide repair templates for telomere elongation. However, the mechanisms for APB assembly and telomere clustering are unknown. We observe that APBs exhibit liquid properties, leading us to hypothesize that APBs condense as liquid droplets to cluster telomeres via coalescence. Furthermore, since DNA damage on telomeres induces APB formation, sumoylation of telomere-binding proteins is important for APB formation, and interactions between SUMO (small ubiquitin-like modifier) and SIM (SUMO interaction motif) can drive liquid condensation, we hypothesized that telomere protein sumoylation, as a result of DNA damage response on shortened/stressed telomere, nucleates APB condensates through SUMO-SIM interactions. To test our hypothesis, we developed an optogenetic approach using a photocaged chemical inducer of protein dimerization. We found that recruiting SUMO or SIM directly to telomeres with dimerization, without DNA damage, induced de novo APB assembly by liquid-liquid phase separation and drove telomere clustering via droplet coalescence in ALT cells. Moreover, such condensation and clustering were also observed in telomerase-positive non-ALT cancer cells that do not sumoylate telomeres or form APBs following DNA damage. Meanwhile, recruiting SUMO ligase to telomeres led to telomere clustering in ALT cells but not in non-ALT cells. These findings suggest that activation of sumoylation is a critical step in enabling APB condensation and telomere clustering in the ALT pathway.

Environmental Control of Cell Behavior

M103

Exogenous and endogenous extra-chromosomal DNA is sorted into a novel cytoplasmic compartment in mammalian cells.

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Extra-chromosomal DNA (ecDNA) of exogenous (like transfected plasmid or viral DNA) or endogenous origin (like telomeric DNA from chromosomes) can be found in every eukaryotic cell type. Transfected plasmids cluster predominantly in one cytoplasmic focus per cell, which partitions asymmetrically over cell divisions (X. Wang, PNAS 2016). To investigate how mammalian cells cluster such ecDNA, we studied the composition of this cluster and its relationship with extra-chromosomal telomeric DNA and membranes. Using the LacI/LacO system and fluorescence in situ hybridization we visualized transfected plasmid DNA as well as telomeric DNA in HeLa and U2OS cells, respectively. Similar to some protein and RNA deposits, we found that an ER-derived double-membrane encloses the plasmid DNA. This membrane is distinguished by the accumulation of Emerin and the presence of Lap2b, both LEM-domain proteins typically found at the nuclear envelope (NE). The membrane does not contain nuclear pore complexes, differentiating it further from the NE. It may protect the plasmid DNA from degradation, since plasmid clusters can persist for more than 120 hours in dividing cells. A similar membrane surrounds small cytoplasmic telomeric DNA. Regularly, this telomeric DNA can colocalize with transfected plasmids into the same membrane-enclosed cytoplasmic compartment, sorting them collectively away from chromosomes. Tethering of the cytoplasmic ecDNA to LEM-domain proteins.
might contribute to their sorting and packaging in the cytoplasm. We tested this hypothesis in two distinct manners. Both a competition approach by overexpressing Emerin’s LEM-domain and deletion of the endogenous LEM-domain of Emerin by CRISPR engineering decreased the ability of cells to form cytoplasmic deposits compared to control transfected cells. Live-cell imaging further supports the notion that Emerin’s LEM-domain facilitates the assembly of these DNA deposits in interphase cells. Thus, mammalian cells differentiate ecDNA from chromosomal DNA, regardless of their origin. EcDNA is collected, packaged, and kept within a specialized membrane compartment, reminiscent of the sorting and storage of aggregating RNAs and proteins. Furthermore, we identified Emerin as being a key player in this process.

M104
Emergence of synchronized multicellular mechanosensing from spatiotemporal integration of heterogeneous single-cell information transfer
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We quantitatively characterized how noisy heterogeneous behavior of individual cells was integrated across a population toward multicellular synchronization by live calcium imaging of monolayers of endothelial cells in response to external mechanical stimuli. We used information-theory to quantify the asymmetric information-transfer between pairs of cells and defined quantitative measures to how single cells receive or transmit information in the multicellular network. We found that cells took different roles in intercellular information-transfer and that this heterogeneity was associated with synchronization. Cells tended to maintain their roles between consecutive cycles of mechanical stimuli and reinforced them over time suggesting the existence of a cellular “memory” in intercellular information transfer. We identified a subpopulation of cells characterized by higher probability of both receiving and transmitting information. These “communication hub” roles were stable - once a cell switched to a “communication hub” role it was less probable to switch to other roles. This stableness property of the cells led to gradual enrichment of communication hubs that was associated with the gradual establishment of synchronization. These results suggest that multicellular synchronization is established by effective information spread in the multicellular network, which may be driven by single cell communication properties: heterogeneity, functional memory and information flow.

M105
Melanoma A375 cells under non-adhesive confinement produces mesenchymal-to-Leader bleb transition and Leader Bleb-based migration to be mediated by Filamin crosslinking protein
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Cancer cells migrating in confined 3D microenvironments mimicking the tissue microenvironment exhibit plasticity of migration modes. Under high confinement, actomyosin contractility and low adhesion, intracellular pressure drives the formation of a large bleb, that mediates mesenchymal-to-leader bleb formation and rapid persistent migration-’leader bleb-based migration’ (LBBM), characterized by an unusual, highly polarized cell morphology with a long (~20 um) sausage-shaped bleb that points in the direction of movement, separated from a smaller (~10-15 um) spherical cell body by a
contractile neck. Actomyosin assembly and retrograde flow along the bleb coupled to non-specific friction with the microenvironment drives mesenchymal-to-leader bleb transition and LBBM. Fundamental organization of organelles nor actin regulatory protein’s role in mediating this unusual mode of motility is known. We demonstrate localization of organelle markers and actin-associated proteins in metastatic human A375 melanoma cells undergoing LBBM. We expressed fluorescent fusion proteins in cells confined to a low adhered 3um space under a polydimethylsiloxane (PDMS) pad and imaged by time-lapse spinning-disk confocal microscopy describing spatial distribution within the cell body and leader bleb, as well as cell motility parameters. We also measure mechanical dynamics of loosely adhered cells using Atomic Force Microscopy (AFM). Most cytoskeleton and membranous organelles examined are localized in the cell body; however, microtubules as well as the Golgi and ER also extend into the leader bleb and the nucleus and centrosome translocate between body and bleb.

We found that actin nucleators localizes towards the leader bleb tip, while crosslinkers are found exclusively in the bleb. Analysis of morphology and motility parameters in cells revealed that α-Actinin-1 and Filamin-A promotes leader bleb formation, speed and directionality. We then tested the hypothesis that Filamin-A is an effector to modulate actin cortex mechanics, thereby mediating bleb-based formation. We find that using AFM, Filamin-A influences cellular mechanics through cortical tension and intracellular pressure. Our study provides the first description of the cellular “anatomy” during mesenchymal-to-leader bleb transition and LBBM, suggesting that actin crosslinkers are important in regulating leader bleb size, speed, and directionality under low adhesive confinement and help mediate cellular mechanics.

M106

Neural crest EMT is controlled via target degradation within P-bodies

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Neural crest cells undergo a tightly regulated epithelial-to-mesenchymal transition (EMT) to delaminate from the neural tube. In the cranial neural crest, we have shown that this developmental EMT program is controlled by temporally restricted expression of the Wnt antagonist, Draxin. A hallmark of Draxin’s function during EMT is its transient expression and rapid downregulation. However, precisely how Draxin expression is regulated has been unclear. Using an in vivo reporter construct and time-lapse imaging to visualize RNA dynamics, we show that the rapid degradation of Draxin mRNA is mediated post-transcriptionally via its 3’-untranslated region (3’-UTR). We observed that that Draxin transcripts are targeted to cytoplasmic processing bodies (P-bodies), which are membrane-less sites of RNA processing and decay. Through time-lapse imaging, we found Draxin mRNA not only co-localizes with a fluorescently-tagged P-body component (DCP1a), but is also rapidly dissolved within P-bodies in migrating neural crest. Furthermore, knockdown of the RNA helicase DDX6 via CRISPR/Cas9, known to dissolve P-bodies, disrupted compartmentalization of Draxin mRNA to P-bodies and it instead appeared broadly cytoplasmic. Importantly, disruption of P-bodies via DDX6 knockdown inhibited endogenous Draxin mRNA degradation and impeded cranial NC EMT in vivo. This work provides the first description of P-bodies in vertebrate neural crest through an adapted RNA live imaging approach and identifies an mRNA that is targeted to and degraded within P-bodies. Together, our data highlight a novel and important role for P-bodies in an intact organismal system— playing an essential role in neural crest EMT via targeted RNA decay. This work was funded by the US National Institutes of Health K99 DE028592 (EJH), K99DE029240 (MLP), R01DE027538, and R01DE027568 (MEB).
Changes in neighbor cell contractility alter the speed and success of epithelial cytokinesis

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A defining feature of cell division is the formation of a cytokinetic furrow, generated by an equatorial actomyosin contractile array, which cleaves a single cell into two daughters. In epithelial tissue, where cells adhere to one another via cell-cell junctions, the formation of a furrow challenges tissue homeostasis by generating mechanical force that pulls on neighboring cells. Apical cell-cell junctions, including tight and adherens junctions, underlie the function of epithelial tissues by creating a selectively permeable barrier between cells. How neighboring epithelial cells respond to the force of furrow ingression in order to maintain tissue homeostasis is not well understood. Previous work in our lab has shown that cell-cell junctions are maintained during cytokinesis in the vertebrate epithelium. Moreover, adherens junctions at the cytokinetic furrow are specifically reinforced through the recruitment of the force-sensing protein, Vinculin. Overexpression of dominant-negative Vinculin increases the rate of furrow closure. This suggests that a force-balance exists between dividing cells and their non-dividing neighbors, which regulates the rate of furrow closure. To investigate the role neighbor cells play during epithelial cytokinesis, we mosaically expressed fluorescent probes for active Rho, F-actin and Myosin II in gastrula-stage *Xenopus laevis* embryos, allowing us to analyze their localization in neighbor cells, independent of their localization in the dividing cell. We find increased accumulation of these contractility factors (active Rho, F-actin, and Myosin II) specifically at junctions neighboring the cytokinetic furrow, suggesting that non-dividing neighbor cells assemble a contractile actomyosin array along the junction in response to mechanical force generated by cytokinetic furrow ingression. We next asked whether actomyosin contractility in neighbor cells could alter the rate of cleavage furrow ingression in the dividing cell. To do this, we adapted the TULIP optogenetic system for use in *Xenopus laevis* embryos. Using light, we activated Rho-mediated contractility specifically in a non-dividing neighbor cell during furrow ingression and found that light-stimulated contractility in neighbor cells was sufficient to pause furrowing for the duration of stimulation. This pause was specific to the side of the furrow adjacent to the light-activated neighbor. Furrow ingression resumed at a normal rate after light stimulation ceased, and cells successfully completed cytokinesis. This suggests that changes in neighbor cell contractility can rapidly alter the speed and success of epithelial cytokinesis. Our work reveals a novel mechanism for the regulation of cytokinesis by neighbor cell contractility in epithelial tissues.

C. elegans reveal a connection between the tissue organizers MLT-4/inversin and the clathrin adaptor AP2

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Primary cilia are antenna-like structures that protrude from the surface of many cell types. Near the proximal end of the cilium is a molecularly distinct region defined by localization of the protein inversin, consequently called the inversin compartment. In vertebrates, mutations in inversin cause *situs inversus* and a form of infantile cystic kidney disease, yet the function of the inversin compartment is unknown. In *C. elegans*, the inversin homolog MLT-4 localizes to repetitive punctate structures that line the junction between non-ciliated cells of the skin, and loss of MLT-4 causes lethal molting defects. Interestingly, in a genetic screen for mutants exhibiting a phenotype called ‘jowls,’ we isolated a novel dominant missense mutation in MLT-4. The jowls phenotype is usually caused by inactive Adaptor
Protein 2 (AP2) complexes, which function during endocytosis as cargo adaptors to link protein cargo and membrane to the clathrin scaffold. Indeed, mutations that generate constitutively active AP2 complexes suppress the MLT-4 mutation. Because our mutation in MLT-4 is a dominant gain-of-function allele that is morphologically similar to AP2 mutants, we have a unique opportunity to connect the tissue patterning function of MLT-4/inversin proteins with the membrane trafficking activities of AP2. We are investigating this using a multipronged approach including genetic analysis, in vivo imaging, and biochemistry.

Host-Pathogen Interactions

**M109**

**Highway to Cell: Rab11a mediates cell-cell spread of influenza A virus genomes via tunneling nanotubes**

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Influenza A virus (IAV) genomes are comprised of 8 negative strand RNA molecules that are packaged into virions in the form of viral ribonucleoproteins (vRNPs). Rab11a plays a crucial role in the transport of vRNPs from the nucleus to the plasma membrane by utilizing the microtubule network, and knockdown of Rab11a or disruption of the microtubule network attenuates virus production. Tunneling nanotubes (TNTs) are F-Actin rich tubules that are used by many viruses including IAV for the transport of viral components, although the mechanism is currently not known. We hypothesize that Rab11a has a dual function in the IAV life cycle. In addition to its well described role in trafficking of vRNPs from the nucleus to sites of virion assembly, we propose a novel function for Rab11a, in which it acts as a vesicular transport hub for IAV genomes using TNTs as molecular highways. To test this hypothesis, we generated HA-deficient reporter viruses which are unable to produce infectious progeny but whose genomes can be replicated and trafficked. This method allows for tracking of cell-cell spread in the absence of conventional infection processes. Using HA-deficient reporter viruses constructed in the A/Panama/2007/99 (H3N2) and A/Netherlands/602/2009 (H1N1) strain backgrounds, we observed the spread of infection to neighboring cells. This spread was abrogated in the presence of Cytochalasin D, an inhibitor of actin polymerization, and in the context of Rab11a genetic knockout. These results demonstrate that direct cell-to-cell spread in the absence of virion production requires both Rab11a and TNTs. Importantly, generation of infectious virus via genome transfer was confirmed following transfer of vRNPs from cells infected with HA-deficient virus to cells stably expressing HA. These data therefore reveal a novel role for Rab11a in the IAV life cycle, which could have significant implications for within-host spread, genome reassortment and immune evasion.

**M110**

**The Cytosol of Intestinal Epithelial Cells Is an Important Reservoir for Salmonella Persistence and Fecal Shedding**

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The intestinal epithelium is a critical defensive barrier that prevents harmful intestinal luminal contents from entering the sterile host body. Upon ingestion, colonization of the intestine by the model enteropathogen *Salmonella enterica* serovar Typhimurium (STm), a major source of foodborne illness,
initiated by bacteria-driven invasion of intestinal epithelial cells. STm can replicate within the membrane bound *Salmonella*-containing vacuole or the cytosol of intestinal epithelial cells. Cytosolic bacteria replicate rapidly, express invasion factors, and induce extrusion of infected epithelial cells into the lumen of the intestine. However, whether they play a distinct role in *Salmonella* pathogenesis is unknown. Here, we investigated the role of cytosolic STm in persistence and shedding, hallmarks of *Salmonella* infections that drive fecal-oral transmission. We generated recombinant STm that self-destruct specifically in the cytosol (STm pCytoKill), but grow normally in other environments, including the *Salmonella*-containing vacuole. First, we measured the infection potential of STm pCytoKill with a 2-monolayer re-infection assay. Infection of naive epithelial cell monolayers by STm pCytoKill was significantly reduced compared to wild-type STm, demonstrating cytosolic STm as an infectious reservoir for secondary infections. Next, we investigated the pathogenic role of cytosolic STm replication in vivo. Immunofluorescence analysis of cecal sections from mice infected with a cytosolic GFP reporter STm strain revealed blooms of released cytosolic bacteria, readily identified by GFP expression, coincident with the appearance of extruded cells in the lumen of the cecum at 2 d pi in an acute enterocolitis model of infection. Notably, we found the capacity of STm pCytoKill for intestinal expansion significantly impaired compared to wild-type STm in this infection model. More importantly, chronic carriage and fecal shedding were completely eliminated in a persistence mouse model of *Salmonella* infection. Together, these data demonstrate that STm replication in the cytosol of intestinal epithelial cells is required for efficient and sustained colonization of the intestinal tract. Thus, the intestinal epithelial cell cytosol is a critical pathogen reservoir that reseeds the intestine for dissemination in feces. Our findings present a potential new target for disease control.

M111

The antiviral sirtuin 3 bridges protein acetylation to mitochondrial integrity and metabolism during human cytomegalovirus infection

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Regulation of mitochondrial structure and function is a central component of numerous viral infections as a virus means to modulate cellular metabolism and immune responses. During infection with human cytomegalovirus (HCMV), mitochondria undergo fragmentation and alterations in composition. Accumulating evidences have placed mitochondrial protein acetylation into the spotlight, given the HCMV-induced global elevation of the mitochondrial acetylome and the antiviral function of the mitochondrial deacetylase SIRT3. Here, we link SIRT3 enzymatic activity to its defense function and regulation of both mitochondrial structural integrity and metabolism during HCMV infection. We establish that SIRT3 deacetylase activity is necessary for suppressing virus production, and that SIRT3 maintains mitochondrial pH and membrane potential during infection. By defining the temporal dynamics of SIRT3-substrate interactions during infection, and overlaying acetylome and proteome information, we find altered SIRT3 associations with the mitochondrial fusion factor OPA1 and acetyl-CoA acyltransferase 2 (ACAA2), concomitant with changed acetylation levels. These alterations point to virus-induced changes in mitochondrial organization and fatty acid metabolism. Using mutagenesis, microscopy, and virology assays, we determine OPA1 modulates mitochondrial morphology of infected cells and inhibits HCMV production, depending on its K931 acetylation state, a site regulated by SIRT3. Furthermore, modulation of SIRT3 protein levels or enzymatic activity is sufficient for regulating mitochondrial filamentous structure. Lastly, we establish a virus restriction function for ACAA2, an enzyme regulating fatty acid beta-oxidation. Altogether, we highlight SIRT3 activity as a regulatory hub.
for mitochondrial acetylation, integrity, metabolism during HCMV infection, and point to global acetylation as a reflection of mitochondrial health.

M112
Manipulating PrP glycan structure to understand toxic signaling pathways driving prion-induced neurodegeneration
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Prion proteins cause an infectious and rapidly progressive neurodegenerative disease characterized by prion aggregates, spongiform encephalopathy, dystrophic neurites, and neuronal death. These processes depend on the neuronal expression of prion protein (PrP\textsuperscript{C}), which exists on the outer leaflet of the cell membrane as a glycosylphosphatidylinositol (GPI)-anchored glycoprotein with two variably occupied N-linked glycosylation sites on its carboxy terminus. Previous work has shown that glycan modifications impact PrP aggregation and neuronal toxicity. To investigate the role of glycans in prion-induced neurotoxicity, we engineered a new knockin mouse model that expresses PrP with an additional glycan. This glycan is sensitive to PNGase F digestion, but not to endoglycosidase H digestion, indicating the presence of a complex N-linked glycan. In contrast to other murine models of prion disease, the brain lesions develop in the absence of PrP aggregates or infectivity, as shown by a fibrillization assay known as RT-QuIC, ThT fluorescence, and inoculation of wild-type mice with brain homogenates. Therefore, this model provides the opportunity to investigate the neurotoxic role of PrP\textsuperscript{C} uncoupled from aggregation. We show that although the extra glycan does not affect PrP\textsuperscript{C} expression or stability, primary hippocampal neurons isolated from these mice display signs of excitotoxicity such as dendritic retraction. Primary cortical neurons isolated from these mice exhibit enhanced sensitivity to glutamate-mediated calcium signaling, indicating that the addition of a third glycan to PrP\textsuperscript{C} affects neurotoxic signaling pathways. Additionally, immunoprecipitation-mass spectrometry (IP-MS) studies of whole brain reveal that introduction of this third glycan significantly alters the PrP interactome as compared to wild-type littermates. These studies hold relevance not only to diseases of prion aggregation, but also to other neurodegenerative diseases characterized by protein aggregation.

M113
Determining the Role of Multiple Endocytic Pathways in Promoting Listeria monocytogenes epithelial cell-to-cell spread
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Listeria monocytogenes is a bacterial pathogen that is able to spread intracellularly, travelling between adjacent cells without exposure to the extracellular space. Recent studies have identified host cell factors that promote L. monocytogenes cell-to-cell spread and have characterized the mechanisms by which some of these factors facilitate spread. Additionally, a population-level study has uncovered heterogenous spreading behavior, where some bacteria take small steps to spread to neighboring cells, while other pioneer bacteria travel farther. To follow up on an RNAi screen which identified cell junction protein, E-cadherin, as a factor promoting L. monocytogenes cell-to-cell spread, we transduced A431D cells, which do not express endogenous E-cadherin, with wild-type E-cadherin or acyto E-cadherin that
lacks E-cadherin’s cytoplasmic domain. Spread efficacy was greater between cells expressing WT E-cadherin than cells expressing αcyto or no E-cadherin, suggesting that E-cadherin’s cytoplasmic domain contributes to spread. Moreover, L. monocytogenes was more likely to spread between two neighboring WT E-cad cells than from a WT E-cad donor to a null E-cad recipient cell. This leads us to conclude that E-cadherin’s cytoplasmic domain participates in spread at the recipient side of cell contacts. In particular, we proposed that E-cadherin promotes protrusion engulfment, where donor cell protrusions containing L. monocytogenes would be internalized into the recipient cell simultaneous with E-cadherin through a caveolin dependent process. In support of this hypothesis, we have evidence showing that blocking caveolin-mediated endocytosis with the drug filipin and preventing E-cadherin’s interaction with caveolin using the caveolin-scaffolding domain peptide, cavtratin, both reduce spread in WT E-cad cells but not αcyto E-cad cells. Additionally, we uncovered a second endocytic pathway that contributes to spread, as inhibiting micropinocytosis with the drug EIPA reduced L. monocytogenes cell-to-cell spread in both WT E-cad cells and αcyto E-cad cells. Furthermore, we found that inhibiting E-cadherin dependent and caveolin-mediated spread produced more compact bacterial foci, while inhibiting micropinocytosis did not alter focus shape. As reducing the number of pioneer bacteria that take longer steps compacts foci, we reasoned that L. monocytogenes cell-to-cell spread between immediate neighbors occurs via micropinocytosis, while pioneer bacteria employ caveolin to spread. Through this work, we have discovered additional mechanisms through which L. monocytogenes spreads from cell to cell and have connected molecular mechanisms describing spread between individual cells with the population-level growth of the bacterial focus over time.

M114
Functional single-cell genomics of human cytomegalovirus infection
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Understanding how host factors and hundreds of viral genes orchestrate the complex life cycle of herpesviruses represents a fundamental problem in virology. Here we show that the sequence of viral gene expression during lytic infection of human cytomegalovirus (HCMV) is controlled by viral factors, whereas host factors determine how fast and to what extent the program can be executed. Using CRISPR screening technology, we first comprehensively measured the functional contribution of each viral gene and identified both the host dependency and restriction factors relevant for productive infection. We then recorded the transcriptomes of tens of thousands of single cells, and monitored how genetic perturbation of critical host and viral factors alters the timing, course, and progression of infection in the cell population. We find that normally, the large majority of cells follow a stereotypical trajectory in viral gene expression space. Perturbing critical host factors minimally affects this trajectory, but can accelerate or stall progression. Conversely, perturbation of viral factors can create distinct abortive trajectories. Our results reveal a dichotomy between the roles of host and viral factors and more generally provide a road map for functional dissection of host-pathogen interactions.
Nutrient Sensing and Energy Metabolism

M115
**Adipose tissue nutrient sensing in the control of oocyte production using Drosophila melanogaster as a model**

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Organismal nutritional status influences reproductive output by impacting gamete production. The energy-intensive process of oogenesis requires coordination of multiple cell types within the ovary in addition to input from other organs. In *Drosophila melanogaster* females, the stem cell-supported ovary sustains robust reproductive capacity, which responds dramatically to dietary changes. Female flies fed suboptimal diets, e.g. protein poor, high sugar, or high fat, display significantly reduced egg production rates. This response to diet is mediated by highly conserved nutrient-sensing pathways that function tissue autonomously, within the ovary, as well as non-autonomously, within the adipose tissue, to modulate germline stem cells (GSCs) and their progeny. By manipulating gene expression specifically in adult adipose tissue, we have shown that amino acid sensing by adipocytes controls GSC maintenance and ovulation via activity of the amino acid response (AAR) pathway and Target of rapamycin (TOR)-mediated signaling, respectively. We have also shown that insulin/insulin-like growth factor signaling (IIS) within adipocytes controls multiple steps of oogenesis in a complex manner. First, adipocyte IIS promotes GSC maintenance via Akt’s inhibition of GSK3β. Interestingly, adipocyte IIS promotes early germline survival via Akt but independent of GSK3β and FOXO, well-known negative regulators of IIS. Lastly, adipocyte IIS promotes germ cell survival later in oogenesis via Akt. While we have begun to decipher the molecular mechanisms of AAR pathway and IIS activity within adipocytes, distinct downstream effectors and their targets remain unclear. Currently, we are using genetic and cell biological tools to 1) explore the involvement of known GSK3β and Akt targets in controlling GSC maintenance and early germline survival, respectively, 2) examining the role if Akt-independent IIS pathways in controlling late germline survival and 3) assessing the contribution of reduced global translation and selective up-regulation of ATF4-dependent transcription, two downstream branches of AAR pathway activity. Thus far, we find that *Drosophila* adipose tissue expresses several Akt and GSK3β targets and that the IIS/Ras/MAPK axis in adipocytes is not involved in late germline cyst survival. In addition, our data indicates that the AAR pathway suppresses translation of adipocyte factors that promote GSC maintenance. Ultimately, we aim to identify adipocyte factors downstream of IIS, AAR pathway, and TOR that modulate the GSC lineage in the *Drosophila* ovary. This work will illuminate how inter-organ communication coordinates organismal nutritional status with oocyte production.

M116
**Pre-mRNA Splicing Control of Mitochondria and Energy Metabolism**

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Oxidative phosphorylation (OXPHOS) and glycolysis are the two major pathways for ATP production in animals. Whereas certain cells and tissues favor one pathway over the other to fulfill their metabolic needs, it is not clear how the balance between OXPHOS and glycolysis is regulated. In particular, while many genetic lesions cause a shift from OXPHOS to glycolysis, we know of only a few genes that cause a shift in the opposite direction. Here, we used genome-wide CRISPR/Cas9 screening to systematically identify genes whose depletion leads to an improved fitness in the absence of glucose. Surprisingly, genes in this category are enriched for components of the pre-mRNA splicing machinery, including U1 snRNP subunits frequently mutated in myelodysplastic syndromes and acute leukemia. We show that these genes promote glycolysis by binding and ensuring splicing of pre-mRNAs encoding key glycolytic enzymes, while they prevent OXPHOS by promoting glutamate export, a key anaplerotic substrate for the TCA cycle. Accordingly, metabolite profiling revealed accumulation of TCA cycle intermediates along with a dramatic increase in OXPHOS activity in cells depleted of these U1 snRNP subunits. Together, these results underscore the regulation of the OXPHOS-glycolysis balance by pre-mRNA splicing and provide a metabolic perspective to our understanding of the disorders caused by mutations in splicing genes.

M117

PCK2 balances TCA cycle flux and mitochondrial respiration to maintain the redox equilibrium in starved lung cancer cells

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Cancer cells permanently face fluctuating nutrient levels and starvation conditions. To ensure survival and fast proliferation, cancer cells have to adapt their metabolism. Gluconeogenesis, in big parts the reverse pathway of glycolysis, has been recently described as survival strategy. Phosphoenolpyruvate carboxykinase (PEPCK, PCK) is the key gluconeogenic enzyme, catalyzing the conversion of oxaloacetate, a tricarboxylic acid (TCA) cycle intermediate, to phosphoenolpyruvate. The mitochondrial isoform of PEPCK, PCK2, is expressed in different tumor types, including lung cancer, and has been linked to pro-survival and pro-proliferative effects in vitro and in vivo. Here we show that silencing of PCK2 under glucose and serum starvation increases the amount of cellular TCA cycle intermediates in two different lung cancer cell lines, A549 and H23. The TCA cycle is connected to mitochondrial respiration via its production of reducing equivalents. Glucose and serum starvation led to increased mitochondrial respiration compared to non-starvation conditions, additionally PCK2 regulates oxidative phosphorylation in H23 and A549 lung cancer cells. Silencing of PCK2 resulted in an augmented basal oxygen consumption rate and maximal mitochondrial respiration whereas this effect was only minor in the case of non-starvation conditions. However, mitochondrial mass and mitochondrial structure remained mostly unchanged. In addition, PCK2 silencing provoked a small increase in mitochondrial superoxide levels. Notably, the amount of reduced glutathione (GSH), a key antioxidant defense
molecule, was substantially decreased by PCK2 silencing. PCK2 silencing significantly reduced the colony forming ability of H23 and A549 cells, an effect partly abolished by addition of different antioxidants and enhanced by treatment with the oxidant H2O2. Key results, such as enhanced respiration, increased mitochondrial superoxide and reduced GSH/GSSG ratio could be mimicked through the addition of the TCA cycle intermediate dimethylmalate. In summary, we show that PCK2 plays a cataplerotic role in lung cancer cells, inhibiting excessive respiration and the formation of ROS under starvation conditions. Accordingly, PCK2 inhibition significantly impaired colony formation by starved lung cancer cells. In conclusion, PCK2 inhibition could potentially be utilized as a therapeutic approach to prevent metabolic adaptation and to enhance the formation of cell-damaging ROS in lung cancer cells.

M118
Maladaptive nutrient signalling sustains the m.3243A>G mtDNA mutation
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Mutations of the mitochondrial genome (mtDNA) cause a range of profoundly debilitating clinical conditions for which treatment options are very limited. Most mtDNA diseases show heteroplasm - tissues express both wild-type and mutant mtDNA. While the level of heteroplasm broadly correlates with disease severity, the relationships between specific mtDNA mutations, heteroplasm, disease phenotype and severity are not well-understood. Among human mtDNA mutations, the m.3243A>G mutation is the most prevalent one of mtDNA diseases and the major cause of mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes syndrome (MELAS). Using live-cell imaging, we found that mitochondrial dysfunction caused by the m.3243A>G mutation increases glucose dependence in the mutant cells, resulting in redox imbalance and oxidative stress. Metabolomics further revealed that the m.3243A>G mutation remodels glucose and lipid metabolism towards increased anabolic biosynthesis and lipid accumulation. Moreover, RNA-sequencing and immunofluorescence showed that the m.3243A>G mutation leads to metabolic changes, promoting the upregulation of the PI3K-Akt-mTORC1 axis in patient-derived cells and tissues. Remarkably, pharmacological inhibition of PI3K, Akt, or mTORC1 for 6-12 weeks activated mitophagy, reduced mtDNA mutant load and rescued cellular bioenergetics. We further established that the reduction of the mutant load is cell-autonomous by long-term cell growth/death analysis and single-cell PCR. In addition, the reduction was prevented by inhibition of mitophagy, showing that mitophagy is necessary for the rescue. Of note, we also examined the effects of PI3K-Akt-mTORC1 inhibition in cells carrying the m.8993T>G mtDNA point mutation (a gift from Dr Minczuk, MRC MBU), in which inhibition of the axis had no impact on mutant load. This suggests that hyperactivation of the PI3K-Akt-mTORC1 axis is relatively disease-specific and perhaps points to mechanisms that define different disease phenotypes between mitochondrial diseases, and suggesting that therapeutic options should be considered separately for each disease related to mtDNA mutations. Together, these data strongly argue that the chronic activation of the PI3K-Akt-mTORC1 axis, presumably as an adaptive response to impaired oxidative metabolism, instead serves as a maladaptive response in the m.3243A>G mutation disease model. Thus, the PI3K-Akt-mTORC1 axis represents a therapeutic target with translational potential that may benefit people suffering from the consequences of the m.3243A>G mutation.
M119
The cell cycle-related kinase AURKA forms a tripartite complex with PHB2 and LC3 to select metabolically-competent mitochondria by mitophagy
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AURKA is a serine/threonine kinase with multiple functions during interphase and cell division. It is frequently overexpressed in epithelial cancers, correlating with poor patient survival and resistance to therapies. Recent evidence showed that AURKA localizes and activates at mitochondria. At this location, AURKA regulates mitochondrial dynamics, and its overexpression boosts mitochondrial ATP levels. However, the way AURKA orchestrates its different mitochondrial functions and interacts with its over 200 mitochondrial partners remains elusive. While exploring how AURKA interacts with the inner mitochondrial membrane (IMM) protein Prohibitin 2/PHB2 - one of the main interactors of the kinase at mitochondria -, we observed a partial loss of the mitochondrial pool upon the overexpression of AURKA in human cells. Quantitative microscopy and complementary biochemical approaches in cellulo and in vivo revealed that this mitochondrial loss recapitulates several features of mitochondrial elimination by mitophagy, i.e. the proteasome-dependent loss of Outer Mitochondrial Membrane (OMM) proteins and the autophagy-dependent degradation of IMM/matrix content. We also determined that such mitochondrial loss is independent of the PINK1/Parkin pathway, but it is PHB2-dependent. In addition, Förster’s Resonance Energy Transfer (FRET) revealed that AURKA phosphorylates PHB2 on Ser39. This modification is needed to accommodate the mitophagy mediator LC3 on PHB2 itself, where it simultaneously interacts both with PHB2 and AURKA. This identifies a “AURKA-PHB2-LC3” tripartite complex triggering mitophagy upon AURKA overexpression. Altering the formation of the tripartite complex using the PHB2 ligand Xanthohumol or the AURKA inhibitor MLN8237 impaired mitophagy and restored normal ATP levels. Our results show a novel mechanism by which overexpressed AURKA, a cancer-like paradigm, triggers mitophagy. When overexpressed, AURKA forms a tripartite complex with PHB2 and LC3 to dispose of metabolically-inefficient mitochondria while preserving the organelles producing high ATP quantities. This mechanism could be a selective advantage for cancers cells to overcome cell death. Blocking the AURKA-PHB2-LC3 tripartite complex - and thereby blocking mitophagy - could represent a promising strategy to lower mitochondrial ATP levels in patients with cancer linked to AURKA overexpression.

M120
Diversity in mTORC1 substrate recruitment enables specificity of metabolic responses to nutritional cues
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The mechanistic target of rapamycin complex 1 (mTORC1) is a master nutrient- and stress-sensing protein kinase that integrates environmental signals to control cell metabolism and growth. The activation of mTORC1 occurs upon its recruitment to the lysosomal membrane, a process modulated by
amino acids through the activation of Rag GTPases, and is mediated by Rheb, a small GTPase whose activity is induced by growth factors. Thus, different nutritional inputs impinge on mTORC1 activation status to induce the phosphorylation of its multiple substrates. However, whether mTORC1 responds to diverse stimuli by differentially phosphorylating specific substrates is poorly understood. Here we show that mTORC1 phosphorylates Transcription Factor EB (TFEB), a master modulator of lysosomal biogenesis and autophagy, via a specific substrate recruitment mechanism that is mediated by Rag GTPases. Owing to this mechanism, mTORC1-mediated phosphorylation of TFEB is insensitive to growth factor-mediated activation of Rheb, unlike other “canonical” mTORC1 substrates S6K and 4E-BP1, but highly sensitive to amino acid-mediated activation of Rag GTPases. Interestingly, we found that the activity of RagC/D GTPases is essential for mTORC1-mediated phosphorylation of TFEB but plays a lesser contribution towards the phosphorylation of S6K and 4E-BP1. This mechanism explains the paradoxical hyperactivation of mTORC1 observed in Birt-Hogg-Dubé (BHD) syndrome, a disorder caused by mutations in the RagC/D activator folliculin (FLCN) and associated with polycystic kidney and renal cell carcinoma. Together, these findings identify a novel mechanism that enables differential phosphorylation of mTORC1 substrates and underlies BHD pathogenesis.

Organelles, Membrane Contact Sites and Lipid Trafficking

M121

Actin polymerization around depolarized mitochondria disrupts ER-mitochondrial contact, delaying Parkin accumulation and mitophagy

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Actin polymerization around depolarized mitochondria disrupts ER-mitochondrial contact, delaying Parkin accumulation and mitophagy

Rajarshi Chakrabarti, Tak Shun Fung & Henry H. Higgs

A growing number of studies suggests that actin polymerization participates in mitochondrial communication and dynamics in mammalian cells, with multiple distinct modes of actin/mitochondria interaction. In one mode (Fung et al (2019) J. Cell Sci.), mitochondrial depolarization causes rapid accumulation of an extensive ‘shell’ of actin filaments around depolarized mitochondria, dependent on Arp2/3 complex. We call this ADIA (Acute Depolymerization-Induced Actin). Here, we show that ADIA requires WAVE complex for Arp2/3 activation, as well as contributions from FMNL family of formins. ADIA is temporally distinct from a second round of actin polymerization that occurs over 1 hr after depolarization (Kruppa et al (2018) Dev. Cell.), which we call PDIA (Prolonged Depolarization-Induced Actin). We show that ADIA does not require myosin 6, further differentiating it from PDIA. Mitochondrial depolarization results in several other events, including Parkin accumulation. ADIA precedes Parkin accumulation, with peak actin polymerization at 2 min whereas Parkin accumulation starts at 45 ± 10 min after depolarization. Surprisingly, inhibition of ADIA by either inhibition of Arp2/3 complex or FMNL knock-down accelerates Parkin accumulation to 29 ± 9 min and 32 ± 8 min, suggesting that ADIA might inhibit Parkin recruitment to mitochondria. Our initial hypothesis was that the actin shell might serve as a barrier to Parkin access. However, experiments using rapamycin-inducible recruitment of a fluorescent protein to the mitochondrial outer membrane did not support this model. Interestingly, we noticed that ADIA promotes detethering of ER from mitochondria upon mitochondrial depolarization, an effect not observed for interactions between mitochondria and lysosomes. To test the effect of ER-mitochondrial tethering on Parkin recruitment, we artificially increased tethering by expression of VAP-B. Increased ER-mitochondrial tethering accelerates Parkin accumulation on depolarized mitochondria to 24 ± 8 min,
phenocopying the effect of Arp2/3 and FMNL inhibition. We propose that ADIA acts as a transient protective mechanism, allowing mitochondria a chance to recover polarity before mitophagic pathways are engaged. On a larger scale, our data show that the purpose of actin polymerization around depolarized mitochondria changes over time, with ADIA delaying mitophagy and PDIA facilitating mitophagy.

M122

New insight into EGFR signaling regulation by the LIN-2/7/10 complex in C. elegans

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Scaffolding proteins play a central role in spatiotemporal regulation of signaling cascades, which ensures proper timing, localization, and activation of signaling during development. One well-characterized example is induction of the vulval cell fate in the nematode Caenorhabditis elegans. Vulval development is initiated by activation of LET-23, the Epidermal Growth Factor Receptor (EGFR) homolog, in the basolateral membrane of the vulval precursor cells (VPCs), which give rise to the vulva. Subsequent activation of the canonical Ras/ERK signaling cascade specifies the vulval cell fate. Basolateral localization of LET-23 EGFR is mediated through an interaction with an evolutionarily conserved complex composed of the scaffolding proteins LIN-7 (Lin7/Veli in mammals; interacts directly with LET-23 EGFR), LIN-2 (CASK), and LIN-10 (APBA1/Mint1) (the LIN-2/7/10 complex). Disruption of the complex leads to exclusive apical receptor localization, loss of signaling, and a vulvaless phenotype. The LIN-2/7/10 complex has been defined biochemically, but where the complex forms and how it regulates localization remains unknown. Using CRISPR/Cas9 to generate fluorescent-fusion proteins, we found that LIN-2 and LIN-7 are recruited to cytosolic punctae by LIN-10, likely representing Golgi ministacks or recycling endosomes. LIN-7 independently colocalizes with LET-23 EGFR at basolateral membranes, and LIN-2/7 associate minimally with LIN-10 in vivo. Furthermore, we found that overexpression of either LIN-7 or LIN-10, but not LIN-2, can bypass the requirement for their complex components and restore LET-23 EGFR activation and vulval development in vulvaless mutants, pointing to a novel complex-independent function for these two proteins. A structure-function analysis of LIN-10 revealed differential requirements for its protein subdomains: whereas its phosphotyrosine binding domain is necessary for rescue of lin-10 mutants, its PDZ domains are specifically required for both the complex-independent function and endomembrane localization of LIN-10. Class I and II Arf GTPases, which regulate Golgi secretion and interact with mammalian homologs of LIN-10, are appealing candidate effectors for the complex-independent function of LIN-10. We found that LIN-10 colocalizes with ARF-1.2 (Class I), and may be working in a similar genetic pathway as LIN-10. Our studies reveal dynamic spatiotemporal regulation and in vivo formation of the LIN-2/7/10 complex. By uncovering the secondary pathways through which LIN-7 and LIN-10 promote LET-23 EGFR signaling, our results offer insight into how cells harness multidomain protein complexes to regulate signal transduction.

M123

Direct PA-binding by Chm7 is required for nuclear envelope surveillance at herniations

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MINISYMPOSIA-81
There is considerable evidence to support that the integrity of the nuclear envelope membranes and the function of nuclear pore complexes (NPCs) are surveilled by components of the endosomal sorting complexes required for transport (ESCRT). The precise mechanisms that contribute to ESCRT recruitment, activation and membrane remodeling at the nuclear envelope remain to be fully defined. We will present data that supports a key role for the local accumulation of phosphatidic acid (PA) in contributing to nuclear envelope recruitment and function of the budding yeast ESCRT, Chm7 (orthologue of CHMP7). Using direct in vitro binding with recombinant Chm7 and liposomes of defined lipid compositions, we will show a direct and specific PA-binding activity of this ESCRT. This PA-binding is mediated through a conserved hydrophobic stretch of amino acids, which confers specific binding to the nuclear envelope in genetic backgrounds where PA levels are elevated. Further, these PA-specific nuclear envelope interactions are independent but are nonetheless required for interaction with the LAP2-emerin-MAN1 (LEM) domain protein, Heh1 (LEM2). Consistent with the functional importance of PA-binding for nuclear envelope surveillance, mutation of the PA-binding motif inhibits recruitment of Chm7 to the nuclear envelope and abolishes Chm7 function in genetic backgrounds where NPC-assembly-associated nuclear envelope herniations or “blebs” occur. In fact, we show using correlative light and electron tomography that a fluorescent PA sensor specifically accumulates at sites of these nuclear envelope herniations. Thus, we suggest that local control of PA metabolism is important for ensuring productive nuclear envelope remodeling by ESCRTs and that its dysregulation may contribute to pathologies associated with defective NPC assembly.

M124

Nir1 is a Novel ER-PM Junction Tether and a Positive Regulator of PM PI(4,5)P2

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Endoplasmic reticulum-plasma membrane (ER-PM) junctions are subcellular loci characterized by the close and stable apposition of the ER to the PM. ER-PM junctions have been demonstrated to facilitate Ca²⁺ signaling/homeostasis as well as lipid signaling and metabolism. However, our knowledge regarding the molecular factors required to maintain and modulate ER-PM junctions is limited. Here, we describe a previously unidentified tether that is required for ER-PM junction maintenance as well as supports non vesicular-mediated PI(4,5)P₂ homeostasis at the PM. While we validated that membrane targeting domains are functionally conserved in Nir proteins, Nir1 is distinct in that it is enriched at ER-PM junctions in resting cells. Manipulation of Nir1 levels in the cell via overexpression or transient knockdown drives remodeling of ER-PM junction properties such as size and density. Additionally, Nir1 lacks a phosphatidylinositol transfer protein (PITP) domain, yet Nir1-deficient cells exhibit reduced PI(4,5)P₂ replenishment at the PM. Further, Nir1 expression potentiates targeting of the lipid transfer protein (LTP) Nir2 to ER-PM junctions during receptor-mediated signaling. Lastly, live-cell and co-immunoprecipitation data identify Nir1 as an interactor of Nir2. Results from this study reveal Nir1’s role as a novel ER-PM junction tether as well as a positive regulator of PM PI(4,5)P₂ and the LTP Nir2. These findings provide greater mechanistic insight into non-vesicular mediated lipid transfer at ER-PM junctions.

M125

Endolysosome motility is restricted by SNX19-mediated membrane contacts with the endoplasmic reticulum
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The ability of endolysosomal organelles to perform their essential functions is tightly coupled to their transport within the cytoplasm. Long-range transport occurs by interactions with the microtubule-based motor proteins, kinesin and dynein, that drive anterograde and retrograde movement of endolysosomes (EL), respectively. It is now appreciated that contacts with the endoplasmic reticulum (ER) influence the movement of ELs, but a mechanistic understanding of how this occurs is lacking. We now show that the protein sorting nexin 19 (SNX19) mediates contacts between the ER and EL and that these contacts restrict EL motility. SNX19 has two N-terminal transmembrane domains (TMD) followed by PXA, PX and PXC domains. We demonstrate by deletion analysis that the TMD anchors SNX19 to the ER, while its PX domain interacts with the endolysosomal phosphoinositide PI(3)P, resulting in the local enrichment of SNX19 at ER-EL membrane contact sites (MCS). Indeed, disruption of the PX domain-PI(3)P interactions prevented the accumulation of SNX19 at ER-EL MCS and redistributed the protein throughout the ER. In contrast, deletion of the PXA or PXC domains on either side of the PX domain, resulted in enhanced enrichment of mutant SNX19 at these MCS, suggesting a regulatory role for these domains. Super-resolution imaging of contacts by 3D-SIM and TEM, a type of correlative light and electron microscopy (CLEM), revealed that these ER contacts most often occurred with multivesicular endosomes. To explore the function of SNX19, we generated SNX19 knock-out (KO) U2OS cells and found that these cells have more dispersed and significantly more motile EL compared to wild type cells, as measured by particle tracking algorithms. In contrast, overexpression of SNX19 significantly reduced EL motility - an effect that was exacerbated by deletion of the PXA or PXC domains. Thus, we identify a novel mechanism for controlling EL positioning and motility that involves tethering to the ER by a sorting nexin. It was previously shown that the related protein SNX14 redistributes to ER-lipid droplet (LD) contact sites upon incubation of cells with oleic acid (OA), and that this redistribution promotes LD biogenesis. Interestingly, we find that SNX19 can also redistribute to ER-LD contacts upon OA stimulation, all the while maintaining ER-EL contacts. SNX14 on the other hand does not contact EL, likely due to a single amino acid substitution in its PX domain. SNX19 therefore has the properties of a dual-MCS protein that may immobilize EL near LD for maintenance of lipid homeostasis. Finally, perturbations in SNX19 expression have been extensively linked to schizophrenia risk, thus, our work raises the possibility that defective MCS underlie a pathological mechanism of this disease.

M126

Last step in the path of LDL cholesterol from lysosome to plasma membrane to ER is governed by phosphatidylserine

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MINISYMPOSIAS-83
Animal cells acquire cholesterol from receptor-mediated uptake of low-density lipoprotein (LDL), which releases cholesterol in lysosomes. The cholesterol moves to the endoplasmic reticulum (ER), where it inhibits production of LDL receptors, completing a feedback loop. Here we performed a CRISPR-Cas9 screen in human SV589 cells for genes required for LDL-derived cholesterol to reach the ER. We identified the gene encoding PTDSS1, an enzyme that synthesizes phosphatidylserine (PS), a phospholipid constituent of the inner layer of the plasma membrane (PM). In PTDSS1-deficient cells where PS is low, LDL cholesterol leaves lysosomes but fails to reach the ER, instead accumulating in the PM. The addition of PS restores cholesterol transport to the ER. We conclude that LDL cholesterol normally moves from lysosomes to the PM. When the PM cholesterol exceeds a threshold, excess cholesterol moves to the ER in a process requiring PS. In the ER, excess cholesterol acts to reduce cholesterol uptake, preventing toxic cholesterol accumulation. These studies reveal that one lipid—PS—controls the movement of another lipid—cholesterol—between cell membranes. We relate these findings to recent evidence indicating that PM-to-ER cholesterol transport is mediated by GRAMD1/Aster proteins that bind PS and cholesterol.

Friday, December 11, 2020

Cell Morphology and Aging

M127

A new concept explaining the cell biology of axons and axon pathology
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To develop remedial strategies for neurodegeneration, we need to improve our understanding of the cell biology of neurons - in particular their axons. Axons are the cable-like, up-to-meter long processes of neurons that wire our nervous system, and are key target sites for degenerative processes. Key roles are played by the axonal cytoskeleton (J Cell Biol 10.1083/jcb.201912081). Based on our studies of over 50 actin- and microtubule (MT)-regulators and incorporating the wider literature (Neural Dev 10.1186/s13064-019-0134-0), we propose the "dependency cycle of local axon homeostasis" as a promising new concept explaining long-term maintenance of axons and the many causes for their pathologies (animated summary: tinyurl.com/y4bd42yd). In a nutshell: (1) cargo transport is essential for axon function and physiology; (2) axonal transport requires MT bundles as essential highways, but also (3) imposes mechanical stress that damages MT bundles; (4) consequently, MT bundles have to be actively maintained; (5) this maintenance depends on materials, components and physiology supplied by transport - thus closing the dependency cycle. Breaking this cycle at any point leads to bundle decay and can explain why trauma, intoxication, various genetic defects or ageing can cause very similar pathologies. Here I will explain genes and mechanisms underpinning this model. Thus, we uncovered a number of mechanisms mediating long-term maintenance of bundled MT arrangements, including roles (a) of spectraplakins in guiding polymerising MTs, (b) of cortical collapse factors in eliminating off-track MTs leaving the bundles, (c) of MT polymerisation machinery in maintaining MT volume and bundle organisation, and (d) of the actin cortex in upholding MT polymerisation. In support of the notion that MT maintenance is dependent on axon physiology, I will demonstrate that loss of kinesin-1 and -3 cause
severe MT bundle aberration through patho-mechanisms that involve harmful ROS production.
Supported by the BBSRC

M128
**Machine learning predicts states of aging based on emergent single-cell mechanical phenotypes**

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The mechanical properties of cells are continuously subject to change in the context of differentiation, chronological age, and malignant transformation. Thus, our work is poised to demonstrate that these mechanical properties can be exploited and may present novel label-free biomarkers of clinically relevant functional cell states. We have implemented the use of a novel mechano-node-pore sensing (mechano-NPS) method, which is a powerful tool in multi-parametric single-cell analysis that simultaneously measures cell diameter, resistance to compressive deformation, transverse deformation under constant strain, and recovery time after deformation. Based on these measurements we have defined a new dimensionless parameter, the whole-cell deformability index (wCDI), which can be utilized to discriminate between different cell types. Mechano-NPS measurements were shown to be remarkably sensitive to pharmacologically induced alterations of the cytoskeleton. We are now utilizing machine learning algorithms to identify distinguishing mechanobiological features of different cell types in a non-biased manner. Indeed, using this approach we are now generating a database of the mechanical phenotypes of normal human mammary epithelial cells (HMEC) derived from primary tissue from women age 16 to 91, whose cells are in a range of states from normal to malignant, and with a number of defined signaling pathway alterations. Our machine learning model predicts the chronological age of tested HMECs with high accuracy and can predict pathways that differ between cell types based on patterns of mechanical measurements. Further, we are investigating the molecular underpinnings of the age dependent mechanical phenotypes. Knockdown experiments of the intermediate filament of keratin 14 (KRT14) partially restored a young mechanical phenotype in HMECs isolated from post-menopausal women. Thus, suggesting a role for KRT14 and the cytoskeletal network in cellular aging. Mechano-NPS has effectively identified mechanical phenotypes that can be exploited to detect and predict cellular properties and its potentially clinically-relevant implications in bias-free disease detection are powerful.

M129
**Cilia on muscle stem cells are critical to maintain regenerative capacity and are lost during aging**

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Adult muscle stem cells (MuSCs) are crucial for skeletal muscle regeneration throughout life. Sarcopenia, the age-dependent loss of skeletal muscle mass and strength, is a major public-health problem that affects an estimated 15% of individuals 65 years or older. During aging, the regenerative capacity of MuSCs decreases, both due to extrinsic changes in the aged microenvironment and due to intrinsic changes in signaling pathways, which reduce MuSC number and function. This decrease in MuSCs reduces the capacity of muscle to respond to exercise and injury and to efficiently repair damage to
muscle fibers. We performed a small molecule library screen and discovered that the proliferation and expansion of aged MuSCs is regulated by signal transduction pathways organized by the primary cilium, a cellular protrusion that serves as a sensitive sensory organelle. Abolishing MuSC cilia in vivo using a Pax7-CreERT2; Ift88fr transgenic mouse model severely impaired injury-induced muscle regeneration. In aged muscle, we found a cell intrinsic defect in MuSC ciliation, which leads to impaired Hedgehog signaling and a decrease in regenerative capacity. This deficit could be overcome by exogenous activation of Hedgehog signaling which promoted MuSC expansion, both in vitro and in vivo. Notably, transient delivery of the small molecule Smoothened agonist (SAG) to muscles of aged mice restored their regenerative capacity, leading to increased strength post-injury. These findings provide fresh insights into the signaling dysfunction in aging and identify the ciliary Hedgehog signaling pathway as a potential therapeutic target to counter the loss of muscle regenerative capacity during aging.

M130
Mitochondrial Homeostasis Is Maintained in the Absence of Autophagy
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Mitochondria not only facilitate ATP production through oxidative phosphorylation (OXPHOS), but also act as signaling hubs controlling key processes like cell death, fatty acid synthesis as well as heme and Fe-S synthesis. Consequently, cells must maintain mitochondrial health and dysregulated mitochondrial homeostasis is implicated in a variety of diseases including cancer, neurodegeneration, and metabolic diseases. Damaged mitochondria are turned over by selective GABARAP/LC3-dependent macroautophagy (mitophagy), which is critical for maintaining mitochondrial homeostasis. Our objective was to identify alternate forms of mitochondrial quality control that can functionally compensate if mitophagy is inactive. To this end, we selected for autophagy-dependent cancer cells that could survive loss of GABARAP/LC3-conjugation and autophagosome formation caused by inactivation of ATG7 and RB1CC1/FIP200. We discovered rare surviving ATG7 and FIP200 KO clones that could adapt to maintain mitochondrial homeostasis after complete gene inactivation. Oxygen consumption rates and metabolomic analysis indicated the autophagy deficient cells maintained mostly functional mitochondria. Utilizing high-resolution microscopy, we showed that the autophagy deficient clones accumulated significantly more hyperfused mitochondria and consequently had an acquired dependence on mitochondrial dynamics for survival. We also discovered that autophagy deficient clones could still deliver damaged mitochondria to lysosomes via SNX9-mediated mitochondrial derived vesicles (MDVs). While confocal imaging could be used to detect the vesicles, we identified a flowcytometry based assay to better quantify delivery of MDVs to lysosomes. Finally, we showed that cells lacking canonical autophagic machinery are dependent on MDVs to maintain mitochondrial function and overall cell viability, particularly in the context of mitochondrial insults. We conclude that highly autophagy-dependent cancer cells can upregulate alternative mechanisms to maintain mitochondrial health and cell survival in the absence of canonical autophagic machinery.

M131
Macrophage activation alters mitochondrial morphology and promotes mitochondrial transfer to cancer cells
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Macrophages contribute to a diverse set of functions including tissue homeostasis, immune responses, and tumor clearance. In some circumstances, macrophages also contribute to tumor progression, however this is not well understood. It has been shown that cells can directly transfer mitochondria to increase survival of recipient cells, however macrophage mitochondrial transfer has not been fully investigated. By co-culturing human macrophages and human cancer cells, we observed that macrophages transfer mitochondria to cancer cells, resulting in enhanced tumor cell proliferation. In a process called macrophage activation, environmental cues dictate macrophages to undergo transcriptional and phenotypic changes, eliciting their diverse functionality. Activated macrophages are commonly classified as M1 or M2 macrophages, which promote inflammatory or pro-tumorigenic processes, respectively. Aiming to better understand how M2 macrophages may contribute to tumor progression, we tested how macrophage activation influenced macrophage mitochondrial transfer and found that M2 macrophages exhibit enhanced mitochondrial transfer rates to tumor cells when compared to M1 macrophages. To investigate how macrophage activation could promote mitochondrial transfer, we sought to determine whether macrophage activation influenced mitochondrial dynamics and morphology. We hypothesized that macrophages with fragmented mitochondrial networks would be more likely to transfer mitochondria than macrophages with highly-fused mitochondrial networks. Through quantitative analyses we determined that M2 macrophages contain a 4-fold increase in the number of small mitochondrial fragments compared to M1 macrophages. Aiming to directly determine how mitochondrial morphology influenced mitochondrial transfer, we knocked down a key driver of mitochondrial fission, Drp1, in macrophages. This genetic perturbation resulted in highly fused mitochondrial morphology, 1.9 fold decrease in mitochondrial fragmentation and did not influence macrophage activation status. By co-culturing Drp1-knock down macrophages with tumor cells, we observed that preventing mitochondrial fragmentation in macrophages reduced mitochondrial transfer rates to cancer cells. These studies suggest that macrophage activation contributes to changes in mitochondrial morphology, and that these changes influence mitochondrial transfer to tumor cells. Investigating how macrophage activation influences macrophage-to-tumor mitochondrial transfer will help further elucidate the diverse role of macrophage function and help characterize an unappreciated mechanism of M2 macrophage associated tumor progression.

M132
Accelerating Drug Discovery through the Power of Cell Morphology
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The morphology of cell structures can reveal intricate and important details about their mechanisms and functions, and microscopists have excelled at identifying interesting phenomena. Yet it is becoming increasingly clear that images of cell structures contain far more information than meets the eye. The tremendous, rich information in cell images can now be captured and quantified by image analysis, including via deep learning, and put to good use for applications in basic biology research and drug discovery. For example, image analysis can reveal how diseases, drugs, and genes affect cells, which can uncover small molecules’ mechanism of action, discover disease-associated phenotypes, identify the functional impact of disease-associated alleles, and identify novel therapeutics. This "image-based profiling", using fluorescence microscopy assays such as Cell Painting or label-free images, can identify leukemic cells, stage the degradation of red blood cells or their infection by malaria, predict the biological impact and toxicity of compounds, identify screenable phenotypes associated with intractable
diseases, detect cancer cells’ response to drugs, and more. Together, these applications are beginning to impact progress in the pharmaceutical industry, as cell morphology takes its place among molecular-omics readouts as a powerful data source for systems biology.

**Evolution and Emerging Models**

**M133**

*C. elegans* reads bacterial non-coding RNAs to learn pathogenic avoidance

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*C. elegans* must distinguish pathogenic from nutritious bacterial food sources among the many bacteria it is exposed to in its environment. Here we show that a single exposure to purified small RNAs isolated from pathogenic *Pseudomonas aeruginosa* (PA14) is sufficient to induce pathogen avoidance, both in the treated animals and in four subsequent generations of progeny. The RNA interference and piRNA pathways, the germline, and the ASI neuron are required for bacterial small RNA-induced avoidance behavior and transgenerational inheritance. A single *P. aeruginosa* non-coding RNA, P11, is both necessary and sufficient to convey learned avoidance of PA14, and its *C. elegans* target, *maco-1*, is required for avoidance. Our results suggest that this ncRNA-dependent mechanism evolved to survey the worm’s microbial environment, use this information to make appropriate behavioral decisions, and pass this information on to its progeny.

**M134**

When plants and animals become one: Organismal and cellular interactions in a vertebrate-alga symbiosis.

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Amphibians have many strategies for protecting their eggs from predation. Some amphibians seek to hide them, others protect them bodily, while others encase them, 70 or more at a time, in an impenetrable jelly mass. That last strategy has its fair share of complications, one of the most serious being limiting availability of oxygen to embryos deep within the egg mass. An innovative way around the limits of oxygen diffusion would be to embed each egg with its own oxygen generator. In the spotted salamander-alga symbiosis, this is just the type of solution that emerged: the eggs of the salamander, *Ambystoma maculatum*, are colonized by a green alga, *Oophila amblystomatis*, that generates oxygen and removes harmful nitrogenous waste from egg capsules throughout the egg mass. The alga is able to grow to a high density using nutrients in the egg, and the salamander embryos get oxygen produced during algal photosynthesis. This relationship is even more intriguing, however, as we relatively recently learned that the alga does more than colonize the empty space in the egg and actually colonizes tissues and enters cells of developing salamander embryos. This vertebrate-alga endosymbiosis is the first such interaction observed in nature. Our work on this symbiosis encompasses many aspects of the interaction between the salamander-host and the algal-symbiont, including: physical aspects of the egg jelly that allow irradiation of the algae, even when the egg mass appears opaque; observations of independent
carbon fixation by both the alga and salamander; examination of gene expression changes in both host and symbiont in endosymbiotic cells; exploration of metabolite transfer during the endosymbiosis; and visualization of the distribution of algae during the course of salamander embryo development. We have evidence that the alga switches from oxidative metabolism to fermentation when it enters host cells, we hypothesize that the alga interacts with the developing host immune system, and we are exploring potential mechanisms of entry for algae into host cells.

M135
Cellular and developmental insights into the evolution of myocytes: insights from sponges
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Whereas a great deal has been learned about the molecular underpinnings of morphological evolution in animals, much less is known about the origin of novel cell and tissue types. Here, we use a sponge (Porifera) model to examine the origin of myocytes; a fundamental animal cell type that provides the contractile force for muscle tissues. Sponges are one of two animal lineages that lack muscle. Even so, they undergo whole-body contractions to clear their internal water canal system of contaminating debris (they are filter-feeders). An outstanding question is whether the contractile modules of myocytes can be traced to the contractile tissue of sponges and help illuminate the evolutionary origin of this fundamental animal cell type. Using the freshwater sponge model, Ephydatia muelleri, we identify an endothelial-like tissue (the endopinacoderm) as a major contributor to contractions and find that this tissue resembles muscle in other animals with respect to both its developmental specification and mechanisms of contraction. Specifically, we characterize large linear actin bundles which align in the basal region of adjoining cells and significantly shorten during a contraction. Moreover, we find that a muscle-specific paralog of Type II myosin heavy chain (stMyHC) is found exclusively at these structures and shortening of the actomyosin bundles correlates with phosphorylation of the myosin regulatory light chain. From a developmental perspective, we find that the formation of contractile bundles depends on myocardin related transcription factor (MRTF), an important myogenic factor in many animals. Pharmacological inhibition of MRTF limits development of these structures and reduces contractile behavior, whereas, activation of MRTF in sponge stem cells (archeocytes) induces contractile bundle development, driving differentiation of the endopinacocytes. Collectively, these findings support that the endopinacoderm is the primary contractile tissue in E. muelleri and that the contractile module of myocytes predates their evolutionary origin.

M136
Evolution of cell contractility and the origin of animal morphogenesis: insights from choanoflagellates
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Contractile cell types are universally present in animals and fundamental to animal life. Contractions of individual, scattered cells underlies amoeboid cell migration, which is prevalent in both adult organisms and embryos, while tissue-scale collective cell contractility underlies both embryonic morphogenesis and adult motricity. However, the origin of animal contractile cell types remains obscure. As the sister-group of animals, choanoflagellates hold the promise of illuminating the evolutionary origins of animal cell biology. Intriguingly, choanoflagellate genomes encode an extensive complement of homologs to animal contractility genes, suggesting the involvement of (yet unidentified) contractile processes in their
life history. We report on the recent discovery of both individual and collective cell contractility in choanoflagellates. Under confinement, the model choanoflagellate Salpingoeca rosetta rapidly undergoes a phenotypic switch from a flagellate to an amoeboid cell phenotype that resemble animal migratory cells in both structure and function. This represents an unexpected expansion of the known phenotypic repertoire of choanoflagellates and suggests an ancient origin for animal crawling cells, in line with the temporal-to-spatial transition hypothesis for the origin of animal cell types. Finally, collective cell contractility has also been recently discovered in a newly discovered colonial choanoflagellate isolated from a Caribbean island, that undergoes rapid and reversible whole-colony inversion in response to external photic and mechanical stimuli.

M137

**Evolutionarily conserved mechanisms governing branched morphology in the aggregatively multicellular Rhizarian amoeba, *Filoreta ramosa***

*S. Guest, S. Dawson; UC Davis, Davis, CA.*

Evolutionary mechanisms of cell organization and multicellularity involve emergent morphological complexity to generate spatial differentiation. While multicellularity has evolved multiple times across the eukaryotic supergroups, the Rhizaria, a clade including numerous multicellular amoebae, remains understudied. Our recently isolated Rhizarian amoeba, *Filoreta ramosa*, exhibits “aggregative multicellularity.” Individual amoebae migrate together and fuse to form a multinucleate syncytial network that develops a complex reticulated pattern using pseudopodial branches. Through mechanisms reminiscent of neuronal growth cones and dendritic arborization, the syncytium can grow to span several centimeters in diameter. Nuclei and organelles undergo rapid and bidirectional long-range transport along microtubules within these branches at rates averaging 4.8µm/sec. Fragmented branches are capable of self-recognition and auto-fusion, facilitating rapid repair, expansion, and dynamic interactions with the environment. How and when did the cytoskeletal mechanisms that drive polarized growth cones and branching morphology evolve in Eukarya? We are using our recently completed *Filoreta* genome combined with super-resolution imaging and morphometric analyses to quantify conserved cytoskeletal elements of syncytial morphology and development. We raised antibodies specifically to *Filoreta* γ-TURC components and EB1 to visualize non-centrosomal microtubule nucleation and polarity in proliferating pseudopodia. We compared morphological perturbations following treatment with cytoskeletal drugs affecting microtubule and actin dynamics. As in neuronal branches, the network is organized with longitudinal microtubule arrays coupled with actin-rich pseudopodial protrusions to initiate branch formation. Cytoskeletal drugs alter network development, underscoring the critical role of microtubule and actin dynamics and their interactions in generating the complex branched morphology in Rhizaria and Metazoa. We predict that the mechanisms governing the intricate cytoskeletal networks in *Filoreta* are emergent properties of simple branch and anastomosis patterns involving conserved cytoskeletal proteins. Thus, the branching mechanisms typified by neuronal outgrowth are conserved in Rhizaria and pre-date the divergence of major eukaryotic lineages.

M138

**Evolutionary principles for the actions of molecular machines***

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Molecular machines are protein complexes that generate force, transport cargo, and maintain ion gradients for the cell. Molecular machines give unique insight into evolution, since evolutionary pressures act directly on quantifiable thermodynamic properties like speed and efficiency. Machines like FoF1-ATPase, which synthesizes an organism’s ATP, and like myosin II, the motor responsible for muscle contraction, use a large fraction of a cell’s energy. So, we expect large evolutionary pressures on their efficiencies. But speed matters, too, in order to rapidly recharge ATP stores and to generate fast muscle contractions in these examples. There is a tradeoff between speed and efficiency, and we study how machines have evolved under these large and opposing evolutionary forces. We compare experimental data on molecular machines to dynamical models that are optimized along coordinates that are plausible evolutionary degrees of freedom. We find evidence that evolution seeks an optimal balance of speed and efficiency, where any further small increase in one of these quantities would come at great expense to the other. This gives insight into how the cell distributes its energy resources. We also identify specific evolutionary degrees of freedom—such as the c-ring structure of FoF1-ATPase, and the ADP-binding pocket of myosin II—that have evolved differently across species in response to different needs and different environments.

**Maintenance of Genome Organization and Integrity**

**M139**

*Synthetic regulatory genomics of mammalian Hox clusters*

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The coordinated expression of *Hox* genes is crucial for embryonic patterning. In mammals, *Hox* genes are organized into compact clusters that are under strong purifying selection. The action of local transcription factor binding and distal enhancer elements have emerged as the two major regulatory modes controlling the initiation and maintenance of *Hox* gene expression. However, a synergistic model describing the relative contribution of these locus intrinsic and extrinsic modes has remained elusive, which will require the ability to manipulate them in concert. Despite the emergence of CRISPR/Cas9 based genome editing, there is a gap in our ability to make multiple, precise edits on the same haplotype or generate large scale complex rearrangements to interrogate the multi-way functional connections between regulatory elements. In the absence of such tools, there has been a focus on defining individual cis regulatory elements that are *necessary* in the endogenous context to control gene expression. To address this limitation, we developed a ‘regulatory reconstitution’ approach that enabled us to identify a minimal set of elements that were *sufficient*, and not just necessary, to specify the dynamic regulation of *HoxA* genes. Bottom-up synthesis of large DNA segments (>100kb) allows for the arbitrary modification, removal and inclusion of elements on the scale that is required to probe gene regulation. We coupled the bottom-up synthesis of mammalian *HoxA* clusters (130-170kb) containing subsets of locus constituents, with their site-specific delivery to an ectopic location in the mouse genome. We analyzed the behavior of these synthetic, ectopic *Hox* clusters in an *in vitro* differentiation system through epigenomic and transcriptomic analyses. We find that a minimal *Hox* cluster is sufficient to specify and establish stable chromatin domains in response to developmental pattering signals, independent of enhancers. Enhancers serve to increase both total transcriptional output from the clusters and to improve the resolution of the chromatin boundary. Thus, our data suggest that compact *Hox* clusters contain all the information to decode and maintain positional information. We expect this
to be a proof-of-principle for ‘Synthetic Regulatory Genomics’ - a broadly generalizable approach for the study of gene regulation in complex genomes.

M140
Arginine methylation promotes siRNA-binding specificity for a spermatogenesis-specific isoform of the Argonaute protein CSR-1
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RNA silencing is a critically important mechanism through which cells regulate gene expression and protect the genome against aberrant RNAs, transposons, and viruses. RNA silencing is mediated by small non-coding RNAs, which are bound by Argonaute proteins and regulate complementary mRNAs at the level of transcription, translation, and RNA stability. CSR-1 is an essential Argonaute protein in C. elegans that binds to a subclass of small interfering RNAs (siRNAs) targeting most germline-expressed genes. Here we demonstrate that CSR-1 has two isoforms with distinct expression patterns; CSR-1B is ubiquitously expressed throughout the germline and during all stages of development while CSR-1A expression is restricted to germ cells undergoing spermatogenesis. Furthermore, CSR-1A associates preferentially with siRNAs mapping to spermatogenesis-specific genes whereas CSR-1B-bound siRNAs map predominantly to oogenesis-specific genes. The specificity of the two CSR-1 isoforms is interesting, considering they share nearly complete sequence homology and co-localize at the P granule, perinuclear germ granules required for RNA regulation and silencing, in both L4 larval and male germlines. We found that the exon unique to CSR-1A is modified at arginine/glycine (RG) motifs by dimethylarginine, and loss of the dimethylarginine results in the loss of CSR-1A specificity for its preferred spermatogenic small RNA partners, resulting in CSR-1A indiscriminately binding to both spermatogenic and oogenic siRNAs. Thus, we have discovered a regulatory mechanism for C. elegans Argonaute proteins that allows for specificity of small RNA binding between similar Argonaute proteins with overlapping temporal and spatial localization.

M141
Building a nuclear state landscape in human iPSCs: integrated analysis of the organization and function of nuclear bodies
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The Allen Institute for Cell Science is developing a state space of stem cell structural signatures to understand the principles by which cells reorganize as they traverse the cell cycle and differentiate. To do this, we have developed a pipeline that generates high-replicate, dynamic image data of cell organization and activities in human induced pluripotent stem cell (hiPSC) lines (the Allen Cell Collection at www.allencell.org). To study the organization of the nucleus at multiple spatial scales, 17 of these lines express an endogenous monoallelic EGFP-tagged protein that represents a particular key nuclear structure. Tagged structures include major nuclear landmarks (nuclear lamina, pores, speckles and nucleoli), chromatin structure (histone H2B, HP1-beta, and EZH2), key proteins in chromatin looping (CTCF, SMC1A), chromatin loci (telomeres and DNA replication sites), RNA polymerase, and two pluripotency transcription factors. We take advantage of the thousands of 3D single cell images collected for each structure and use our image analysis tools combined with deep learning-based label free technology to create integrated models and analyses of three key nuclear reference structures: the lamina, nucleoli and speckles. We find that the total number of speckles scales with nuclear volume,
suggesting that new speckles are created, either de novo or by fission, as cells progress through interphase. We also find a scaling relationship between the size of nucleoli and the size of speckles that is independent of each of their scaling with nuclear size, indicating a mechanism by which speckles and nucleoli coordinate their total size. Further, in collaboration with the 4D Nucleome, these integrated models are being expanded to incorporate models of the 3D genome that are computationally generated by combining image-based spatial constraints with 3D genome mapping assays. We are also investigating the functional role of these key nuclear structures with an initial focus on the nucleolus. We performed a CRISPRi pooled screen using a cell line with 2 nucleolar markers tagged with fluorescent proteins as well as the CRISPRi machinery. We screened for genes that perturbed nucleolar morphology as measured by a flow based assay and are in the process of validating the hits by live cell imaging. The primary hits from the screen include genes that regulate both transcription and epigenetics, preliminarily hinting at a relationship between nucleolar structure and regulation of gene expression in hiPSCs. In summary, we presented new integrated models of 4D nucleome organization in hiPSCs along with analysis and a functional screen that together suggest new regulatory connections between nuclear bodies as well as new insights into the connection between their morphology and function.

M142
DNA damage induces divergent response in chromatin dynamics at break sites and in undamaged regions of the cell nucleus
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Dynamic changes in chromatin organization maintain proper nuclear functions that include gene expression, DNA replication and genome maintenance. The movements of chromatin fibers are thought to play important roles in the regulation of these fundamental processes. Yet the mechanisms controlling chromatin mobility are still poorly understood in mammalian cells. To map and characterize chromatin motions throughout cell nuclei, we developed a method based on structured illumination. This method allows us to track photoactivated histone microdomains and to relate the mobility of each microdomain to the presence or absence of DNA breaks. We demonstrate a transient drop in global chromatin motions after DNA damage induced by chemotherapeutic drugs or by ionizing radiation. Break sites nonetheless appear to move faster, which may facilitate the DSB repair process while preventing genomic translocations by insulating damaged chromatin. These results have been confirmed by computational simulations, showing that the motions observed are minimally influenced by noise. This spatially divergent response of chromatin to DNA damage was also confirmed by tracking stretches of chromatin labeled with fluorescent nucleotides and by analyzing motions of single nucleosomes. Finally, we discovered that adjacent chromatin microdomains exhibit significantly correlated movements, indicative of chromatin cohesion at the microscale. DNA damage reduced chromatin cohesion, interpreted again as insulation of damaged chromatin. We anticipate that our results will spur the development of more accurate biophysical models of chromatin and lead to a better understanding of the biogenesis of genomic translocations.
“When bigger is not better”: Whole genome duplication generates DNA damage by compromising DNA replication dynamics.

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Polyploidy results from the gain of complete chromosome sets. Polyploidization can be developmentally programmed to sustain cell and tissue functions. Conversely, when non-programmed, polyploidization generates high levels of genetic instability. Although elimination pathways are described, a growing amount of evidence shows that non-programmed polyploid cells can escape these pathways and contribute to pathological conditions, including cancers. Indeed, about 40% of human tumors have experienced whole genome duplications (WGD), which appear to fuel highly abnormal karyotypes favoring tumor evolution. However, the molecular mechanisms linking WGD to genetic instability remain poorly understood. To investigate this question, we combined in vivo approach in Drosophila melanogaster with mechanistic approach in the human non-tumoral RPE-1 cell line. We first developed several strategies to generate mono- or multinucleated polyploid cells. Independently of the strategy used and of the number of nuclei, we observed DNA damage generation, revealed by γH2AX foci, within the first interphase upon polyploidization. Interestingly, γH2AX signal covered large part of the nucleus, suggesting that DNA damage is not generated at a specific region of the genome. By synchronizing polyploid cells in G1, we fully prevented DNA damage, whereas cells released in S-phase accumulated γH2AX foci and replication stress markers. More importantly, inhibition of DNA replication fully prevented DNA damage in polyploid cells. Previous data suggested that DNA damage in polyploid cells resulted from errors during mitosis leading to cell cycle arrest in the next G1. Here, we show that even before mitosis, polyploid cells experience high levels of DNA damage, causing by the first DNA replication. These observations are crucial since such genetic instability could promote the acquisition of mutations allowing the proliferation of unstable polyploid clones. Now, by characterizing DNA replication dynamics using 4D quantitative live imaging, we show that full activation of replication sites is delayed in polyploid cells. Moreover, we screen for DNA replication factors and identified a group of proteins, including MCM2, that does not scale up with DNA amount in polyploid cells, suggesting that some replication factors could be limiting generating then DNA damage. This model implies that physiological polyploid cells, that do not experience genetic instability, are able to scale up factors essential to prevent DNA damage. In support of this model, we observe that some proteins that maintain genetic stability are more expressed in physiological polyploid tissues in vivo in Drosophila brain compare to our model of non-programmed polyploidy.

Sonic hedgehog accelerates DNA replication and causes replication stress

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Cancer is a multi-stage disease caused by sequential mutations; however, the molecular mechanism generating cancer-initiating mutations for many cancers is not well understood. Using the developing cerebellum as model system, here we delineate a molecular mechanism for tumor initiation in medulloblastoma (MB), the most frequent malignant pediatric brain tumor. Activation of the Sonic
hedgehog (SHH) pathway is frequent in MB, with mutations in the tumor suppressor PTCH1 (a negative regulator of SHH signaling) being the most common initiating event for SHH-MB. However, how SHH as a developmental mitogen promotes early carcinogenesis in the cells of origin, granule cerebellar progenitors (GCPs), remains to be determined. Here we report that physiological exposure of GCPs to Shh causes a distinct form of DNA replication stress, altering DNA replication dynamics to increase both origin firing and fork velocity. Shh promotes DNA helicase loading and activation in GCPs, with increased levels of Cdc7-dependent replication origin firing. S-phase duration is reduced and hyper-recombination consequently occurs. Such elevated recombination causes copy-number neutral LOH, an event seen frequently at the PTCH1/ptch1 locus. Moreover, Cdc7 inhibition to attenuate origin firing in a MB mouse model is sufficient to reduce somatic recombination and preneoplastic tumor formation. We therefore establish that tissue-specific replication stress induced by Shh acts to promote LOH, which in tumor-prone Ptch1+/‐ GCPs can result in loss of this tumor suppressor, as an early cancer initiating event.

Neuronal Trafficking and Cell Polarity

M145

E-cadherin-mediated cell contacts underlie epithelial symmetry breaking in vivo

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Epithelia provide resiliency to organs and act as environmental barriers, functions that depend on establishing polarized apical surfaces facing the exterior and basolateral surfaces oriented toward the body. Achieving this polarity requires symmetry breaking, the process by which apico-basolateral axis orientation is initially defined. Epithelial symmetry breaking must occur at both the cellular level (every cell adopts polarity) and at the tissue level (cells align their individual polarity axes to generate a polarized epithelium). While critical for tissue function, the molecular signals underlying epithelial symmetry breaking at these two scales are poorly understood. To determine such mechanisms we use the C. elegans embryonic intestine, a simple epithelium where cell and tissue symmetry breaking are temporally separable. Apical proteins initially coalesce as puncta at intestinal (E) cell membranes before undergoing a tissue-wide migration to the intestinal midline to define the apical surface. We find that these puncta localize to E/E homotypic contacts and are excluded from contacts between E and non-E neighbors, thus we hypothesized that symmetry breaking is informed by tissue-wide contact asymmetry created by the stereotypic arrangement of intestinal precursors. Consistently, altering this arrangement using mutants with ectopic E/E contacts is sufficient to change apical protein localization. To explore modes of E/E contact recognition, we tested a role for the adhesion protein E-cadherin/HMR-1, which we found localized at homotypic puncta ahead of apical proteins. Through intestine-specific protein depletion (HMR-1<sup>edl(1)</sup>), we show that HMR-1 is required for cellular symmetry breaking as apical puncta formation is significantly delayed in HMR-1<sup>edl(1)</sup> embryos. HMR-1 further plays a role in tissue symmetry breaking as the apical puncta that eventually form are misoriented and fail to migrate to the midline. Surprisingly, these defects ultimately correct as mislocalized puncta are swept to the midline through an apparent HMR-1-independent mechanism, resulting in severely delayed but viable animals. These data support a model in which E-cadherin contributes to: 1) cellular symmetry breaking by designating homotypic contacts and creating a platform for apical proteins at the onset of polarization; and 2) tissue symmetry breaking by guiding apical proteins to the tissue midline while also highlighting a role for redundant mechanisms in this process. As internal epithelia must interpret their environment to break symmetry despite being surrounded by other cells, this model offers a logical framework for how such
contact asymmetries can inform polarization through an E-cadherin based mechanism that likely plays a similar role in other tissues.

M146

**Cell-cycle-dependent cues temporally regulate Cdc42 activity at growth sites in fission yeast**

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Cells undergo polarized growth to acquire a shape that promotes function. In fission yeast, polarized cell growth is activated by the Morphogenesis Orb6 (MOR) pathway and the small GTPase Cdc42. After cell division, the MOR pathway first promotes cell separation, the final step in cytokinesis, and then promotes polarized growth at the cell ends. It is unclear how the ends initiate growth after the cells undergo separation. It is plausible that the MOR pathway activates end growth only if it successfully enables cell separation. To test this, we developed a system whereby we delay cytokinesis, while mitosis progresses, via a temporary Latrunculin A (LatA) treatment. Cells treated with LatA during mitosis, when allowed to recover, initiate old end growth without cell separation. We call this the PrESS phenotype – polar elongation sans cell separation. PrESS cells show Cdc42 re-activation at the ends often before the completion of cytokinesis, showing that growth occurs independently of cell separation. Additionally, we find that Cdc42 regulators, Cdc42 itself, and trafficking machinery are siphoned away from the cell middle and appear at the cell ends, suggesting a competition between the cell middle and the ends. This competition leads to cell separation failure since the requisite digestive enzymes are not secreted to promote septum digestion. To identify how initiation of Cdc42 activation at cell ends is regulated after cell division, we performed a candidate screen with Cdc42 regulators. We find that loss of the Cdc42 inactivator Rga4 increases the frequency of the PrESS phenotype. Moreover, we show that the distribution of Rga4 along the cortex changes according to the cell-cycle stage, displaying a punctate appearance mostly relegated to the cell sides during G2, and a more diffuse appearance that extends to the cell ends during mitosis. We hypothesized that growth at cell ends requires simultaneous MOR pathway activation, which promotes protein synthesis, and Rga4 removal from the ends after mitosis. To test this, we constitutively activated the MOR pathway in an rga4 deletion mutant. Cells constitutively activating the MOR pathway often lyse due to premature synthesis and delivery of digestive enzymes to the division site. We find that deletion of rga4 in these cells enhances cell end growth while preventing cell separation and rescuing lysis, thus recapitulating the PrESS phenotype. Therefore, we propose that Rga4 is removed from the cell ends during cell separation in a cell-cycle-dependent manner, allowing Cdc42 activation and growth at these ends.

M147

**Coupling between protrusion dynamics and polarized secretion steers persistent cell migration**

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When cells migrate, they need to coordinate multiple signaling pathways in space and time to orchestrate the dynamics of their cytoskeleton and the distribution of their internal organelles. Yet, it remains unclear how the different signaling programs are coupled, and to what extent a proper cell polarity is required for migration. Such questions are difficult to answer because of the numerous feedbacks in the cell signaling circuitry. In this work, using dynamic micropatterning, optogenetics,
pharmacological and trafficking assays, and live-cell imaging, we found that a feedback between RhoGTPases’ biochemical gradients -that dictate local membrane activity- and cell internal organization -that defines the global polarity axis of the cell- allows cell migration to be persistent. We first demonstrated that Golgi complex positioning is instructive for the selection of the direction of movement using a well-controlled dynamic micropatterning assay. Second, by pharmacologically dispersing Golgi complex and disrupting polarized secretion, we show that persistent migration breaks down and becomes random. Third, we found evidence that the Golgi orientation biases protruding activity, most likely by directing the secretion of adhesive molecules. Fourth, by applying sustained optogenetic perturbations, we prove that a localized Cdc42 RhoGTPase gradient is able to orient the Golgi complex. Taken together, we propose a model of persistent cell migration based on the coupling between protrusion dynamics, driven by RhoGTPases’ activities, and polarized secretion, biased by organelle positioning.

M148

**Autophagosomal maturity coordinates dynein effector scaffolding in axons**

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Autophagy is a degradative pathway required for neuronal homeostasis; autophagy defects are observed in neurodegenerative diseases including Parkinson’s and Huntington’s disease. In neurons, autophagosomes form constitutively at the axon terminal and mature by fusing with lysosomes during dynein-mediated transport to the soma. However, it is unknown how the dynein-autophagosome interaction is regulated during this maturation. We now identify a series of handoffs between dynein effectors as autophagosomes transit along the axons of primary murine hippocampal neurons. Through both live-cell imaging and proximity ligation assays, we find the scaffold protein JIP1 is significantly enriched on nascent autophagosomes in the distal axon, where it interacts with the dynein complex and is required to initiate the long-range transport of autophagosomes. The transit of autophagosomes along the axon shaft requires neurodegeneration-associated protein Huntingtin and its interacting partner HAP1. In vitro binding and single molecule motility assays demonstrate that HAP1 induces microtubule binding of the dynein-dynactin complex and activates dynein-driven motility. HAP1 interacts with components of dynein and dynactin via both canonical and noncanonical interaction sites. Point mutations in HAP1 that disrupt these binding sites have a dominant negative effect on autophagosomal transport. Surprisingly, we find that in addition to HAP1, the lysosomal motor scaffold JIP3 is also associated with most axonal autophagosomes. However, JIP3 specifically regulates the transport of mature, acidified autolysosomes in the proximal axon, within 200 µm of the soma. Inhibiting autophagosomal transport is known to disrupt their maturation; we now find that inhibiting autophagosomal maturation pharmacologically via Bafilomycin A1 treatment or genetically via a dominant negative syntaxin-17 mutant disrupts the recruitment and activity of dynein effectors. Thus maturation state and cofactor activation are tightly linked. Collectively, these results describe a novel maturation-based motor effector handoff on neuronal autophagosomes key to autophagosomal motility, cargo degradation, and ultimately axonal health. *Supported by NIH grant R35 GM126950.*
M149
A novel role of presynaptic periactive zone proteins in extracellular vesicle trafficking
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Extracellular vesicles (EVs) are small, endosomally-derived, membrane bound vesicles that can transport cargoes between cells, and are important for cell-cell communication in the nervous system. However, it remains unclear how specific endosomal cargoes are sorted for release through the EV pathway. Much of our knowledge of EVs comes from in vitro or cell culture-based studies, which are unable to fully recapitulate the process of endogenous EV cargo trafficking across complex tissues, such as the nervous system. To overcome these limitations, we have developed tools to use the Drosophila neuromuscular junction (NMJ) as a model system to study the trafficking of EV cargoes in vivo from the presynaptic neuron to the postsynaptic muscle. Through a directed genetic screen, we identified an unexpected role for presynaptic periactive zone (PAZ) membrane remodeling proteins, which have canonical roles in clathrin-mediated endocytosis, in regulating the traffic of EV cargoes at the Drosophila NMJ. PAZ mutants, including those lacking synaptojanin, dynamin, endophilin, and the F-BAR/SH3 protein Nervous Wreck (Nwk) exhibit a local and dramatic decrease in the levels of the EV cargoes Synaptotagmin-4 (Syt4) and Amyloid Precursor Protein (APP) at presynaptic terminals. Further, this decrease is sufficient to abolish Syt4 function and reduce APP toxicity, suggesting that loss of specific EV cargoes may play unrecognized roles in canonical phenotypes of PAZ mutants. Interestingly, this novel EV cargo traffic defect is genetically separable from the well-established functions of these proteins in synaptic vesicle recycling and synaptic growth. Our data suggests a novel clathrin-dependent molecular mechanism that protects EV cargoes from local degradation at synapses, and promotes their release and function in EVs.

M150
A kink in brain development: Altering tubulin’s conformational transitions disrupts neuronal morphology and migration
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Human brain malformations are increasingly linked to heterozygous mutations in TUBA1A α-tubulin, yet we do not understand how these mutations impact microtubule function or lead to developmental defects. We hypothesize that ‘tubulinopathy’ mutations act dominantly to subvert the normal regulation of microtubule activity, and may therefore provide an opportunity to gain new insight into basic tubulin function and the regulation of microtubule networks during neuronal migration. Here we investigate mutations from two patients with distinct grades of brain malformations and alternative substitutions at the same V409 residue of TUBA1A. We find that the ectopic expression of either patient allele, TUBA1A-V409A or -V409I, dominantly disrupts neuronal migration in the developing mouse brain. In its free state, the tubulin heterodimer is in a kinked conformation, but must subsequently straighten as it is assembled into the microtubule polymer state. Interestingly, V409 resides within the α-tubulin H11’ helix, positioned at the hinge point between the kinked and straight conformations of the tubulin heterodimer. Our computational modeling predicts that V409A or V409I substitutions perturb the kinked conformation of the tubulin heterodimer, indicating the potential importance of V409 in establishing a kinked state. To test these predictions, we modeled the V409A and V409I mutants in budding yeast α-tubulin and examined effects on XMAP215/Stu2, which selectively binds kinked heterodimer via its TOG domains. We show that both mutants decrease the localization of
XMAP215/Stu2 to microtubule plus-end. Furthermore, both mutants exhibit weaker binding to Stu2 TOG domains in vitro, compared to wild type tubulin. Despite their decreased interaction with XMAP215/Stu2, we find that both mutants increase microtubule polymerization rates and decrease catastrophe frequencies in vivo. Neuronal morphogenesis is highly sensitive to shifts in the balance of how much tubulin exists in the free versus the polymer state, as tipping the scale too far in either direction is detrimental to neurons. Our data in budding yeast suggest that the V409 mutants spend an increased amount of time in the polymer state, compared to wild type tubulin. Accordingly, the ectopic expression of TUBA1A-V409A or -V409I in cultured rat cortical neurons perturbs cellular morphology by increasing the number of secondary and tertiary neurite branches. Taken together, these data indicate that V409 mutants disrupt the kinked tubulin conformational state and lead to increased microtubule polymer in the cell, resulting in aberrant neurite branching that is detrimental to radial neuronal migration. Our work thus highlights a critical role for establishing proper tubulin conformations in migrating neurons.

Physical Principles of Cytoskeleton-Membrane Interactions

M151
Cell surface mechanics gate stem cell differentiation
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Cell differentiation typically occurs with concomitant shape transitions to enable specialized functions. To adopt a different shape, cells need to change the mechanical properties of their surface. However, whether conversely cell surface mechanics control the process of differentiation has been relatively unexplored. Here, we show that membrane mechanics gate the exit from naïve pluripotency of mouse embryonic stem cells. By measuring membrane tension during early differentiation, we find that naïve stem cells release their plasma membrane from the underlying actin cortex when transitioning to a primed state. Mechanically tethering the plasma membrane to the cortex by enhancing Ezrin activity or expressing a synthetic signalling-inert linker, we demonstrate that preventing this detachment forces stem cells to retain their naïve pluripotent identity. We thus identify a decrease in membrane-to-cortex attachment as a new cell-intrinsic mechanism that is essential for stem cells to exit pluripotency.

M152
Membrane Ruffling Allows Cells to Sense and Respond to Changes in Viscosity
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Throughout the body, cells are immersed in extracellular fluids (ECF) that are typically orders of magnitude more viscous than water and normal cell culture medium. Changes in the viscosity of biological fluids are associated with physiological processes and diseases ranging from aging to wound healing to cancer. However, unlike the material properties of the extracellular matrix, the effects of viscous fluids on cell behavior have not been thoroughly studied. Treating cells with synthetic viscous ECF, we find that single cells in 2D exhibit a dramatic response to viscous fluid, doubling in spread area, migrating nearly two-fold faster, and generating significantly stronger traction forces. This behavior is
conserved across several cell types tested, including NIH 3T3, MDA-MB-231, and HEK293. Our characterization of this response to viscosity leads us to propose a mechanism by which membrane ruffling acts as a sensor of viscosity. We simulated elevated ECF viscosity by adding methylcellulose, an inert thickening agent, to culture medium to increase the viscosity up to a maximum of 11,000 times that of water at low shear. Immediately upon addition of viscous medium, membrane ruffling at the cell’s edge is curtailed and cell spread area begins to expand. We observed that the spread area plateaus at around 200% of its original size after roughly 30 minutes. This behavior is viscosity-dependent, with more viscous solutions leading to greater suppression of membrane ruffling and larger increases in spreading area. Counterintuitively, single cells migrate nearly two times faster in very viscous medium, and this speed increase persists for at least 24 hours. The strong correlation between viscosity-dependent spreading expansion and cell speed is reminiscent of a previously proposed model, in which strong cell-ECM adhesion promotes cell spreading and, subsequently, motility. Disrupting integrin engagement to ECM or actin polymerization via drug inhibition profoundly perturbs the increase in cell motility and spreading in response to viscous fluid. Therefore, we deduce that the resistance imposed by viscous ECF suppresses membrane ruffling, facilitating greater integrin engagement with ECM and more efficient actin-driven leading edge protrusion. Ultimately, this allows the cell to generate stronger forces and migrate faster. The implications of these findings are two-fold. First, all reported values indicate ECF is much more viscous than that of cell culture medium, and our study suggests the effects of viscosity should be factored in when studying cellular behaviors. Second, understanding the role of ECF viscosity in various diseases could inspire treatments that modulate local viscosity to affect cell behavior.

M153
Mechanisms of actin force production in clathrin-mediated endocytosis revealed by integrating mathematical modeling with in situ cryo-electron tomography
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Many essential cellular processes, including clathrin-mediated endocytosis (CME), depend on the function of actin filament networks. During CME, the plasma membrane is deformed, forming clathrin-coated vesicles (CCVs) containing cargo. Membrane remodeling is supported by actin filament assembly, but its mode of function remains elusive. We previously used an experimentally constrained mathematical model to find that a minimal endocytic actin network can self-organize, bend, and produce sufficient force at sites of CME for pit internalization (Akamatsu et al., eLife 2020). The study revealed gaps in knowledge of the structural organization of actin filaments in CME necessary to understand its endocytic function. Here we used cryo-electron tomography (cryo-ET) of intact mammalian cells to directly visualize networks of individual actin filaments at CME sites and CCVs, and used mathematical modeling to identify their mechanistic functions. Surprisingly, actin networks at CME sites consisted of both branched and unbranched filaments. Endocytic actin filament lengths were exponentially distributed (90 ± 80 nm), which was predicted by simulations of our previously published model and can be explained by stochastic filament capping. In contrast to previous studies, actin filaments localized not only in short branches around the neck of the pit but also in long filaments stretching between the coat and the plasma membrane. The branched filaments were arranged in multiple discrete clusters, in agreement with model simulations, implying that actin networks arise from
several distinct “founding” mother filaments. CME actin branched at a $70 \pm 8^\circ$ angle, indicating stiffer branch junctions than previously assumed. Simulations suggested that endocytic success is robust to these variations. We determined actin filament polarity. Branched filament plus ends were oriented to allow for plasma membrane deformation and CCV transport. Finally, we identified long proteins ~60 nm in length resembling the actin-CME linker Hip1R, both in the clathrin-coated area and in the neck of the pit. This neck localization of Hip1R linkers might direct actin polymerization-mediated force generation toward the neck of CME sites for vesicle scission. We are currently testing this hypothesis using mathematical modeling. Taken together, our results reveal the complex actin filament organization at endocytic sites in unprecedented structural and mechanistic detail. By combining mathematical modeling and in situ cryo-ET, deeper insights into actin mechanism during CME were achieved than either approach alone. Such a collaborative approach is necessary going forward to gain a mechanistic understanding of the interplay of ensembles of force-producing protein complexes in cellular processes.

M154  
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M154  
Spectrins stabilize extracellular and intracellular polymer networks to regulate endothelial shear responses  
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Shear stress is a frictional force exerted on the vascular lumen by flowing blood. Endothelial cells in the vessel wall sense and respond to this force by secreting vasoactive compounds and by undergoing extensive cytoskeletal remodelling to orient themselves in a direction parallel to the shear axis. Previous work has identified that luminal glycopolymers are involved in shear-sensing and that shear responses require the rearrangement and activation of basolateral integrins. However, a unifying mechanism for how these directional signals are transmitted from the apical to the basolateral cell surface is lacking. We identified a luminal spectrin network in the endothelium that is required for normal cellular alignment along the shear axis. Using single molecule tracking in combination with super-resolution microscopy, we found that this network is required for the immobilization and apical retention of the transmembrane glycoprotein CD44. By stabilizing CD44, spectrin also regulated the surface density of hyaluronic acid, an abundant component of the endothelial glycocalyx that is required for shear-sensing. Interestingly, spectrin depletion resulted in a significant increase in the elastic modulus of endothelial cells as measured by atomic force microscopy. This suggested that spectrin also has a role in regulating the gross cytoskeletal composition of these cells. Importantly, we found that spectrin interacts with and stabilizes actin and vimentin filaments involved in force transduction. Taken together, our observations support the notion that spectrins play a central role in stabilizing mechanosensory and mechanoresponsive machinery in endothelial cells to coordinate responses to shear stress.

M155  
The balance between actomyosin contractility and microtubule polymerization regulates hierarchical protrusions that govern efficient fibroblast-collagen interactions  
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Fibroblasts undergo a critical transformation from an initially inactive state to a morphologically different and contractile state after several hours of being embedded within a physiologically relevant three-dimensional (3D) fibrous collagen-based extracellular matrix (ECM). However, little is known
about the critical mechanisms by which fibroblasts adapt themselves and their microenvironment in the earliest stage of cell–matrix interaction. We identified the mechanisms by which fibroblasts interact with their 3D collagen fibrous matrices in the early stages of cell–matrix interaction and showed that fibroblasts use energetically efficient hierarchical micro/nanoscaled protrusions in these stages as the primary means for the transformation and adaptation. Using a strain mapping technique together with a computational model, we have calculated the 3D strain and stress fields generated by cell protrusions and have found that while cell protrusions grow and compress collagen fibers by their tips, they are able to maintain the tension at the cell–matrix interface by pulling on the matrix by their sides. We found that actomyosin contractility in these protrusions in the early stages of cell–matrix interaction restricts the growth of microtubules by applying compressive forces on them. Consistent with this observation, fibroblasts that never experience tension at the cell–matrix interface are not able to induce any compressive forces on protrusion microtubules and subsequently exhibit a fully dendritic morphology with microtubule-rich thin and long protrusions. Taken together, our results show that actomyosin contractility and microtubules work in concert in the early stages of cell–matrix interaction to adapt fibroblasts and their microenvironment to one another. These early-stage interactions result in responses to disruption of the microtubule network and/or actomyosin contractility that are opposite to well-known responses to late-stage disruption and reveal insight into the ways that cells adapt themselves and their ECM recursively.

M156
**Actin polymerization controlled by mechanical force protects the cytoskeleton from damage and facilitates stress fiber repair**

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Cells are truly the ultimate “smart material” fine-tuning their mechanical properties to match the mechanical demands of their environments. Such plasticity of cellular mechanics underlines a wide range of cell and tissue behaviors such as allowing cells to migrate through narrow spaces, to resist blood shear forces, and to reinforce force bearing tissues. Moreover, the plasticity of cell mechanics, which relies for the most part on the regulation of the structure and dynamics of actomyosin cytoskeleton, plays a protective role and safeguards the cell against mechanical damage. Yet, the molecular players implicated in adaptive changes in cell mechanics are unknown. By combining live-cell imaging and pharmacological manipulations, we assessed how suppression of major actin elongation factors affects the dynamics of actin polymerization at integrin-based focal adhesions, force bearing linkages between the actin cytoskeleton and extracellular matrix. We found that inhibiting formins but not Arp2/3, significantly decreased the rate of actin polymerization at focal adhesions. By depleting individual formins with siRNA, we identified mDia1 as the major actin elongation factor at focal adhesions. To determine whether Dia1 activity is regulated by mechanical forces, we assessed the effect of myosin inhibition on the rate of actin polymerization in the control and mDia1 depleted cells. Analysis of control cells has revealed a dose-dependent decrease in actin polymerization rate at focal adhesions as myosin contractility was suppressed by ≈90%. In contrast, the rate of actin polymerization in mDia1 depleted cells was not affected by myosin inhibition, suggesting that myosin-generated tension modulates mDia1 activity. Furthermore, by using a super-localization microscopy we showed that actin polymerization at focal adhesions exhibits pulsatile dynamics where the spikes of Dia1 activity are
triggered by mechanical force. By combining mathematical modeling and laser nano-surgery, we showed that suppression of force-dependent actin polymerization at focal adhesions results in two-fold increase in mechanical tension on the stress fibers. To assess how this increase in mechanical tension affects the integrity and repair of actin cytoskeleton we used flashes of the LIM domain protein zyxin as a marker for cytoskeleton damage. We found that suppression of actin polymerization at focal adhesions increases the magnitude of spontaneous stress fiber damage and decreases the efficiency of zyxin-mediated stress fiber repair. As the actin cytoskeleton as a whole is subject to complex mechanical constraints in living cells, these results demonstrate the key role of mDia1 in fine-tuning actin organization to prevent cytoskeleton damage.

**Pushing and Pulling Within and Between Cells**

**M157**

The ESCRT machinery counteracts Nesprin-2G-mediated cytoskeletal forces during nuclear envelope repair

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Nuclear envelope ruptures during interphase (NERDI) cause transient loss of nuclear-cytoplasmic compartmentalisation and failure to efficiently repair these ruptures contributes to genome instability and pro-inflammatory responses. Thus, uncovering the mechanisms that underpin rupture repair is a fundamental question in cell biology, and a better understanding of this process has broad implications for human diseases such as cancer and autoimmunity. NERDIs usually occur at sites where the membrane is weakened due to defects in lamina organisation and rupture is induced through contractile actin fibres that increase pressure on the nucleus via the linker of nucleoskeleton and cytoskeleton (LINC) complex, the signalling platform that transfers mechanical forces from the cytoskeleton to the nucleus. Recent studies have identified several cellular components required for nuclear envelope (NE) re-sealing, including integral membrane proteins of the Lap2-emerin-MAN1 (LEM) family, the protein barrier-to-autointegration factor (BAF) and the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, a membrane remodelling pathway highly conserved in eukaryotes. However, a key remaining question is how local regulation of compressive forces at the NE is coordinated with membrane remodelling to re-establish nuclear compartmentalization. Here we describe the ESCRT-associated protein BROX as an essential factor for NE homeostasis that regulates the mechanical properties of the NE during repair. At a mechanistic level, we show that BROX binds Nesprin-2G, a main component of the LINC complex, and that this interaction promotes the removal of Nesprin-2G from compression sites through ubiquitination. BROX also induces changes in Nesprin-2G levels to release cytoskeleton-mediated compressive forces on the nuclear surface, thereby facilitating efficient membrane resealing and protecting genetic material from damage.

**M158**

Tension promotes kinetochore-microtubule release by Aurora B kinase

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To ensure accurate chromosome segregation, interactions between kinetochores and microtubules are regulated by a combination of mechanics and biochemistry. Tension provides a signal to discriminate attachment errors from bi-oriented kinetochores with sisters correctly attached to opposite spindle poles. Biochemically, Aurora B kinase phosphorylates kinetochores to destabilize interactions with microtubules. To link mechanics and biochemistry, current models regard tension as an input signal to locally regulate Aurora B activity. Here we show that the outcome of kinetochore phosphorylation depends on tension. Using optogenetics to manipulate Aurora B at individual kinetochores, we find that kinase activity promotes microtubule release when tension is high. Conversely, when tension is low, Aurora B activity promotes depolymerization of kinetochore-microtubules while maintaining attachment. Thus, phosphorylation converts a catch-bond, in which tension stabilizes attachments, to a slip-bond that releases microtubules under tension. We propose that tension is a signal inducing distinct error-correction mechanisms, with release or depolymerization advantageous for typical errors characterized by high or low tension, respectively.

M159
Caveolae control membrane tension during cytokinesis and promote successful abscission
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Cytokinesis is the last step of cell division and leads to the physical separation of the daughter cells connected by a thin intercellular bridge. The final step of cytokinesis, abscission, requires the assembly of ESCRT-III filaments at the midbody localized at the center of the bridge to constrict and eventually fuse the plasma membrane at the abscission site. It has been previously described that high tension at the intercellular bridge inhibits abscission due to impaired ESCRT-III polymerization to the abscission sites (1). However, beside myosin-II, proteins regulating tension during cytokinesis remain elusive. A proteome analysis of intact, detergent-free “midbody remnants” carried out in the lab recently revealed an unexpected enrichment of constitutive proteins of caveolae at the midbody (2). Many functions have been associated to caveolae in interphase cells, in particular buffering plasma membrane tension upon mechanical stretch. However, no function for caveolae in cytokinesis has been reported so far. Immunofluorescence and time-lapse microscopy revealed that the caveolae components Caveolin1 and Cavin1 dynamically colocalized at the midbody in cytokinetic bridges. This suggests that bona-fide caveolae are present at the midbody. Functionally, Caveolin1 and Cavin1 were found to selectively promote the abscission step. Indeed, CRISPR/Cas9- or RNAi-mediated depletion of either Cavin1 or Caveolin1 caused a delay in abscission, measured by time-lapse microscopy. In addition, ESCRT-III recruitment at the abscission site was decreased, which likely explains the observed cytokinetic defects. Immunofluorescence and time-lapse microscopy revealed that the caveolae components Caveolin1 and Cavin1 dynamically colocalized at the midbody in cytokinetic bridges. This suggests that bona-fide caveolae are present at the midbody. Functionally, Caveolin1 and Cavin1 were found to selectively promote the abscission step. Indeed, CRISPR/Cas9- or RNAi-mediated depletion of either Cavin1 or Caveolin1 caused a delay in abscission, measured by time-lapse microscopy. In addition, ESCRT-III recruitment at the abscission site was decreased, which likely explains the observed cytokinetic defects. Interestingly, the cytokinetic defects resulting from Cavin1/Caveolin1 depletion could be fully rescued by lowering cellular tension, for instance by using the ROCK inhibitor Y27632. In order to directly assess the tension state within intercellular bridges after Cavin1 depletion, we performed laser ablation that revealed an increase in the recoiling velocity of experimentally severed bridges. This demonstrates that bridges are under higher tension after caveolae depletion. We propose that caveolae are important for abscission by regulating membrane tension and thus ESCRT-III polymerization to the abscission site. This work represents the first reported function of caveolae in cell division. (1) Lafaurie-Janvore et al. Science, 2013 (2) Addi et al. Nature Communications, 2020
M160

**Mechanism of cell-cell fusion induced by a bacterial pathogen**

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Cell-cell fusion is important for many biological processes including fertilization, development, bone maintenance, and immunity. Cell-cell fusion also contributes to microbial pathogenesis. For example, bacterial pathogens in the pseudomallei group of *Burkholderia* species, the causative agents of melioidosis and glanders, invade mammalian host cells, live in the cytosol, and spread directly from one host cell to another by inducing cell-cell fusion. The requirements for cell-cell fusion induced by one species in this group, *B. thailandensis*, include intracellular bacterial actin-based (or flagellar) motility and a bacterial protein secretion apparatus called the type VI secretion system. However, the temporal and spatial stages, as well as the molecular mechanisms of cell-cell fusion induced by *B. thailandensis* remain unknown. Using live cell imaging, we found that bacteria first use actin-based motility to push on the host cell plasma membrane to form protrusions that extend into neighboring cells. Then, fusion pores form within these membrane protrusions, either along their length (40%) or at the leading tip (60%). Finally, fusion pores expand to create a fused, multinucleated cell. Membrane protrusions formed by *B. thailandensis* likely help mediate membrane fusion by promoting the key molecular steps leading to fusion pore formation, including fusion protein localization and membrane apposition. In support of this notion, a *B. thailandensis* mutant deficient in a component of the type IV secretion system (VgrG5) enters into protrusions that get engulfed by neighboring cells rather than inducing cell-cell fusion. Interestingly, the *vgrg5* mutant cannot be functionally rescued by over-expression of VgrG5 in the host cell or by secretion of VgrG5 by neighboring wild type bacteria. This finding suggests that VgrG5 must be secreted locally by bacteria in protrusions to localize and/or function to promote fusion. Further study of cell-cell fusion induced by *Burkholderia* may lead to a better understanding of the basic mechanisms of cell-cell fusion in infected and uninfected cells.

M161

**Coordinated trafficking of VE-cadherin for endothelial migration and angiogenesis.**

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Angiogenic sprouting relies on collective migration and coordinated rearrangements of endothelial leader and follower cells. VE-cadherin-based adherens junctions have emerged as key cell-cell contacts that transmit forces between cells and trigger signals during collective cell migration in angiogenesis. However, the underlying molecular mechanisms that govern these processes and their functional importance for vascular development still remain unknown. We previously showed that the F-BAR protein PACSIN2 is recruited to tensile asymmetric adherens junctions between leader and follower cells. Here, we show that PACSIN2 mediates the formation of endothelial sprouts during angiogenesis by coordinating collective migration. We discovered that PACSIN2 recruits the recycling regulators EHD4 to the rear end of asymmetric adherens junctions to form a recycling endosome-like tubular structure. The junctional PACSIN2/EHD4 complex controls VE-cadherin trafficking and thereby coordinates polarized endothelial migration and angiogenesis. Our findings reveal a novel molecular event at force-dependent asymmetric adherens junctions that is active during the tug-of-war between endothelial leader and follower cells and allows for junction-based endothelial guidance during in angiogenesis.
Canoe/Afadin and Abl are required for mechanotransduction at tricellular junctions

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A universal property of cells is the ability to detect and respond to mechanical stimuli, such as the stiffness of the extracellular environment, shear and osmotic stress from fluid flow, and forces generated by neighboring cells. During development, epithelial structure is generated by the dynamic reorganization of cells in response to mechanical forces. In particular, tricellular junctions where three cells meet are actively remodeled as cells rearrange and represent sites of concerted adherens junction reorganization under tension. How tricellular junctions sense and respond to physiological forces, and how these processes influence the dynamics of cell behavior during epithelial remodeling, are not well understood. We demonstrate a tissue-wide pattern of enhanced tyrosine phosphorylation at tricellular junctions in living Drosophila embryos during convergent extension, and show that this enrichment requires cytoskeletal tension. We identify a novel mechanotransduction pathway that contributes to this pattern involving the Abl tyrosine kinase and the conserved actin-binding protein Canoe/Afadin. We show that the levels of Canoe and tyrosine phosphorylation at tricellular junctions are dynamically modulated in response to actomyosin forces. In addition, Canoe rapidly dissociates from tricellular junctions when tension is acutely released using laser ablation. This localization is regulated by Abl-dependent phosphorylation of a highly conserved tyrosine in the Canoe actin-binding domain. Preventing phosphorylation at this site disrupts Canoe mechanosensitivity and destabilizes tricellular adhesion. Conversely, anchoring Canoe at tricellular junctions independently of mechanical inputs aberrantly stabilizes adhesion, arresting cell rearrangement. These results demonstrate that Canoe is a mechanotransducer that dynamically reinforces tricellular adhesion under tension during epithelial remodeling.

Signaling and Metabolic Regulation in Health and Disease

Elucidating the integration of metabolism with oncogenic signals in a Drosophila tumour model

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Sustained proliferative signals and deregulated cellular energetics are two hallmarks of cancer. However, cross talk between growth signals and metabolic states during cancer progression remains elusive. Using Drosophila melanogaster (the fruit fly) as the genetic model organism, we set out to elucidate the molecular basis underlying the integration of metabolic pathways with oncogenic signals in tumor cells. Previously, we established an in vivo tumor model using overexpression of an oncogenic protein kinase known as Homeodomain-interacting protein kinase (Hipk). We reported that the tumor model manifests neoplastic overgrowth, tumor invasion and induction of the epithelial-to-mesenchymal transition (EMT) program, mimicking human carcinomas. Here, combining the powers of fly genetics and metabolic imaging, we find that tumor cells with elevated Hipk acquire distinct metabolic profiles - robust aerobic glycolysis (the Warburg effect) and active mitochondrial metabolism. Mechanistically, elevated Hipk drives transcriptional upregulation of the oncogene MYC, likely through convergence of multiple signaling cascades. MYC induces expression of phosphofructokinase-2 (PFK-2 or PFKFB) among other glycolytic genes. PFK-2 catalyzes the synthesis of fructose 2,6-bisphosphate, which acts as a potent
allosteric activator of phosphofructokinase (PFK) and thus stimulates glycolytic flux. PFK-2 and PFK in turn are required to sustain MYC protein accumulation post-transcriptionally, forming a positive feedback loop. Disruption of the loop abrogates tumorous growth. Most recently we have found that downstream of the feedback loop, elevated Hipk causes tumor cells to accumulate hyperfused networks of mitochondria. These tumor mitochondria are metabolically active and do not overproduce reactive oxygen species, unlike many other tumor models which are characterized by elevated ROS. We find that targeted inhibition of the respiratory chain and the mitochondrial quality control system reduce tumorous growth. All in all, we perform a comprehensive functional characterization of the metabolic profile in a Drosophila tumor model. Our work illustrates extensive cross talk among oncogenic signals, aerobic glycolysis and mitochondrial metabolism, and more importantly, identifies several metabolic vulnerabilities that could be exploited for cancer treatment.

M163
Temporal changes in lipid metabolism control neural crest epithelial-to-mesenchymal transition and plasma membrane fluidity

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Epithelial-to-mesenchymal transition (EMT) during development requires dramatic changes in gene expression, cell adhesion, and membrane protrusions. These cellular processes are intricately linked to fluctuations in plasma membrane dynamics and content; yet, precisely how lipid composition is altered to facilitate these changes remains poorly understood. To ask how lipid composition regulates plasma membrane dynamics in vivo, we examined the membranes of chicken neural crest cells which undergo a temporally-regulated EMT. Using live cell imaging and fluorescence correlation spectroscopy to measure plasma membrane dynamics, we found that migratory neural crest cells have increased membrane fluidity compared to their premigratory precursors. In parsing how increased membrane fluidity is achieved, we observed that expression of the sphingomyelin modifying enzyme, neutral sphingomyelinase 2 (nSMase2), coincides with the onset EMT. Through ex vivo loss-of-function analysis, we found that nSMase2 is necessary for increased membrane fluidity during EMT, and surprisingly, for directional cell migration. Further, we discovered that nSMase2 is required to activate the Wnt and BMP signaling cascades, raising the possibility that sphingomyelin metabolism enhances the endocytosis of activated signaling complexes. We assayed endocytic activity in control and nSMase2-deficient cells and found that sphingomyelin metabolism is required for endocytosis. Further, endocytosis inhibition phenocopied nSMase2 effects on neural crest EMT, consistent with functioning downstream of nSMase2 activity. Together, these data indicate that sphingomyelin metabolism initiates endocytosis to enable cell signaling, increase plasma membrane fluidity, and facilitate directional migration. These results highlight how subtle changes in lipid composition alter the biophysical properties of the plasma membrane to have broad-reaching effects on critical processes such as EMT. This work was funded by NIH grants K99DE029240 (MLP), K99DE028592 (EJH), R01DE027538, and R01DE027568 (MEB) and by the Company of Biologists Travelling Fellowship (MLP).

M165
Lysosome signaling induces mitochondrial itaconate to suppress permissive bacterial growth niches
Mitochondria are signaling organelles that regulate a wide variety of cellular functions and can dictate cell fate. Recent evidence from innate immune cells confer a role for TCA cycle intermediates, as signaling molecules. Upon activation macrophage rapidly rewire TCA cycle integrity and increase the abundance of several TCA intermediates, including citrate, succinate, fumarate and the accessory TCA metabolite itaconate. First cellular functions of select metabolites have been identified, and include the regulation of cytokine production, epigenetic memory and anti-microbial activity. While we are beginning to unravel how TCA-derived metabolites instruct immune cell function, the mechanisms controlling intermediate abundance are unclear. Using a combined live cell imaging/RNA sequencing approach, we made the unexpected finding that lysosomal stress signaling, driven by the lysosomal transcription factor EB (TFEB) controls mitochondrial TCA cycle integrity. TFEB activation, induced by bacterial signals, controls the expression of the TCA cycle accessory enzyme Irg1. Once expressed, the mitochondrial enzyme Irg1 synthesizes itaconate from cis-Aconitate. We show that this TCA cycle programming has major consequences for macrophage biology. When exposed to complex milieus of opposing inflammatory signals, as often found in inflamed and infected tissues, itaconate actively limited the induction of an anti-inflammatory phenotype, thus strengthening the pro-inflammatory state of macrophages. This pathway was important for infections with *Salmonella Typhimurium*, an intracellular bacterium that forms permissive growth niches inside macrophages biased toward an anti-inflammatory phenotype. Activation of the TFEB-itaconate axis selectively restricted hyperproliferating Salmonella subpopulations in macrophages, emphasizing the special role of this pathway in the defense against intracellular bacteria that reside in a lysosome-like compartment. In summary, our data define an unexpected role of an inter-organelar communication pathway between lysosomes and mitochondria to induce itaconate production. We further provide novel insight into the function of itaconate in counteracting immune escape of the intracellular bacteria *Salmonella Typhimurium*.
profiling, we uncover a pronounced proteolytic impairment of NPC lysosomes that is compounded by depletion of luminal hydrolases and enhanced susceptibility to membrane damage. Genetic and pharmacologic mTORC1 inhibition restores lysosomal proteolysis and lysosomal membrane integrity without correcting cholesterol storage, implicating aberrant mTORC1 as a pathogenic driver downstream of cholesterol accumulation. Consistently, mTORC1 inhibition reverses mitochondrial dysfunction in a neuronal model of NPC. Thus, cholesterol-mTORC1 signaling controls organelle homeostasis and is a targetable pathway in NPC.

M167

Trypanosoma brucei Tim50, a Mitochondrial Protein Translocase, Acts as a Virulence Factor for the Parasite

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Trypanosoma brucei belongs to a family of unicellular parasitic protozoa that cause debilitating diseases in humans world-wide. These parasites are transmitted through insect vectors. During its digenetic life cycle, T. brucei adopts in dramatically different host environments by changing their metabolic pattern and mitochondrial activities. Like other eukaryotes, T. brucei imports hundreds of proteins into its single mitochondrion via receptor/translocase complexes of the mitochondrial outer and inner membranes, known as ATOMs and TbTims. TbTim50, a relatively conserved Tim protein in T. brucei possesses a pair of characteristic DXDX(T/V) phosphatase signature motifs found in haloacid dehydrogenase (HAD) superfamily. The recombinant TbTim50 showed both a dual-specific protein phosphatase as well as a phosphatidic acid (PA) phosphatase activity. We found that besides its involvement in mitochondrial protein import, TbTim50 participates in other non-canonical functions. TbTim50 knockdown (KD) in the procyclic form (PF) of T. brucei that dwells in the insect vector increased cellular AMP, activated AMP-activated protein kinase (AMPK) by phosphorylation, and increased the levels of PIP39 that belongs to the same group of protein phosphatases as TbTim50 and is developmentally regulated. PIP39 is localized in the glycosomes, which are modified peroxisomes that compartmentalize many glycolytic enzymes in T. brucei. TbTim50 KD in PF caused the parasite to become more tolerant to exogenous oxidative agents. Label-free quantitative proteomics analysis indicates upregulation of certain glycosomal proteins in this parasite. Interestingly, knocking down both TbTim50 and PIP39 reduced this tolerance to wild-type levels, indicating that inter organellar communication mediated by these phosphatases is important for cellular adaptation. TbTim50 is also expressed in the mammalian infective bloodstream form (BF) of the parasite and is required to maintain mitochondrial membrane potential. Importantly, mice infected with the TbTim50-KD BF of T. brucei survived and the parasite number in blood remained below detection levels for more than 30 days. Depletion of TbTim50 also increased AMPK phosphorylation in this form and transform T. brucei towards a non-replicating stumpy BF, thus reduced infectivity, suggesting an additional role of TbTim50 in parasite development. Supported by NIH grants 1RO1AI125662 and 2SC1GM081146

M168

HD-PTP deficient mouse exhibits lipodystrophy.

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The Endosomal Sorting Complexes Required for Transport (ESCRTs) drive reverse topology membrane remodeling events including the formation of intraluminal vesicles within multivesicular bodies, the budding of retroviruses from the plasma membrane, and the scission of the cytokinetic bridge. The ESCRTs have also been implicated in repairing membrane ablations, biogenesis of extracellular vesicles, and autophagy. The Bro1 Domain Family proteins, including mammalian HD-PTP (product of PTPN23) and ALIX (product of PDCD6IP), are ESCRT-associated factors that facilitate ESCRT-mediated processes. Whereas ALIX knockout mice are viable with a normal lifespan [1, 2], HD-PTP knockout mice are embryonic lethal [3] and HD-PTP heterozygote animals exhibit increased tumorigenesis [4]. To gain insights into the biological processes facilitated by HD-PTP, a hypomorphic allele has been generated. Homozygous hypomorphic HD-PTP mice (HD-PTPh/h) are viable but exhibit lipodystrophy, reduced size, and decreased survival. Analysis of 14-day inguinal adipose tissue indicated reduced expression of adipogenesis markers in the HD-PTPh/h mouse, and HD-PTP knockout preadipocytes similarly display reduced adipogenesis in vitro. Defects in insulin-stimulated signaling were apparent in differentiated HD-PTP knockout adipocytes and HD-PTPh/h inguinal adipose tissue in vitro, correlating with reduced levels of insulin-signaling hallmarks observed in adult HD-PTPh/h inguinal adipose tissue in vivo. Whereas the ESCRT machinery have been suggested to downregulate signaling, these results indicate that HD-PTP promotes insulin-induced signaling in, as well as differentiation of, inguinal adipose tissue. In addition, HD-PTP deficient fibroblasts exhibited reduced lipid droplet formation. These results revealed unexpected roles for HD-PTP in promoting fat accumulation in mammalian cells through supporting insulin signaling, adipogenesis, and lipid droplet formation. 1. Campos, Y., et al., Alix-mediated assembly of the actomyosin-tight junction polarity complex preserves epithelial polarity and epithelial barrier. Nat Commun, 2016. 7: p. 11876. 2. Laporte, M.H., et al., Alix is required during development for normal growth of the mouse brain. Sci Rep, 2017. 7: p. 44767. 3. Gingras, M.C., et al., Expression analysis and essential role of the putative tyrosine phosphatase His-domain-containing protein tyrosine phosphatase (HD-PTP). Int J Dev Biol, 2009. 53(7): p. 1069-74. 4. Manteghi, S., et al., Haploinsufficiency of the ESCRT Component HD-PTP Predisposes to Cancer. Cell Rep, 2016. 15(9): p. 1893-900.
Monday, December 7, 2020

**Cells Migrating in Complex Environments**

**SG1**

**Molecular cues controlling cell migration during germinal center responses**

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Germinal centers (GCs) are crucial sites of antibody diversification and selection. They are dynamic structures composed of actively migrating GC B cells and T follicular helper (Tfh) cells as well as follicular dendritic cells (FDCs) and macrophages. Confinement of these cells within individual GCs is thought to be important for ensuring efficient B cell selection and growth regulation and may also contribute to response diversity by allowing separate evolution of antigen-reactive clones. We have shown that two G-protein coupled receptors, sphingosine-1-phosphate receptor-2 (S1PR2) and the orphan receptor P2RY8, signal via Ga13 and ArhGEF1 to promote GC B cell confinement and growth regulation. Loss of this pathway is associated with the development of GC-type diffuse large B cell lymphoma in mice and humans. We have recently de-orphanized the P2RY8 receptor, identifying S-geranylgeranyl-L-glutathione (Ggg) as a novel intercellular signaling lipid that is involved in mediating GC B cell and Tfh cell confinement and growth regulation. Our ongoing work on the mechanisms underlying S1PR2- and P2RY8-mediated control of cell migration will be presented.

**SG2**

**Cancer-associated fibroblasts use supracellular contractility to compress and shape tumors**

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The tumor microenvironment plays an essential role in tumor progression. One of the most abundant cell types in the tumor microenvironment is cancer-associated fibroblasts (CAFs). Besides biochemical signals, mechanical forces that CAFs exert the extracellular matrix also play a role in tumor progression. CAFs have enhanced contractility and capacity to remodel the ECM, increasing cancer cell invasion. However, at the early stage of tumor progression, CAFs accumulate at the tumor periphery before the onset of invasion, forming a continuous and cohesive layer that surrounds the tumor. Here we investigated if this capsule made of CAFs function as a passive physical barrier that prevents tumor expansion or if possibly CAFs use their contractility to confine cancer cells. Using co-culture of patient-derived cancer cells and primary CAFs, traction force microscopy and mouse models, we show that CAFs organize into supracellular rings around cancer cells and by exerting mechanical forces, they actively compress and shape tumors.

**SG3**

**Epithelial cell division opens the door for macrophage tissue invasion in the Drosophila embryo**

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Tissue-resident macrophages are indispensable in embryo- and organogenesis, and also influence the progression of major types of tumors in both invertebrates and vertebrates [1,2]. These functions rely
on their ability to invade into interfaces formed by tissues, yet how surrounding tissues influence these capabilities in vivo has been the focus of little study. We study such invasive migration in the early Drosophila embryo, where macrophages migrate along the inner basal side of an epithelia and eventually separate this ectodermal layer from the underlying mesoderm, thus invading between these tissues [3,4]. Ectoderm-mesoderm attachment is mediated by a thin extracellular matrix (ECM). Previous experiments imply that the time for macrophage entry is influenced by the mechanical resistance of the surrounding cells [4]; however, what determines the choice of when macrophages start to invade remained mechanistically unclear. Here we show that breaching of the ectoderm-mesoderm barrier by the first macrophage always correlates with the mitotic rounding or division of the ectodermal cell at the entry site. This correlation holds even when the timing of division is altered genetically or pharmacologically: increasing the division rate fosters invasion, and decreasing division frequency impedes it. If ectodermal divisions are completely blocked, macrophages cannot invade the tissue. It is known from in vitro work that cells gradually lose focal adhesions during mitotic rounding until only weak reticular contacts remain [6]. In our in vivo context, the facilitation of invasion by division appears to act through the dissolution of these same focal adhesions in the basally-dividing ectoderm cells flanking macrophage entry; we observe the disappearance of Vinculin-mCherry-marked ECM attachments facing the mesoderm just prior to macrophage advancement. Loosening the ecto-meso attachment by knocking down focal adhesion components specifically in the ectoderm through RNAi of Vinculin, Talin, or beta-PS Integrin facilitates macrophage invasion. Thus, we show that focal adhesions at the tissue edge prevent cells separation by a migrating cell, and that adhesion loss caused by division is required for initial breaching of this barrier. Our study demonstrates how cell division at a tissue edge influences cell invasion into a confined environment in vivo. These results may also be relevant for immune cell infiltration of solid tumors and cancer cell invasion into confluent tissues. [1] Wood W. et al. Dev Cell.;40(3):221-233 (2017)[2] Ovchinnikov D, Genesis 46:447-462 (2008)[3] Siekhaus D.E., et. al, Nat. Cell Biol., 12(6), pp. 605-610, (2010)[4] Ratheesh A. et al., Developmental Cell, 45, 331-346 (2018)[5] Lock J. et al. Nat Cell Biol.;20(11):1290-1302. (2018)

SG4

Cell clusters adopt a collective amoeboid mode of migration in confined non-adhesive environments

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Cell migration is essential to most living organisms. Single cell migration involves two distinct mechanisms, either a focal adhesion- and traction-dependent mesenchymal motility or an adhesion-independent but contractility-driven propulsive amoeboid locomotion. Cohesive migration of a group of cells, also called collective cell migration, has been only described as an adhesion- and traction-dependent mode of locomotion where the driving forces are mostly exerted at the front by leader cells. Here, by studying primary cancer specimens and cell lines from colorectal cancer, we demonstrate the existence of a second mode of collective migration which does not require adhesion to the surroundings and relies on a polarised supracellular contractility. Cell clusters confined into non-adhesive microchannels migrate in a rounded morphology, independently of the formation of focal adhesions or protruding leader cells, and lacking internal flow of cells, ruling-out classical traction-driven collective migration. Like single cells migrating in an amoeboid fashion, the clusters display a supracellular actin cortex with myosin II enriched at the rear. Using pharmacological inhibitors and optogenetics, we show that this polarised actomyosin activity powers migration and propels the clusters. This new mode of
migration, that we named collective amoeboid, could be enabled by intrinsic or extrinsic neoplastic features to enable the metastatic spread of cancers.

SG5

Localized glucose import and ATP production fuels cell invasion through basement membrane barriers
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Cell invasion across basement membrane (BM) occurs in normal developmental processes, immune surveillance, and acquisition of this behavior is the defining step that initiates metastatic disease. Anchor Cell (AC) invasion in C. elegans is a simple in vivo model to visualize and genetically dissect cell invasion events in real-time. The pathways and mechanisms promoting AC invasion are shared with cancer cells, including expression of matrix-metalloproteinases (MMPs) that breakdown BM and F-actin rich membrane protrusions, termed invadosomes, that breach BM. We recently demonstrated that mitochondria are enriched at the invasive cell membrane and produce ATP to fuel polymerization of branched F-actin networks required for invadosome mediated BM invasion. To further understand how cell metabolism powers invasion, we are taking a multipronged approach in investigating mechanisms of nutrient acquisition, mitochondria regulation, and ATP dynamics. Using a ratiometric ATP/ADP sensor (Perceval-HR) we found that ATP levels spike when the AC breaches the BM and then quickly resolve to pre-invasion levels after invasion is complete. Through an AC RNA-seq profile, we further discovered the increased expression of mitochondrial Complex I/III/IV components, suggesting that the AC may maximize ATP production by upregulating the I/III/IV ETC pathway (2.5 ATP/NADH substrate) rather than using II/III/IV (1.5 ATP/succinate substrate). Consistent with this, AC-specific RNAi-directed loss of Complex I, but not Complex II encoding genes, blocks invasion. Further, preliminary results using CRISPR/Cas9 to fuse mNeonGreen (mNG) to ETC components show increased levels of Complex I/III/IV specifically within AC mitochondria, suggesting the invasive AC harbors high capacity mitochondria for increased ATP production. Finally, to determine the carbon source(s) for ATP generation, we completed a focused RNAi screen targeting carbon import and lipid storage systems and identified FGT-1, the GLUT2/3 (glucose transporter) ortholog. A FGT-1::mNG reporter and glucose (visualized using Green Glifon4000) are enriched at the AC invasive cell membrane. FGT-1 is required for invasion and its loss reduces polymerized F-actin and invadosome numbers. Interestingly, absence of FGT-1 also results in loss of mitochondria and ATP at the invasive cell membrane, indicating that polarized glucose import establishes localized ATP production. Taken together, these results reveal how integrated energy acquisition, uptake, and usage are a necessary and dynamic aspect of cellular metabolism that drives invasive behavior.

SG6

Dissemination of Ras^{V12}-transformed Cells Requires the Mechanosensitive Channel Piezo
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Dissemination of transformed cells is a key process in metastasis. Despite its importance, how transformed cells disseminate from an intact tissue and enter the circulation is poorly understood. Here, we use a fully developed tissue, Drosophila midgut, and describe the morphologically distinct steps and the cellular events occurring over the course of Ras^{V12}-transformed cell dissemination. Notably, Ras^{V12}-
transformed cells formed the Actin- and Cortactin-rich invasive protrusions that were important for breaching the extracellular matrix (ECM) and visceral muscle. Furthermore, we uncovered the essential roles of the mechanosensory channel Piezo in orchestrating dissemination of RasV12-transformed cells. Collectively, our study establishes an in vivo model for studying how transformed cells migrate out from a complex tissue and provides unique insights into the roles of Piezo in invasive cell behavior.

SG7

**Epithelial-mesenchymal plasticity is required for metastasis in triple negative breast cancer models.**

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The roles of epithelial-mesenchymal plasticity in metastasis has been controversial, including both the epithelial-mesenchymal transition (EMT) and the reverse mesenchymal-epithelial transition (MET). A major barrier to progress has been the lack of physiological metastasis models with a spontaneous EMT-MET cycle. We characterized two genetically engineered mouse models (GEMM) of breast cancer, two triple-negative breast cancer (TNBC) patient-derived xenografts, and patient tumor samples for their epithelial vs. mesenchymal character. We demonstrated that cancer cells in luminal models typically retain an epithelial differentiation state, while cancer cells in TNBC models display a hybrid E/M state in vivo, and lead invasion in organoids ex vivo. We showed using single-cell RNA sequencing that basal breast cancer cells undergo EMT during invasion. Knocking down the mesenchymal marker vimentin, which is acquired during EMT, suppressed invasion in multiple TNBC models, and metastasis formation in vivo, indicating that EMT is required for metastasis. Consistent with a MET in the distant organs, we demonstrated at single-cell resolution that basal breast cancer cells undergo MET during metastatic outgrowth. Knocking down vimentin at this stage, increased colony formation and metastasis in vivo, demonstrating the importance of MET.

SG8

**Investigating principles of homeostatic vascular remodeling via 4D imaging of live mice**

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Our organs depend on an organized vascular network that supplies them with nutrients and soluble factors via the bloodstream. The endothelial cells (ECs) that line our blood vessels dictate crucial aspects of vascular function and morphogenesis. However, there are still many open questions concerning the cellular mechanisms by which the vasculature is able to establish and maintain homeostatic conditions. The challenge in addressing these questions is the inability to follow the same tissues and cells over time. To overcome this roadblock, we have established an intravital imaging approach to track and manipulate the same ECs in live mice to ask how vessel remodeling is orchestrated as a vascular plexus is established and the mechanisms in place to maintain vascular homeostasis. Longitudinal imaging of vessel remodeling of the dermal vascular plexus in neonatal mouse skin reveals that vessel regression significantly outpaces new vessel growth (angiogenesis) during postnatal development, and that vessel regression is accelerated in mutant mice lacking the actin cytoskeletal remodeler Rac1 in ECs. Tracking of single labeled cells shows that the vast majority of capillary ECs undergo elongation and interestingly, participate in luminal migration during this developmental period. Furthermore, we find that regression
events are not mediated by apoptosis but rather the coordinated repositioning and integration of ECs into existing vessels. Intriguingly, the rate of EC migration gradually decreases and eventually arrests in adulthood with adult ECs displaying positional stability even over a period of 4-weeks. We next sought to understand the mechanisms by which the adult vascular plexus is able to maintain vascular function and integrity during homeostasis. By carrying out targeted laser ablations to inflict localized subcellular damage, we find unexpectedly that ECs do not respond with proliferation but instead rapidly compensate for their neighbors by elongation or migration towards the injury site to mediate vascular repair. Intriguingly, we also observe that injury inflicted on the plasma membrane of individual ECs results in a pinching off and discarding of damaged membrane in what is likely an exocytic mechanism of plasmalemmal self-repair. This is a phenomenon that has not previously been described in ECs and we are currently investigating the role that caveolae (enriched in ECs) could be playing in the self-repair process by utilizing Caveolin-1 knockout mice. Overall, this study sheds light upon fundamental mechanisms by which ECs maintain and control vascular homeostasis in a live mammal.

SG9
Compromised nuclear envelope integrity leads to TREX1-dependent DNA damage and tumor cell invasion

The nucleus is the largest and stiffest organelle of the cell, thus its deformability becomes a critical factor affecting cell behavior and function when cells experience strong spatial confinement. While mutations leading to a fragile envelope of the cell nucleus are well known to cause diseases such as muscular dystrophies or accelerated aging, the pathophysiological consequences of the recently discovered mechanically induced nuclear envelope (NE) ruptures in cells harboring no mutation are less known. In this study, we demonstrated a causal relationship between confinement-induced NE rupture, DNA damage and specific long-term cellular phenotypes: senescence in non-transformed cells, and an invasive phenotype including increased collagen degradation in human breast cancer cells, both in vitro and in a mouse xenograft model of breast cancer progression. Strikingly, we identified the cytosolic nuclease TREX1 as a key factor responsible for DNA damage following NE rupture. We show that these phenotypic changes are due to the presence of chronic DNA damage and activation of the ATM kinase. Importantly, TREX1 depletion was sufficient to abolish the DNA damage in mechanically challenged nuclei and to suppress the phenotypes associated with the loss of NE integrity. Additionally, our results show that TREX1-dependent DNA damage induced by physical confinement of tumor cells inside the mammary duct drives the progression of in situ breast carcinoma to the invasive stage. We also analyzed the association of TREX1 expression levels in breast cancer with overall survival using public datasets. This analysis revealed that high level of TREX1 expression is significantly associated with reduced probability of patient survival. Thus, targeting this nuclease might be an effective therapeutic strategy to curb tumor progression. My findings suggest, for the first time, an enzymatic origin for DNA damage occurring in response to mechanical constraints. We anticipate that in normal tissues, this would trigger cellular senescence, contributing to developmental programs, tissue homeostasis, maybe serving as a
cell-crowding sensor to regulate cell density, or even participating in tissue aging. This phenomenon could be exacerbated by mutations causing fragile nuclei, leading to degenerative diseases. In contrast, in tumor cells, in which the senescence checkpoint has been lost, it would lead to aberrant invasiveness, promoting tumor growth and invasion. Hence, we propose that DNA damage in mechanically challenged nuclei could affect the pathophysiology of crowded tissues by modulating proliferation and extracellular matrix degradation of normal and transformed cells.

Cytoskeletal Ensembles Across Scales

SG10
Intertwined orthogonal contractile fibers across heart ventricular walls
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Heart is made of specialized muscle cells called cardiomyocytes, which contains an array of subcellular structures sarcomere. These sarcomeres contract in coordinated fashion to generate a unidirectional wringing motion, essential for effective blood pumping throughout an organism. From its shape and electrical propagation patterns, researchers have speculated that the cardiomyocytes are organized as contractile fibers (myofibers) across heart walls. Further, several models converge to suggest that these myofibers are arranged in a helicoid fashion across different regions of heart. These models are derived from low-resolution diffusion-tensor imaging and modeling, it is still not clear how the cardiomyocytes are organized at cellular level and whether there is a helical arrangement of muscle fibers in heart. Here we report the first reconstruction of myocyte geometry across the entire ventricular wall of mouse heart, at micron scale. We achieve this by clearing the mouse heart and the entire short axis and long axis sections stained with cell membrane specific fluorescent dyes were subjected to light microscopy based deep imaging. We then apply computer vision methods to estimate the cell orientation from the composite image stacks of the whole area of heart tissue section. Thus, the imaging and computational analysis yields ~ 3 order magnitude gain in resolution compared to the existing models that describe myofiber organization of heart ventricles. Our reconstructions at the cellular scale in intact tissue reveal entirely new features of the structural arrangement of cardiomyocytes, including, sharp transitions of cell/myofiber orientations and an array of myofibers orthogonal to the myocardium (or circumferential fibers). Combinedly from our data, we hypothesize that the intertwined orthogonal fiber system is important for coordinating contraction, electrical wave propagation and the opening-closing cycle of atrioventricular valves. Our work provides organizational principles that will be invaluable for understanding the 3D architecture that make up heart tissue and their dysfunction during cardiomyopathy disease progression.

SG11
Reconstitution of Excitable and Oscillatory Rho Waves
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Cell-free reconstitution of biological systems is an essential tool for uncovering the molecular mechanisms that underlie cytoskeletal function. Here we describe the development of an ex vivo system
that reconstitutes cellular cortical excitability on a supported lipid bilayer. In cells, cortical excitability is a phenomenon characterized by waves of active Rho (Rho-GTP) and actin polymerization that are thought to “prime” cells to initiate actomyosin-dependent events such as cytokinesis and cell migration. Our group has previously characterized cortical excitability in developing *Xenopus laevis* embryos and starfish oocytes. We found that cortical waves are amplified by overexpression of the RhoGEF Ect2 and inhibited by actin filaments (F-actin). These results suggest that cortical excitability exists as an activator-inhibitor system, where both positive (Ect2-dependent) and negative (F-actin-dependent) feedback loops exist simultaneously to induce wave formation and propagation. We have now generated an *ex vivo* system using actin-intact *Xenopus* egg extract and a supported lipid bilayer that successfully reconstitutes both excitatory and oscillatory active Rho waves. Upon addition to a lipid bilayer, egg extract generates a robust, excitable wave of Rho-GTP followed by actin polymerization. This first excitable wave has a phase shift between the Rho-GTP and F-actin that is comparable to *in vivo* cortical waves. This wave is quickly followed by the formation of an increasingly dense F-actin network and oscillatory active Rho waves. The periodicity of these oscillatory waves changes over time, which is in contrast to the stable period that has been characterized in cells. Both excitable and oscillatory waves are mediated by Rho signaling, as the addition of C3 transferase, a Rho inhibitor, blocks the formation of any *ex vivo* wave patterning. Reconstituted oscillatory Rho-GTP waves are also highly sensitive to the presence of F-actin. Surprisingly, the addition of the actin depolymerizer Latrunculin induces the rapid termination of oscillatory Rho-GTP patterning on the bilayer, whereas *in vivo*, Latrunculin addition amplifies Rho-GTP patterning. The addition of select factors that destabilize actomyosin (including SMIFH2, H-1152, Swinholide A, and Cofilin) also induces the rapid termination of oscillatory Rho-GTP patterning on the bilayer. Differences between reconstituted active Rho waves and cellular cortical waves offer a unique opportunity to investigate the various ways in which Rho-GTP and cytoskeletal patterning are regulated.

**SG12**

*Visco-elastic properties of bulk cytoplasm maintain the mitotic spindle in the center of large cells*


Mitotic spindles are large macromolecular assemblies that reside in the cytoplasm to specify the position of the division plane. Spindle position is largely admitted to result from forces of astral microtubules (MTs) that connect spindle poles to the cell surface. In large sea urchin eggs, metaphase spindles are stable in the cell center, but small compared to cell size so that astral MTs do not reach the surface. Using *in vivo* magnetic tweezers to displace or rotate spindles with calibrated forces and torques, we find that in spite of their lack of interaction with the cortex, spindles are held in the cell center by large viscoelastic forces, which partially restore their centered position upon displacement. Centering elasticities and time-scales are independent of MTs and dynein, and have similar magnitudes to that of pure cytoplasm, and can even move back a passive magnetized oil droplet with a size similar to the spindle to its initial position. Bulk cytoplasm viscoelasticity may thus constitute an essential cellular element for maintaining spindles in the cell center.
SG13

**Opposing motors provide mechanical and functional robustness in the mammalian spindle**

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The metaphase spindle reaches a steady-state shape, despite continuous microtubule turnover and motor activity. How motors together give rise to the mammalian spindle’s emergent architecture, mechanics, and function remains poorly understood. The motors dynein and Eg5 are each key to bipolar spindle formation: dynein mediates contractile microtubule minus-end clustering, and Eg5 drives extensile microtubule sliding. Yet when both are inhibited, the spindle can still establish its normal shape. What, then, are the roles of these opposing motors? To answer this question, we generate doubly inhibited spindles by knocking out dynein’s microtubule end-targeting factor NuMA and acutely inhibiting Eg5. These spindles not only attain a shape and size indistinguishable from controls, but also enter anaphase. However, under mechanical compression these spindles are more fragile: they deform more easily and fail structurally. Further, they exhibit reduced microtubule dynamics and internal organization, and lagging chromosomes in anaphase. Thus, while these opposing motor activities are not required for the spindle’s shape, they are essential to its mechanical and functional robustness, which we propose occurs through increased spindle dynamics and organization. Together, this work may provide insight into the robustness of other active, self-organizing cellular structures.

SG14

**Visualizing Microtubule Array Remodeling at Single Microtubule and Protofilament Resolution by Atomic Force Microscopy**

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Assembly and disassembly of self-organized structures such as cytoskeletal arrays, membrane domains and biological condensates are critical for cellular function. The dynamics of the individual units of these structures are invisible to optical and electron-microscopy. We employ Atomic Force Microscopy to bridge this spatial-temporal resolution gap and image the remodeling of large microtubule arrays. Our model systems are crosslinked-microtubule bundles and axonemes, and two conserved depolymerases. This imaging modality reveals depolymerization reaction intermediates at single microtubule and protofilament level, including lattice defect propagation. The data indicates that dichotomy in depolymerizing kinesins, which enables either rapid array remodeling or fine length control, arises from differences in activity at the protofilament level. This study highlights the power of AFM in visualizing the dynamics of micron-sized self-organized structures.

SG15

**Actin cables, clouds, and comet tails organize mitochondrial networks in mitosis**

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Symmetric cell division requires even partitioning of genetic information and cytoplasmic contents between daughter cells. While the mechanisms coordinating the replication and segregation of the genome have been extensively explored, the process by which organelles are apportioned remains less well-understood. Here, we identify multiple cytoplasmic actin assemblies that play distinct but convergent roles in mitochondrial organization in mitosis. First, we find a dense network of subcortical actin cables crisscrossing individual mitochondria in mitotic cells. This meshwork templates uniform 3D-mitochondrial positioning about the spindle and ensures the equal segregation of mitochondrial mass in cytokinesis. Second, we identify a CDC42/N-WASP/Arp2/3-dependent wave of actin filaments reversibly assembling on the surface of mitochondria to direct their motility. Within this wave mitochondria are either confined within branched-actin clouds or propelled by elongated, multi-tailed actin comets. Together, clouds and comet tails promote randomly oriented bursts of movement, which, over the duration of mitosis, effectively shuffles mitochondrial position in the mother cell to ensure their random and unbiased partitioning between daughter cells.

SG16
Asymmetric Inheritance of Keratin Intermediate Filaments Regulates Fate in the Early Mammalian Embryo
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How a single-celled zygote develops into a complex multicellular organism with specialized cell lineages remains a central question in biology. During the earliest stages of mammalian development, the preimplantation mouse embryo undergoes the first cell fate decision to produce two cell lineages of the blastocyst: an outer trophectoderm layer that forms the placenta, and an inner cell mass that generates the fetus along with other extraembryonic tissues. Although the actin and microtubule cytoskeletal networks are known to play key roles in orchestrating blastocyst formation, the function of the third major class of cytoskeletal filaments - intermediate filaments - is virtually unknown. We thus established live imaging and computational segmentation approaches to study keratin intermediate filament dynamics during the development of the preimplantation mouse embryo. In contrast to actin and microtubules, keratins are expressed heterogeneously beginning in a small subset of cells in the early embryo, where they localize to the apical cell cortex. During the asymmetric cell divisions generating inner and outer cells of the embryo, we find that these early-assembling keratins retain an apical localization in the mitotic cell, and become asymmetrically inherited by the outer daughter cell. There, keratins stabilize the cortex to promote apical polarization and Yap-dependent Cdx2 expression to specify the first trophectoderm cells of the embryo. Our findings identify a new early function for keratin intermediate filaments as asymmetrically inherited fate determinants in mammalian embryogenesis, and support a model in which early cell-cell heterogeneities prior to inner-outter cell segregation can bias lineage fate via the differential regulation of keratins in the mammalian embryo.
SG17

Anchoring of cortical actin pools by the late endocytic pathway during subcellular tube guidance

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The actin cytoskeleton participates in a wide range of cellular processes, from endocytosis to cell migration. Nucleation and anchoring at different subcellular locations allows cells to undergo cell shape changes that are important for morphogenesis. Terminal cells of the Drosophila respiratory system form a subcellular tube by invaginating the apical plasma membrane, and at least three distinct actin pools are involved in regulating their development: In the basal domain (1), in filopodia (2), and in the apical membrane domain (3). Disrupting any of these pools affects subcellular tube guidance, but why is this and what mediates the crosstalk among them is not known. We report here that actin also assembles around vesicles of the late endocytic pathway, which are mostly present towards the tip of the growing subcellular tube. We use live imaging to show that actin bundles bridge the apical and basal membranes through the late endosomes at the tip. Disturbing late endosome formation or actin nucleation at late endocytic compartments (using genetic constructs or laser ablations) affects the directionality of tube growth, uncoupling it from the direction of cell elongation. These findings highlight a role for late endosomes in regulating cell morphogenesis by organizing the actin cytoskeleton, besides their conventional role in membrane and protein trafficking.

SG18

The Drosophila spectraplakin Short stop integrates cell-matrix adhesion during cell migration

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The spectraplakin family of proteins includes ACF7/MACF1 and BPAG1/dystonin in mammals, VAB-10 in C. elegans, Magellan in zebrafish, and Short stop (Shot), the sole Drosophila member. Spectraplakins are giant cytoskeletal associated proteins that can bind actin, microtubules, and intermediate filaments and thus are positioned to coordinate the activity of the entire cytoskeletal network. Fittingly, spectraplakins have been implicated in the regulation of cell migration where they help maintain coordination between actin and growing microtubules. In this study, we examined the role of Shot during cell migration using two systems: the in vitro migration of Drosophila derived tissue culture cells, and in vivo through the migration of border cells. We find that RNAi depletion of Shot increases the rate of random cell migration in two different Drosophila tissue culture cell lines as well as the rate of wound closure during scratch-wound assays. This increase in cell migration prompted us to analyze focal adhesion dynamics as cell-matrix adhesions play a critical role in regulating cell migration speeds. We found that the rates of focal adhesion assembly and disassembly were faster in Shot depleted cells as compared to control treatments. Furthermore, this regulation of focal adhesion dynamics may be dependent on Shot being in an "open" confirmation. Finally, using Drosophila border cells as an in vivo model for cell migration we found that RNAi depletion also led to precocious border cell migration as compared to staged controls. Collectively, these results suggest that spectraplakins not only function to cross-link the cytoskeleton but may also regulate cell-matrix adhesion.
SG19

**Septins mediate actin filament capture and polymerization along microtubules**

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Coordination of the actin and microtubule networks is essential for cellular morphogenesis and underlies fundamental processes including cell migration and division. However, the mechanisms and molecules that mediate actin-microtubule crosstalk are little understood. Septins are a family of multimerizing GTPases, which associate with actin filaments and microtubules, but it is unknown whether septins link directly the organization and dynamics of actin and microtubules. High-resolution imaging showed that septins localize to areas of actin filament and microtubule overlap in the growth cones of primary rat hippocampal neurons and ventral membranes and filopodia of COS-7 cells. Using purified recombinant SEPT2/6/7 complexes, we found that SEPT2/6/7 crosslinks in solution taxol-stabilized microtubules and phalloidin-stable actin filaments into elongated hybrid bundles. Decoration of immobilized taxol-stabilized microtubules with increasing concentrations of SEPT2/6/7 showed that pre-polymerized actin filaments are captured and aligned with microtubules in a concentration-dependent manner. Using TIRF microscopy and stable SEPT2/6/7-coated microtubules, we tested whether septins enhance the capture of actin ends that polymerize from immobilized actin seeds. In the absence of septins, only a small percentage (<10%) of actin ends was captured and polymerized along microtubules at collision angles of <30 degrees. Strikingly, ~40% of actin polymerizing ends co-aligned and polymerized along microtubules with SEPT2/6/7. Similarly, addition of soluble actin into chambers with SEPT2/6/7-coated microtubules resulted in a microtubule-templated actin polymerization with actin ends extending linearly from the lattice of immobilized stable microtubules. Collectively, these findings show that septins can directly crosslink actin filaments and microtubules, and mediate the capture and polymerization of dynamic actin ends on microtubule lattices. We posit that septins provide a novel mode of coordination between actin filaments and microtubules during cell protrusion and migration.

SG20

**Reciprocal regulation between cytoskeletal elements and microRNA-31 during cell division**

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Proper cell division is critical for all biological systems, and its disruption is catastrophic for the organism. Cell division is dependent on the precise choreographic movements of mitotic apparatus, which is a highly dynamic structure that consists of a large complex of microtubules, proteins, and actin. We observed that miR-31 targets are involved with the regulation of actin dynamics (*Fascin, Rab35, and Gelsolin*). They are localized and enriched in the perinuclear region in non-dividing cells and associate with mitotic spindles in *dividing* cells in the sea urchin embryo. This striking oscillation of localization of miR-31 and its targets suggests that these RNAs may play a direct role in spindle assembly, and/or cell cycle progression. To determine the importance of microtubules in miR-31, its targets, and actin dynamics, we treated the embryos with microtubule disruptors (nocodazole) and observed a decrease in the localization of *miR-31* and *Fascin* in the spindle midzone. Nocodazole also caused a decrease in actin localized in the spindle midzone. In the presence of microtubule stabilizers, hexylene glycol, and Taxol, we observed *miR-31, Fascin,* and *Rab35* are further enriched at the mitotic spindles. These results
suggest that microtubules regulate the localization and potentially the stability of these transcripts. Additionally, we observed more cytoplasmic actin in hexylene glycol and Taxol treated embryos, indicating that microtubules could be important for the formation of actin. To examine the function of miR-31 during cell division, we microinjected miR-31 inhibitors into zygotes. We found that miR-31 inhibition resulted in more extensive astral microtubules and less or incorrectly directed interpolar and kinetochore microtubules in cleavage stage embryos compared to the control. These results indicate that miR-31 regulates the cytoskeleton-rich mitotic spindle structure during mitosis. Thus, the cytoskeletal microtubules regulate the localization of miR-31 and its targets, and miR-31 regulates the microtubule structures. Since proper spindle formation is a prerequisite for faithful segregation of chromosomes, understanding additional regulatory factors of cell division will be important for cancer, growth, and development.

SG21

Tropomyosin/actin filaments are required for merging of microtubule asters in mitotic spindle assembly

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An increasing number of studies have demonstrated an important role for the actin cytoskeleton in mitotic spindle assembly in mammalian cells through its interaction with the microtubule network and its dynamic association with spindle poles and the cell cortex during mitotic progression. We have identified a specific population of cortical actin filaments containing tropomyosin Tpm3.1 that is required for the merging of microtubule asters to form a bipolar mitotic spindle via regulating the localization of the NuMA-dynein-dynactin complex to the poles. This mechanism is revealed by exposing HeLa cells to low dose vincristine to induce supernumerary microtubule asters immediately after nuclear envelope breakdown (NEBD). Live-cell imaging was performed to track the behaviour of these microtubule asters in the presence and absence of small molecule inhibitors of Tpm3.1. Many of the asters caused by vincristine single-drug treatment quickly merge with the two centrosome-organized spindle poles; whereas the remainder aggregate into large spindle poles resulting in a multi-polar spindle. Over time the extra acentrosomal poles move towards and combine with the centrosomal poles to form a functional bipolar spindle. The combination of anti-Tpm3.1 compounds with vincristine treatment causes an increase in the number of microtubule asters upon NEBD. Furthermore, the combination disrupts the movement of the asters towards centrosomes, resulting in a persistent multi-polar spindle over a long time period up to 16 h or until cell death. Our fixed-cell imaging results show that those microtubule asters are associated with the NuMA-dynein-dynactin complex, which has been known to cluster microtubules or microtubule asters into spindle poles during early mitosis. Moreover, we found that dynactin and dynein co-immunoprecipitated with Tpm3.1 in mitotic cell extracts, indicating a potential interaction between Tpm3.1 and these motor proteins during mitosis. Taken together, our findings suggest the possibility that microtubule motors are using Tpm3.1-containing actin filaments to facilitate the movement of microtubule asters to centrosomes during bipolar spindle formation.
Reconstituting Cell Biology

SG22
The limits to (actin) growth
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Eukaryotic cells determine their shape and organization through the actin cytoskeleton that forms a variety of networks. Common to all actin structures is their growth from soluble subunits that assemble into filaments. Assembly of functional actin networks requires control over the speed at which actin filaments grow. How this can be achieved at the high and variable levels of soluble actin subunits found in cells is unclear. We have developed new methods to visualize actin growth at physiological subunit concentrations for the first time. We discover that under these conditions, filament growth is limited by profilin dissociating from the filament end and the elongation speed becomes insensitive to the free subunit concentration. Profilin release can be directly promoted by formin actin polymerases even at saturating profilin-actin concentrations. We demonstrate that mammalian cells indeed operate at the limit to actin filament growth imposed by profilin and formins. Our results reveal how synergy between profilin and formins generates robust filament growth rates that are insensitive to changes in the soluble subunit concentration.

SG23
Filament nucleation tunes mechanical memory in actomyosin networks
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Incorporating growth into contemporary material functionality presents a grand challenge in materials design. The F-actin cytoskeleton is an active polymer network which serves as the mechanical scaffolding for eukaryotic cells, growing and remodeling in order to determine changes in cell shape. Nucleated from the membrane, filaments polymerize and grow into a dense network whose dynamics of assembly and disassembly, or ‘turnover’, coordinates both fluidity and rigidity. Here, we vary the extent of F-actin nucleation from a membrane surface in a biomimetic model of the cytoskeleton constructed from purified protein. We find that nucleation of F-actin mediates the accumulation and dissipation of polymerization-induced F-actin bending energy. At high and low nucleation, bending energies are low and easily relaxed yielding an isotropic material. However, at an intermediate critical nucleation, stresses are not relaxed by turnover and the internal energy accumulates 100-fold. In this case, high filament curvatures template further assembly of F-actin, driving the formation and stabilization of vortex-like topological defects. Thus, nucleation coordinates mechanical and chemical timescales to encode shape memory into active materials.
SG24

Spatial control of membrane traffic - deciphering and reconstituting the septin GTPase code

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Spatial control of membrane traffic is essential for the morphogenesis and maintenance of polarized cell types such as epithelia and neurons, and their physiological functions in tissue homeostasis and neurotransmission. Many advances have been made in understanding membrane traffic at points of origin (protein sorting, vesicle formation) and destination (vesicle docking/fusion), but how movement on cytoskeletal polymers (microtubules, actin) is spatially oriented and regulated en route to destination is little understood. The septin GTPases comprise a large family of GTP-binding proteins, which multimerize into higher order structures that associate with spatially distinct populations of microtubules and actin filaments, and membrane domains. Septins assemble into complexes of variable subunit combination and localization, and thereby, may constitute a code for the spatial organization of cytoskeletal tracks and the navigation of molecular motors and their cargo. In vitro reconstitution of microtubule motor motility and microtubule-actin dynamics with septin complexes and filaments has yielded novel insights into the function of septins as spatial regulators. Here, we highlight recent and new evidence on the regulation kinesin and dynein motors by microtubule-associated septin paralogs (SEPT9) and complexes (SEPT2/6/7, SEPT5/7/11). We show that the motility of kinesin-3/KIF1A is differentially regulated by septin complexes and paralogs, while the kinesin-1/KIF5 and dynein-dynactin motors are more broadly inhibited by microtubule-associated septins. Interestingly, however, SEPT5/7/11 appears to function distinctly in the microtubule tethering of kinesins (KIF5, KIF1A) and dynein by blocking detachment from microtubules. Reconstitution experiments also point to functions for microtubule-associated septins in the capture and positioning of microtubules and actin filaments, which impact the directionality of membrane traffic indirectly and independently of motor-driven transport. Overall, our results suggest that septins provide a spatial code for the organization and regulation of membrane traffic, whose complexity and significance have only begun to emerge.

SG25

Microtubule lattice defects promote catastrophes

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Microtubules are dynamic cytoskeletal polymers that can spontaneously switch between phases of growth and shrinkage. The probability of transition from growth to shrinkage, termed catastrophe, increases with microtubule age, but the underlying mechanisms are poorly understood. Here, we set out to investigate whether microtubule lattice defects formed during polymerization can affect growth at the plus end. To generate microtubules with lattice defects, we used microtubule-stabilizing agents which promote formation of polymers with different protofilament numbers. By employing different agents during nucleation of stable microtubule seeds and the subsequent polymerization phase, we could reproducibly induce switches in protofilament number. Such switches led to frequent catastrophes, which were not observed when microtubules were grown in the same polymerization conditions but without a protofilament number mismatch. Microtubule severing at the site of the defect
was sufficient to suppress catastrophes. We conclude that structural defects within microtubule lattice have propagating properties that can affect the dynamic state of the plus end.

SG26
**Regulation of microtubule dynamics by TOG domain proteins**
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Regulation of microtubule dynamics is essential in fundamental cellular processes such as cell division, cell migration and neuronal development. XMAP215/chTOG and CLASP are two families of prominent microtubule regulators which contain characteristic TOG domains - helical repeat modules that bind tubulin heterodimers. Although the regulatory functions of both XMAP215 and CLASP families critically depend on their TOG domains, the respective effects of XMAP215 and CLASPs on microtubule dynamics are strikingly distinct. XMAP215 is a potent microtubule polymerase, capable of promoting an order-of-magnitude increase in microtubule growth rates in vitro. Interestingly, XMAP215 simultaneously promotes microtubule catastrophe, a transition from microtubule growth to shrinkage. In contrast, CLASPs strongly suppress microtubule catastrophe and promote microtubule rescue, the transition in which a shrinking microtubule rapidly switches back to growth, without affecting growth and shrinkage rates. Here, I will present our recent insights into the regulatory mechanisms of the two TOG-domain protein families.

SG27
**Structural and material design principles of centrosomes**
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Cell division demands precise movement of chromosomes via microtubule-mediated forces. In mitotic cells, centrosomes nucleate robust microtubule arrays and act as predominant force-coordinating centers. It remains a mystery how thousands of nanometer-scale centrosome proteins self-organize to create a micron-scale structure that 1) is dynamic, 2) is able to nucleate thousands of microtubules and pattern them into an astral array, and 3) is able to resist microtubule-mediated forces without suffering mechanical failure. We have developed an in vitro reconstitution system to address how molecular-level interactions between centrosome proteins generate the functional ultrastructure and material properties of assembled centrosomes in *C. elegans*. Our results suggest that the centrosome is an amorphous, composite hydrogel whose strength is determined by phospho-tunable connections between scaffolding proteins and dispersed cross-linking proteins.

SG28
**Reconstitution of mitotic chromosome scaling using Xenopus egg extracts**
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During the rapid and reductive cleavage divisions of a developing embryo, subcellular structures such as the nucleus and mitotic spindle scale with decreasing cell size through a combination of biochemical and physical mechanisms. Mitotic chromosome size has also been shown to decrease during development, but this process remains a mystery, partly due to the major challenge of isolating and accurately measuring mitotic chromosome dimensions from embryos at different stages of development.
Previously, we developed an approach using the *Xenopus* in vitro system that reconstitutes mitotic chromosome scaling in vitro by combining G2-arrested nuclei isolated from various stages of embryogenesis with metaphase-arrested egg extract. To identify the underlying molecular mechanisms, we are characterizing the differences between replicated mitotic chromosomes formed upon addition of sperm nuclei and chromosomes formed using nuclei from blastula-stage embryos, which are 50% shorter. Current work addresses two key questions: (1) What are the biochemical or physical characteristics of the embryo nuclei that set chromosome size at a particular developmental stage and (2) how does the architecture of a mitotic chromosome change as it shrinks in size? To begin tackling the first question, we have analyzed the abundance of three factors known to play a role in setting chromosome size: condensin I, topoisomerase 2 and linker histone H1. We found that compared to the longer replicated sperm chromosomes, condensin I and topoisomerase II are less abundant on shorter chromosomes assembled from embryo nuclei, while histone H1 shows the opposite trend. Current experiments focus on how localization of these factors is differentially regulated. To address the second question, we used Hi-C to investigate whether the changes in size between sperm and embryo chromosomes are accompanied by alterations in their physical organization. Combining the Hi-C data with polymer brush models of the mitotic chromosome indicates that the shortening of the embryo chromosomes is due to differences in DNA loop size and DNA stacking along the central axis. Taken together, our results provide new insight into how the biochemical and physical properties of mitotic chromosomes are modulated during development.

**SG29**

*Controlled Metabolic Cascades for the Protein Synthesis in an Artificial Cell*

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Due to the extreme complexity of a living cell, the notion of mimicking cell minimally includes; (1) construction of cellular membranes, providing cellular space and functionalities at interfaces; (2) chemical and biological reactions in cellular structures and (3) construction of a cytoplasmic environment (including macromolecular crowding and compartmentalization of biomolecular components). In nature, photosynthesis provides that energy gradient across lipid membranes though transmembrane proteins, whereby the light energy is converted to chemical energy, to generate energy for incorporation of carbon dioxide. We designed, built, and tested a light-harvesting encapsulated organelle system that provides both a sustainable energy source and a means of controlling intracellular reactions. Independent activation of the two photoconverters, which respond to different light wavelengths, allowed dynamic regulation of ATP synthesis: red light facilitated and green light impeded ATP synthesis. Recently we applied the artificial organelles to simulate a ubiquitous process in cells—cytoskeleton formation through ATP-dependent actin polymerization. Optical stimulation initiated ATP synthesis and induced ATP-dependent actin polymerization, leading to growth of three-dimensional actin filaments. Red light facilitated and green light impeded filament growth via independent PSII and PR activation. The resulting system was able to induce and control cascading metabolic reactions as well as exhibit cell-like behaviour. We envision that our bottom-up approach could be used to create isolated modules representing additional organelles. Introducing networks of proteins and organelles into artificial cell-like environments may contribute to achieving the long-standing goal of building a cell de novo.
SG30

**Mechanism of ER-associated protein degradation (ERAD)**

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Misfolded luminal endoplasmic reticulum (ER) proteins undergo ER-associated degradation (ERAD-L): they are retro-translocated into the cytosol, poly-ubiquitinated, and degraded by the proteasome. ERAD-L is mediated by the Hrd1 complex (composed of Hrd1, Hrd3, Der1, Usa1, and Yos9), but the mechanism of retro-translocation is poorly understood. Hrd1 is a multi-spanning ubiquitin ligase that can mediate retro-translocation in the absence of the other components when overexpressed in vivo or when reconstituted into proteoliposomes. Hrd1 is activated by auto-ubiquitination within its RING-finger domain. In wild-type cells, Hrd1 associates on its luminal side with Hrd3 and on its cytosolic side with Usa1, which in turn recruits the multi-spanning membrane protein Der1, a protein related to rhomboid proteases. Yos9 is a luminal protein that binds to Hrd3. We have determined a structure of the entire active Hrd1 complex by cryo-EM analysis of two sub-complexes. Hrd3 and Yos9 jointly create a luminal binding site that recognizes glycosylated substrates. The rhomboid-like Der1 protein and Hrd1 form two “half-channels” with luminal and cytosolic cavities, respectively, which have lateral gates facing one another in a thinned membrane region. Together with crosslinking and molecular dynamics simulation results, the structures suggest how a polypeptide loop of an ERAD-L substrate is moved through the ER membrane. In this model, Der1 and Hrd1 each stabilize one strand of the substrate loop, while the tip of the loop moves through the thinned membrane region. The loop can then slide back and forth until a suitable lysine emerges on the cytosolic side and becomes poly-ubiquitinated. In the next step, the Cdc48 ATPase complex moves the poly-ubiquitinated substrate into the cytosol. Cryo-EM structures show that the Cdc48 complex initiates substrate processing by unfolding a ubiquitin molecule. Inducing the unfolding of ubiquitin allows the Cdc48 ATPase complex to process a broad range of substrates.

SG31

**Structural and molecular determinants of dynamics in P granules of C. elegans.**

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The germline of sexually reproducing animals contains conserved non-membrane-bound compartments containing proteins and RNAs called ‘germ granules’. Mutations that disrupt assembly of germ granules result in sterility. A germ granule in C. elegans, called ‘P granules’, has been shown to assemble via liquid-liquid phase separation (LLPS) of proteins and RNAs from the surrounding cytoplasm in adult gonads. Approximately 85% of proteins that concentrate in P granules are involved in different aspects of RNA metabolism, suggesting that the P granule phase could process and/or store RNA. To elucidate the underlying molecular mechanisms, it is important to understand the biophysical nature of the P granule phase. Specifically, the molecular and structural determinants that regulate diffusion rates within the P granule phase remains unclear. We used an in vitro reconstitution-based approach to address this question and studied the LLPS behavior of the protein PGL-3, one of the most abundant proteins in P granules. We found that a folded domain of PGL-3 drives LLPS, and dynamics within phase-separated condensates correlates with alpha-helicity of the folded domain. Next, we investigated the effect on dynamics in condensates when other components of P granules are added to condensates of
PGL-3. Our findings suggest that the complex composition of P granules favor fast dynamics within the phase.

SG32
Surviving stem cell injury
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Adult stem cells are the workhorses of regenerative organs. Their capacity to replace multiple specialized cell types is critical for rapidly restoring function to old or damaged tissue, and makes them a powerful tool for regenerative medicine. The stem cells that power the remarkable regeneration capacity of the mammalian small intestine are housed at the base of invaginations of the epithelium, called crypts. We are using live imaging of organoids to dissect the relationship between the crypt structure and the stem cells it contains. By visualizing and perturbing the process of crypt regeneration after stem cell damage, we identify a new cell type that contributes to the crypt rebuilding process, and are currently dissecting the underlying signaling requirements. Together, this work reveals a mechanism to sustain the intestinal epithelium in chronic injury conditions.

Remodeling and Reshaping Membranes

SG33
Mechanism of ER-associated organelle division
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The past several years have revealed that ER membrane contact sites (MCS) play a major role in regulating the biogenesis and dynamics of other cytoplasmic organelles. We have made the surprising discovery that MCS formed between dynamic ER tubules and multiple organelles including mitochondria, early and late endosomes, and even RNP granules define the position where these organelles undergo division. Our current goals are to identify the machinery and mechanism behind this ER-associated organelle division process. How can an ER tubule at a MCS trigger the division of another organelle and how is this process regulated in time? How can ER MCS regulate the division of so many different types of organelles? What is common and what is unique at the ER MCS that regulate organelle division. Here we will discuss our new data aimed at unraveling the molecular contributions of factors that localize to ER MCS and provide insight into their mechanistic roles.

SG34
LEM2 phase separation promotes ESCRT-mediated nuclear envelope reformation.
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During cell division, remodelling of the nuclear envelope enables chromosome segregation by the mitotic spindle. The reformation of sealed nuclei requires ESCRTs (endosomal sorting complexes required for transport) and LEM2, a transmembrane ESCRT adaptor. Here we show how the ability of LEM2 to condense on microtubules governs the activation of ESCRTs and coordinated spindle disassembly. The LEM motif of LEM2 binds BAF, conferring on LEM2 an affinity for chromatin, while an adjacent low-complexity domain (LCD) promotes LEM2 phase separation. A proline-arginine-rich sequence within the LCD binds to microtubules and targets condensation of LEM2 to spindle
microtubules that traverse the nascent nuclear envelope. Furthermore, the winged-helix domain of LEM2 activates the ESCRT-II/ESCRT-III hybrid protein CHMP7 to form co-oligomeric rings. Disruption of these events in human cells prevented the recruitment of downstream ESCRTs, compromised spindle disassembly, and led to defects in nuclear integrity and DNA damage. We propose that during nuclear reassembly LEM2 condenses into a liquid-like phase and coassembles with CHMP7 to form a macromolecular O-ring seal at the confluence between membranes, chromatin and the spindle. The properties of LEM2 described here, and the homologous architectures of related inner nuclear membrane proteins, suggest that phase separation may contribute to other critical envelope functions, including interphase repair and chromatin organization.

SG35
Regulation of organelle remodelling by the ESCRT machinery
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The Endosomal Sorting Complex Required for Transport-III (ESCRT-III) machinery has emerged as a key player in the remodelling of cellular membranes and organelles. While more commonly associated with the biogenesis of multivesicular bodies during interphase, during mitosis this machinery is redeployed to allow regeneration of a sealed nuclear envelope during mitotic exit and completion of the abscission phase of cytokinesis. ESCRT-III assembly at the nuclear envelope occurs transiently during mitotic exit and CHMP7, an ER-localised ESCRT-II/ESCRT-III hybrid protein, initiates assembly in a manner dependent upon the Inner Nuclear Membrane (INM) protein LEM2. Whilst classical nucleocytoplasmic transport mechanisms have been proposed to separate LEM2 and CHMP7 during interphase, it is unclear how CHMP7 assembly is suppressed in mitosis when NE and ER identities are mixed. We will discuss how CHMP7 functions to disaggregate LEM2 clusters at the reforming nuclear envelope and will examine how cell cycle control mechanisms act to during mitotic exit to allow spatiotemporal control over CHMP7s assembly, aiding subsequent nuclear regeneration.

SG36
Structure of the yeast seipin Fld1 reveals determinants for LD biogenesis
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Structure of the yeast seipin Fld1 reveals determinants for LD biogenesisYoel Klug*, Justin C. Deme*, Susan M. Lea, Pedro CarvalhoSir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK*equal contribution Lipid droplets (LDs) are ubiquitous storage organelles for neutral lipids, mainly triacylglycerols and steryl esters. LDs have a unique structure consisting of a phospholipid monolayer enclosing a neutral lipid core. While the mechanisms of LD biogenesis remain elusive, there is evidence that LDs form at the endoplasmic reticulum (ER). In the most popular model, LDs assemble at discrete ER domains by the budding of neutral lipid lenses formed in between of the two leaflets of the ER bilayer. Seipin, a protein encoded by the BSCL2 gene, has emerged as a key LD biogenesis factor. Importantly, BSCL2 mutations associated with Berardinelli–Seip congenital syndrome are the most frequent cause of familial lipodystrophies. Seipin is an ER integral membrane protein predicted to have two transmembrane segments near the N and C termini separated by an extended luminal domain where the disease-causing mutations frequently fall. In a variety of cellular and animal
models, seipin mutations result in aberrant numbers and morphology of LDs. Consistent with a role in LD formation, seipin localizes as discrete foci that coincide with the ER sites of LDs assembly. However, despite intense research and the links to disease, the mechanisms by which seipin contributes to LD formation are unclear. In yeast, the seipin homolog Fld1 functions together with Ldb16, another ER resident membrane protein, and deletion of either Fld1 or Ldb16 results in seipin loss of function. To gain insight into seipin functions we determined the structure of the yeast protein using cryogenic-electron microscopy (cryo-EM). Yeast seipin forms a homodecamer with the luminal domains assembling a ring like structure. The luminal domain adopts a b-sandwich fold, typical of certain lipid-binding proteins. A similar architecture was recently reported for human and fly seipin, however the role of seipin luminal domain and whether it has lipid-binding activity have remained elusive. Combining mutagenesis, in vivo assays and biophysical approaches we have probed our structural data. Our findings lead to new mechanistic insights into the role of seipin during LD formation. Since the structural elements dissected for yeast seipin are conserved, we expect our findings to provide insight into the function of human seipin.

SG37
Mechanisms directing the morphology of the early secretory pathway
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Components of the cytoplasm and the nucleoplasm can undergo a “liquid-liquid” phase separation, forming dynamic and functionally specialized domains that lack any membrane. We report here that membrane-containing organelles, such as the Golgi, are structured by a two-dimensional variation of this same principle, in which rod-like proteins (‘golgins’ and golgin-like proteins) favor lamellar liquid geometries rather than the spherical geometries observed for phase-separating intrinsically disordered proteins. The Golgi is surrounded by a 50 nm, ribosome-excluding ‘cocoon’, termed the Golgi matrix, which consists of a network of 50-400 nm long, coiled-coil domain-containing tethers (golgins) and Rab GTPases. Overexpression of GM130 in mammalian cells results in the formation of dynamic, droplet-like structures that absorb endogenous GM130. Concordantly, pure GM130 phase-separates in vitro, exhibiting protein concentrations within dynamic condensates that agree with the local concentration of endogenous GM130 at the Golgi, whereas other rod-like coiled-coil proteins do not phase separate under these conditions. Electron tomography of recombinant GM130 in solution revealed the presence of sub-micron sheets of monodisperse <100 nm thick condensates that exhibited smooth borders, resembling the interfaces formed by fluids due to surface tension. Furthermore, TIRF microscopy revealed that these sheets grow to form flat, dynamic, and micron-sized condensates that are on the scale of Golgi ministacks. Thus far, we have obtained evidence that 7 of the 11 principal mammalian golgins (GM130, GMAP210, golgin-160, golgin-245, golgin-97, GCC88, GCC185) form dynamic condensates upon overexpression in vivo and/or as recombinant proteins in vitro. This suggests that, beyond their classical role as vesicle tethers, a liquid-like condensation of golgins might represent a central feature responsible for organizing the underlying membrane, and could help explain why the morphology of the Golgi stack is conserved across all higher eukaryotes. Interestingly, proteins with golgin-like architecture are not restricted to the Golgi stack, e.g. the ER residents TANGO1 and cTAGE-5. Morphologically, the ER-Golgi interface appears as a synapse-like, ribosome-excluding zone that is conserved across taxa. Preliminary data supports liquid-like condensation of the cytoplasmic domain of TANGO1 in vitro, and phases of TANGO1 and GM130 spontaneously unmix on a membrane surface. We
therefore suggest that interactions among liquid-like TANGO1/cTAGE5 and cis-face golgins contribute to generating a diffusion-limited space that enables efficient exchange of material between the two organelles.

SG38

Membrane Fission: Insights from Reconstituting Organelle Form and Chemistry
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The lipid bilayer is highly resilient to rupture and explains why it was selected over the course of evolution to serve a barrier function. Yet fission, or the splitting of a membrane compartment, is a central theme in biology that manifests during cell division, organelle biogenesis and vesicular transport. Fission involves the local application of forces to bend and constrict or thin down a membrane tube. Since bending requires the bilayer to deviate from its preferred planar configuration, fission is energetically unfavorable. Using reconstitution approaches that involve biochemical screens, we have discovered novel proteins that catalyze fission and have elucidated their mechanism and cellular functions. My talk will describe these recent developments.

SG39

Structure of the complete, membrane-assembled COPII coat reveals a complex interaction network.
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The COPII coat mediates Endoplasmic Reticulum (ER) to Golgi trafficking of thousands of proteins. Five essential coat components assemble on the ER membrane to induce the formation of transport vesicles while selectively recruiting cargo proteins. Individual COPII proteins interact dynamically through multiple interfaces to assemble into inner and outer coat layers, forming coats of varied architectures. We have used cryo-electron tomography (cryo-ET) and subtomogram averaging to visualise the complete membrane-bound COPII coat to unprecedented detail, revealing a complex interaction network that includes a number of previously unknown interfaces. We identify essential interactions, and functionally characterise their contribution to COPII assembly and membrane remodelling. We find that multiple finely-tuned interfaces contribute to coat organization required to remodel membranes, depending additionally on membrane properties. Our work sheds light on how COPII transports the diverse range of cargo molecules required by the cell.

SG40

Membrane shaping proteins control lamellipodia dynamics and cell migration
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Cell motility has a critical role in a range of biological processes including development, immunity and disease. Navigation through complex and ever-changing environments often relies on the activity of actin-rich protrusions at the leading edge, also referred to as lamellipodia. Its dynamics determines motion persistence, which is a key parameter for directed cell migration. Lamellipodia are known to exhibit areas of continuously rearranging membrane curvature. However, whether and how curvature-sensitive membrane shaping proteins contribute to leading edge dynamics and function, remains poorly understood. Neutrophils display one of the most dynamic and prominent lamellipodia among all
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vertebrate cells, and are emerging as a key model system to investigate the role of membrane curvature in the context of cell migration. We followed the transition of neutrophil-like cells from a blast into a differentiated, lamellipodia-forming state using RNAseq, and identified the most highly enriched curvature-sensitive membrane remodeling proteins in this process. After probing their localization to assess enrichment at the leading edge, we generated CRISPR/Cas9 knockout cell lines of promising candidates. We quantitatively assessed changes in cell shape, membrane tension and actin polymerization, focusing on their contribution to motility in various types of environments. Using a combination of machine learning-based segmentation for time-resolved TIRF microscopy and microfluidics, we reveal that motion persistence and directionality, in both freely moving and environmentally constrained cells, strongly depend on the activity of particular BAR domain proteins. Specifically, we show that such proteins control lamellipodia dynamics by regulating WAVE2-driven actin polymerization. KO cells migrate faster and are more persistent during unobstructed migration, but fall short when a change in direction is required. In summary, we show that curvature-sensitive and membrane remodeling BAR domain proteins are key to direct cell motility in complex environments by controlling lamellipodia dynamics.

SG41
Membrane flows pattern the distribution of membrane-associated proteins around sites of secretion
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Cell patterning and polarization is a fundamental biological problem. Rod-shaped fission yeast cells form polar active zones of polarized secretion, and lateral regions with distinct lipid and protein composition. We show that exocytosis within a broader zone of endocytosis at cell poles causes membrane flows that pattern cell sides. Using a CRY2PHR-CIBN-based optogenetic tool that forms large, initially homogeneously distributed complexes at the plasma membrane upon blue light stimulation, we observed that these complexes are rapidly cleared from the growing poles. In back-and-forth mathematical simulations and experimental validation with synthetic probes and endogenous proteins, we show that a concentrated zone of exocytosis within a broader zone of endocytosis causes membrane flows leading to depletion of membrane-associated proteins from the secretion point. Depletion strongly depends on slow rates of protein diffusion and membrane detachment, which are reduced upon complex formation. The phenomenon is likely general as several proteins that normally form membrane-associated condensates at cell sides re-localize to cell poles upon condensate dissolution. We conclude that a zone of polarized secretion also patterns adjacent lateral regions through membrane flows, and speculate that protein patterning may be acutely modulated in cells by regulation of membrane affinity.

SG42
Adaptor protein complexes: ancient sorting machines
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The last eukaryotic common ancestor must have had five distinct adaptor protein (AP) complexes, as well as two related complexes, COPI and TSET, all of which are present in every branch of modern eukaryotes. Although we still don’t understand what all of them do, studies in one organism can help to uncover their functions in all organisms. Two of the most mysterious AP complexes are AP-1, one of the
first to be discovered, and AP-5, the one that was most recently discovered. We have been using a combination of live cell imaging and subcellular fractionation to investigate AP-1. Our findings so far indicate that it is primarily involved in post-endocytic trafficking rather than biosynthetic trafficking. For AP-5, we have been concentrating on how it is recruited onto membranes. We find that recruitment is by coincidence detection, requiring both the phosphatidylinositol PI3P and Rag GTPases. The Rag involvement uncovers unexpected links between AP-5 and nutrient sensing.

SG43

Multiscale modeling of membrane bending
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Membrane bending is ubiquitous in many cellular phenomena. Over the past couple of decades, biochemical reconstitution experiments have revealed that a variety of membrane-protein interactions can lead to curvature generation. To gain physical insights into how the mechanics and energetics of different membrane bending mechanisms can impact biological processes, mathematical and computational models of membrane bending have been developed. In the past few years, we have developed many models of membrane bending as influenced by membrane-protein interactions. Ongoing developments include linking coarse-grained molecular dynamic simulations with continuum descriptions of the lipid bilayer taking into account the interplay between protein diffusion and membrane bending. In this work, I will discuss these models, insights gained from them, and possible solutions for integrating different modeling approaches. The predictive utility of these models will be discussed in the context of experimental observations and implications for understanding the physics of remodeling and reshaping membranes.

SG44

Ancient trafficking machinery lost in animals and fungi: Rise of the Jotnarlogs
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The membrane-trafficking system is a defining facet of eukaryotic cells. The best-known organelles and major protein families of this system are largely conserved across the vast diversity of eukaryotes, implying both ancient organization and functional unity. Nonetheless, intriguing variation exists that speaks to the evolutionary forces that have shaped the endomembrane system in eukaryotes and highlights ways in which membrane trafficking in protists differs from that in our best-understood models systems. Novel members of protein families are present across eukaryotes but have been lost in humans and yeast. Sufficient examples of such machinery (coined Jotnarlogs) now exist to suggest that this is not just a curiosity, but a phenomenon that needs exploration with careful evolutionary analysis, new model systems and a broader perspective of eukaryotic cell biological diversity. These proteins may well hold clues to understanding differences in cellular function in organisms that are of pressing importance for planetary health.

Single-Cell Signaling and Regulatory Networks in Cell Biology

SG45

Temporal organization of the cell cycle
B. Novak; University of Oxford, Oxford, UNITED KINGDOM.
In order to maintain genome integrity and an effective nucleocytoplasmic ratio from one generation to the next, cells carefully monitor progression through their replication-division cycle and fix any errors before they jeopardize the progeny of the cellular reproduction process. These error-surveillance and correction mechanisms operate at distinct ‘checkpoints’ in the cell division cycle, where a growing cell must ‘decide’ whether it must wait for errors to be corrected or it may proceed to the next phase of the cell cycle. Once a decision is made to proceed, the cell unequivocally enters into a qualitatively different biochemical state, which makes cell cycle transitions switch-like and irreversible. These characteristics of cell cycle transitions are best explained by bistable switches with different activation and inactivation thresholds, resulting in a hysteresis effect. Almost 30 years ago, John Tyson and I proposed that the activity of the mitosis-inducing protein kinase, Cdk1:CycB, is controlled by an underlying bistable switch generated by positive feedbacks involving inhibitory phosphorylations of the kinase subunit. Numerous predictions of this model were experimentally verified by different groups, but more importantly bistability has become a paradigm for all cell cycle transitions. Consideration of the mechanistic details of various transitions suggests a general design principle in the form of a generic network motif underlying each transition. At the heart of the generic motif is a double-negative loop between a cell cycle activator and its inhibitor that creates two alternative stable steady states (pre- and post-transition). At each transition, the activator of the previous transition helps the activator of the current transition to eliminate its inhibitor, which drives the cell into the next stage of the cycle. After the transition, the current activator down-regulates the previous one (negative feedback loop), but the bistable mechanism allows the current activator to maintain its activity. I will argue that the mechanism governing progression through the cell cycle is a sequence of bistable switches, embodied by distinct network motifs of coupled negative and double-negative feedback loops, arranged in a circular fashion.

**SG46**

**Combining optogenetics with network perturbations highlights different regulatory mechanisms of the ERK signaling pathway**

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Mitogen-Activated Protein Kinase (MAPK) signaling networks enable cells to convert extra-cellular inputs such as a growth factor binding to receptor tyrosine kinases (RTKs) into specific cellular outputs. This occurs through production of dynamic ERK activity states that are encoded in the network structure. Despite numerous studies, we still miss crucial information about how different molecular components are wired with a specific network topology that can interpret different input strengths and durations to produce different dynamic ERK states. To explore the MAPK signaling network topology in a scalable fashion, we have built synthetic circuits that consist of an optogenetic RTK (optoFGFR1) or GEF (optoSOS) allowing us to activate ERK at different levels in the MAPK cascade with light pulses of different intensities, durations and frequencies. Single-cell ERK dynamics is then recorded using a spectrally compatible biosensor (ERK-KTR). Combined with automated live imaging and image analysis, our system provides the opportunity to investigate ERK dynamics in response to perturbations with an unsurpassed throughput. We use this system to explore how multiple inputs (soluble GFs + light inputs) compete to control ERK dynamics and to perform a siRNA screen targeting 50 components of the MAPK network. We observe that the latter is extraordinarily resilient against these perturbations. Performing
this screen under different stimulation conditions and in response to activation at the level of the receptor (optoFGFR1) or at the level of Ras (optoSOS) suggests sources of robustness that originate from different feedbacks in the cascade. We also identify the adaptor Grb2 as a weak node in the MAPK cascade, of which perturbation can more efficiently shut down ERK activity than other nodes. Due to its scalability, our system allows for comprehensive analysis of the MAPK network.

SG47

**Studying signaling dynamics without live-cell microscopy**

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Complex signaling dynamics are ubiquitous in cell signaling, including pulses in ion concentrations (e.g. intracellular Ca²⁺), kinase activity (e.g. Erk), transcription factor levels (e.g. NF-kB; p53), and more. One fundamental limitation in the study of endogenous dynamics has been the requirement to watch individual cells over time on a microscope. This has made it impossible to monitor dynamics in any tissue that cannot be imaged at single-cell resolution and high temporal resolution, limiting our ability to trace the eventual fate of pulsing cells or to perform large-scale screens for components of the dynamics-generating circuitry. Here we describe a solution to this challenge for the Erk pathway: an engineered gene circuit that can be used to discriminate Erk-pulsing cells from those that activate Erk to constant high or low levels. We characterize our pulse detector, termed "reader of Erk activity dynamics" (READer), using in silico modeling and in cultured mammalian cells harboring spontaneous, endogenous Erk pulses. The simplicity and generality of our circuit suggests that it could be useful for defining the causes and consequences of signaling dynamics across a broad range of contexts.

SG48

**A switch in p53 dynamics marks cells that escape from DSB-induced cell cycle arrest**

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Cell signaling networks ensure proper responses to internal or external stimuli. The dynamics of signaling pathways, or how attributes of signaling molecules change over time, have been shown to play an important role in ensuring the fidelity of the response. In most systems, however, whether and how the dynamics of signaling pathways might evolve over time has been poorly studied. DNA damage induces a complex response that is largely orchestrated by the transcription factor p53, whose dynamics influence whether a damaged cell will arrest and repair the damage, or initiate cell death. We investigated whether p53 dynamics can change over time in single cells, and if so, what mechanism controls the shift between dynamics. Following DNA damage by irradiation, p53 levels oscillate, resulting in induction of DNA damage-responsive checkpoints and cell-cycle arrest. Using time-lapse single-cell microscopy complemented by biochemical approaches we have found that a subset of cells switches from oscillating to sustained p53 dynamics several days after undergoing damage. The switch was triggered by an escape from cell cycle arrest in the presence of damaged DNA, which activates the Caspase-2 - PIDDosome, a complex that stabilizes p53 by inactivating its negative regulator MDM2. This
work defines a molecular pathway that is activated if the canonical checkpoints fail to halt mitosis in the presence of damaged DNA.

SG49

**Our first choices - decoding signals during developmental transitions**

*S. Santos*; The Francis Crick Institute, London, UNITED KINGDOM.

During early development, extrinsic triggers prompt a collection of pluripotent cells in the blastocyst to begin the dramatic and long process of differentiation that gives rise to the tissues of the three germ layers (endoderm, mesoderm and ectoderm). Precise temporal control during these early fate-choices is paramount and impacts on the success of differentiation. Changes in morphology, gene expression signatures and epigenetic patterns and cell division cycles are believed to mark the point of no-return in fate choices. However, when and how cells irreversibly commit to differentiation is a fundamental, yet unanswered question. Poised to differentiate, embryonic stem (ES) cells are an invaluable model to address this question. Given appropriate differentiation cues, ES cells can recapitulate in vitro all the hallmark events that occur during differentiation and are our system of choice to understand fate decisions at the single cell level. During Silvia’s talk she will share two stories that illustrate how the Santos lab combines single cell imaging, genomic approaches and mathematical modeling to study how cells encode fate choices during early development.

SG50

**Integrating decades of data into a predictive computational model of RTK-dependent gene regulation**

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Terminal patterning of the early Drosophila embryo is a well-established experimental model for gene expression induced by spatially localized extracellular signals. A spatiotemporal pulse of ligand acts through a uniformly expressed receptor tyrosine kinase (RTK) to establish transient expression of a handful of target genes. Specifically in this system, the RTK signaling leads to phosphorylation of Capicua (Cic), a transcriptional repressor, that binds to specific sites in DNA near target genes. Inductive RTK signalling appears in many developmental contexts across model organisms, but the Drosophila embryo is the only system with tractable genetics amenable to quantitative live imaging, biochemistry, spatiotemporal signal perturbations and more. Over the past decade, we have generated many types of datasets such as cell cycle dynamics that populate the embryo with a field of cells, shuttling of a gene repressor into and out of the nucleus, binding of the repressor to DNA, and target gene activation patterns. By now, do we have a complete enough model of this system to predict transcriptional responses? We demonstrate that we have reached this point by designing a multi-scale computational model that integrates dynamics of RTK activation, Capicua phosphorylation, nucleocytoplasmic transport, and DNA binding of Capicua, and a stochastic model of transcription of genes. We run the model on the surface of a prolate spheroid with nuclei going through mitotic divisions and arranged with statistical properties similar to the real embryo. Our model contains a dozen of uncertain parameters, yet remarkably we find that all of the parameters are well constrained by fitting the model output to experimental results using the Markov Chain Monte Carlo (MCMC) method. Furthermore, we use our model to predict the transcriptional response to an optogenetic perturbation that has not been made before, and to explore aspects of gene regulation that are challenging to manipulate experimentally. We
have trained our model on one RTK-responsive gene, and are now using it to understand how
dogenous stimuli control nested expression of a more complex network of gene targets important for
patterning and morphogenesis in the embryo.

SG51

Models for intracellular signaling in cell motility
L. Edelstein-Keshet; University of British Columbia, Dept of Mathematics, Vancouver, BC, CANADA.

Actin-based eukaryotic cell motility is regulated by intracellular signalling with small GTPases (Sac, Rho, Cdc42) at its hub. In this talk, I will survey work done in my group to understand how GTPase properties contribute to spontaneous cell polarity, and to a number of observed migratory phenotypes. I will focus on how modelling of GTPase interactions has helped to understand experimental observations in single-cell settings. A combination of analysis and computation reveals how a single GTPase ‘module’, fine tuned by many inputs and feedbacks, explains diverse cell behaviour, including persistent migration, as well as front-back cyclic switching.

SG52

Nutrient signaling, stress response, and interorganelle communication are noncanonical determinants of cell fate
E. Wood; UT Southwestern Medical Center, Dallas, TX.

Isogenic cells can manifest distinct cellular fates for a single stress, however the nongenetic mechanisms driving such fates remain poorly understood. Here we utilize the response of individual budding yeast cells to acute glucose removal (AGR) to unveil the nongenetic determinants in cellular heterogeneity. We use a time-lapse imaging platform coupled to a microfluidics device and a computational analysis pipeline that allows us to continuously track single cells before, during, and after AGR for 16 hrs. We show that individual cells respond differentially to AGR: upon glucose replenishment, some cells proliferate and resume budding (quiescent cells) while others do not resume budding despite being metabolically active (senescent cells). We simultaneously tracked nutrient signaling, cell cycle, stress, metabolic, and interorganelle contact site markers, dissecting key cellular determinants for response to AGR. We show that all cells halt or delay their cell cycle upon AGR regardless of the cell cycle stage they are in. Senescent cells activate stress responses upon AGR, but achieve a distinct cellular response compared to quiescent cells. In particular, senescent cells display altered interorganelle crosstalk as evidenced by their inability to expand their nucleus-vacuole junctions (NVJ) and exhibited defects in endomembrane trafficking. However, senescent cells remain responsive to environmental cues throughout and after AGR stress, yet fail to proliferate upon glucose replenishment. In line with this, both senescent and quiescent cells respond to nutrient deprivation by up-regulating lipid storage pathways in the form of lipid droplets (LDs), although we surprisingly find that LDs are not operationally required for post-AGR cell fates. Remarkably, we demonstrate that the abundance of the nutrient signaling related kinase Rim15 before AGR predicts cell fate after AGR. Finally, we accurately predicted the fates of individual cells during AGR hours before their cell fates manifest, by applying the Bayesian method of statistical evidence to our single-cell measurements. We also show that cells ‘decide’ on their fates within 3-4 hours of the initiation of the AGR. Collectively, our work pioneers a shift in focus from cell fate-specific signaling to other noncanonical factors related to nutrient signaling, interorganelle
communication, and stress response and shows that cellular responses are predisposed to particular fates well before the fates manifest.

SG53

**Why cells arrest when stress (and why you should give a "flux" about it)**

H. El-Samad, A. Bonny; University of California, San Francisco, San Francisco, CA.

The ability of a cell to mount a robust response to an environmental perturbation is paramount to its survival. While cells deploy a spectrum of specialized counter-measures to deal with stress, a near constant feature of these responses is a down-regulation or arrest of the cell cycle. It has been widely assumed that this modulation of the cell cycle is instrumental in facilitating a timely response towards cellular adaptation. Here, we directly investigate the role of cell cycle arrest in the hyperosmotic shock response of the model organism *S. cerevisiae* by deleting the osmoshock-stabilized cell cycle inhibitor Sic1, thus enabling concurrent stress response activation and cell cycle progression. We find that removal of stress-induced cell cycle arrest accelerated the adaptive response to osmotic shock instead of delaying it. A combination of time time-lapse microscopy, genetic perturbations and quantitative mass spectrometry experiments suggest that unabated cell cycle progression during stress enables the liquidation of internal glycogen stores, which are then shunted into the osmotic shock response to fuel a faster adaptation. Therefore, osmo-adaptation in wild type cells is delayed because cell cycle arrest diminishes the ability of the cell to tap its glycogen stores. However, acceleration of osmo-adaptation in mutant cells that do not arrest comes at the cost of acute sensitivity to a subsequent osmo-stress. This indicates that despite the ostensible advantage faster adaptation poses, there is a trade-off between the short-term benefit of faster adaptation and the vulnerability it poses to subsequent insults. We discuss how cell cycle arrest might act as a carbon flux valve to regulate the amount of material that is devoted to osmotic shock, balancing short term adaptation with long-term robustness.

SG54

**Signaling adaptation mediates rapid escape from BRAF inhibition in single melanoma cells**

C. Tian, C. Yang, T. Hoffman, N. Jacobsen, S. Spencer; University of Colorado Boulder, Boulder, CO.

A non-genetic, reversible, drug-tolerant state has been reported both in the clinic and preclinical models, yet little is known about the inception of drug tolerance, in particular the timing and plasticity involved in cancer cell adaptation. Here we use time-lapse imaging and EllipTrack, a new automated cell tracking pipeline optimized for long-term tracking of hard-to-track cancer cells with minimal errors, to follow thousands of individual melanoma cells over the first four days of treatment with BRAF inhibitor. We find that while the majority of cells enter a CDK2-low quiescence, a subpopulation of cells can escape from drug action within three days to re-enter cell cycle. This escapee subpopulation reverts to a drug-sensitive state upon drug withdrawal, and cell lineages derived via EllipTrack-based cell tracking contain both escapees and non-escapees. These data suggest that rapid drug-induced signaling rewiring events, rather than genetic mutations, promote the ability of cells to divide in the presence of drug. To
identify regulators involved in escape from BRAF inhibition, we perform single-cell RNA sequencing and computationally reconstruct the transcriptomic landscape in escapees by aligning the inferred trajectory of single-cell transcriptomes to the cell lineages measured by time-lapse imaging. We find that escapees display an AXL high/MITF low invasive gene signature and up-regulate multiple stress response pathways that enable their ability to cycle in drug. We further demonstrate that escapees are prone to mutagenesis yet outgrow non-escapees, suggesting that they could be the seed population driving the acquisition of permanent drug resistance. Together, this work provides real-time visualization of the extent of heterogeneity and plasticity in cancer-cell drug responses, and uncovers rewiring events that enable proliferation in drug, a key step on the road to drug resistance.

**SG55**

**Oscillatory timeseries data sheds light on mechanisms of circadian timekeeping**

**J. Kim;** Korea Advanced Institute of Science and Technology, Daejeon, KOREA, REPUBLIC OF.

The circadian (~24h) clock is a self-sustained oscillator, which times diverse behavioral, physiological, and developmental process including our sleep cycle. The key oscillatory mechanism of the clock is a transcriptional-translational negative feedback loop. The suppression of the feedback loop occurs at the same time every day to maintain circadian period although it involves daily nuclear entry of thousands of clock molecules after diffusion through a crowded cytoplasm. Furthermore, the circadian period is robust to environmental temperature change altering reaction rates, which has been a mystery for last 60 years. In this talk, I will describe how we identified underlying molecular mechanisms for such robust rhythms by analyzing the spatio-temporal timeseries data of clock molecules.

**The Nuclear Envelope and Nucleoporins: Influencers of Cellular Structure and the Genome**

**SG56**

**Models and mechanisms of mechanotransduction across the nuclear envelope**

**M. C. King, I. V. Surovtsev, J. F. Williams, H. Nguyen, E. Carley, I. Jalilian;** Department of Cell Biology, Yale School of Medicine, New Haven, CT.

Cells and tissue integrate biochemical and mechanical signals to regulate gene expression in response to their environment. While it is well established that numerous mechanosensitive transcription factors translocate into the nucleus in response to mechanical cues, the contexts and mechanisms by which direct force transmission from the cytoskeleton to the nucleus impacts gene expression is poorly understood. Moreover, while the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex provides a mechanism for force transmission, how tension on the LINC complex remodels the nuclear lamina and/or chromatin to influence its state is unknown. We have leveraged multiple model systems to provide new insights towards our understanding of direct mechanotransduction. Using mouse and tissue culture models we have identified a feedback loop between integrin engagement, tension on the LINC complex, and gene expression that regulates the production of extracellular matrix components. Our work suggests that this pathway involves alterations in the composition of the nuclear lamina in response to tension. Working in fission yeast, we demonstrate that force transduction to chromatin at the nuclear periphery drives the deformation of phase-separated heterochromatic domains, suggesting that force itself may alter the responsiveness of the chromatin to transcriptional regulators. Taken
together, our ongoing work reveals new concepts for how forces are translated to define transcriptional outcomes within the nucleus.

SG57

**Alterations to nuclear pore complexes occur with force driven localization to Actin-LINC-Lamin nuclear lines**

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Multicellular organisms require a high degree of coordination between cells and tissues in order to execute complex developmental processes and organ functions. Much of this coordination is driven by mechanical signals transduced to and mediated by chemical signals. A current challenge in cell biology is understanding how cells sense and respond to mechanical cues to achieve diverse physiologic responses. Actin-LINC-Lamin (ALL) nuclear lines (also known as TAN lines) form on the dorsal surface of the nucleus in response to mechanical force and provide a direct mechanical linkage between the actin cytoskeleton and the laminar nucleoskeleton. Actin stress fibers are supramolecular assemblies that respond to and transmit forces between the extracellular space and other elements of the cytoskeleton. They are dynamic bundles of actin polymers able to contract and relax, stretch, break and repair in response to mechanical force input. Our lab has made the novel observation that Nuclear Pore Complexes (NPCs), macromolecular complexes essential for selective regulation of transport in and out of the nucleus, accumulate at ALL nuclear lines in response to mechanical force. To better understand how mechanical signals are transduced, we studied the localization and composition of NPCs in relation to acto-myosin stress fibers following mechanical stimulation. We elicited stress fiber activation by stretching single living cells on a confocal microscope imaging stage. Tracking protein dynamics in real time, we observed the coalescence of Nesprin2G and Sun2 in forming (ALL) nuclear lines, followed by subsequent colocalization of NPCs with labeled nucleoporins. Specifically, intensity measurements (obtained by tracking a variety of nucleoporins, including POM121, Nup153, and TPR) showed increased presence of nucleoporins proximal to the ALL nuclear lines. Additionally, comparison with NPCs prior to their ALL assimilation showed that their fluorescent signal intensified concomitantly with assimilation, raising the possibility that NPCs undergo compositional or morphological changes when associated with ALL nuclear lines. This work shows dynamic changes in the characteristics of NPCs with force dependent assimilation at ALL nuclear lines.

SG58

**Actin assembly ruptures the nuclear envelope by prying the lamina away from nuclear pores and nuclear membranes in starfish oocytes**

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In metazoas, the nuclear envelope (NE), composed of nuclear membranes, pores (NPCs) and the underlying network of lamina filaments, disassembles and reassembles in every cell division. A phase of sudden rupture is a conserved feature of NE breakdown (NEBD), a key cell-cycle event essential for
timely spindle assembly and faithful chromosome segregation. The nucleus of oocytes is unusually large and densely decorated with NPCs that are stockpiled for embryonic development. We found that the rupture of this specialized NE in the starfish oocyte is mediated by an Arp2/3-nucleated F-actin ‘shell’, in contrast to microtubule-driven tearing in mammalian fibroblasts. Through correlated live-cell, super-resolution and electron microscopy, we show that actin is nucleated within the lamina, sprouting filopodia-like spikes towards the nuclear membranes. These F-actin spikes protrude pore-free nuclear membranes, whereas the adjoining stretches of membrane accumulate NPCs that are associated with the still-intact lamina. Packed NPCs sort into a distinct membrane network, while breaks appear in ER-like, pore-free regions. We reveal a new function for actin-mediated membrane shaping in nuclear rupture that is likely to have implications in other contexts, such as nuclear rupture observed in cancer cells.

SG59
Nup98-dependent transcriptional memory is established independently of transcription.
P. Pascual-Garcia, S. Little, M. Capelson; University of Pennsylvania, Philadelphia, PA.

Cellular ability to mount an enhanced transcriptional response upon repeated exposure to external cues has been termed transcriptional memory, which can be maintained epigenetically through cell divisions. This phenomenon of increased gene reactivation upon repeated stimulus is conserved, and in multiple species, has been found to depend on a nuclear pore component Nup98. The majority of mechanistic knowledge on transcriptional memory has been derived from bulk molecular assays. To gain an alternative perspective on the mechanism and on the contribution of Nup98, we set out to examine single-cell population dynamics of transcriptional memory by monitoring transcriptional behavior of individual Drosophila cells upon initial and subsequent exposures to steroid hormone ecdysone. To this end, we combined single-molecule RNA FISH with mathematical modeling, and found that upon hormone exposure, cells rapidly activate a low-level transcriptional response, but simultaneously, begin a slow transitioning into a specialized memory state, characterized by a high rate of expression. Strikingly, our modeling predicted that this transition between non-memory and memory states is independent from transcription during initial activation, and we were able to confirm this prediction experimentally by showing that inhibiting transcription during initial ecdysone exposure did not interfere with memory establishment. Together, our findings reveal that Nup98’s role in transcriptional memory is to stabilize the forward rate of conversion from low to high expressing state, and that induced genes engage in two separate behaviors - transcription itself and the establishment of epigenetically propagated transcriptional memory.

SG60
Transcription and chromatin architecture regulation by nucleoporin proteins
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The nuclear pore complex (NPC) components, nucleoporins (Nups), have been proposed to mediate spatial and temporal organization of chromatin during gene regulation. Nevertheless, we have little understanding on the molecular mechanisms that underlie Nup-mediated chromatin structure and transcription in mammals. Here, we show that Nucleoporin 153 (NUP153) interacts with the chromatin architectural proteins, CTCF and cohesin, and mediates their binding across cis-regulatory elements and TAD boundaries in mouse embryonic stem (ES) cells. NUP153 depletion results in altered CTCF and
cohesin occupancy and differential gene expression. This function of NUP153 is most prevalent at the developmental genes that show bivalent chromatin state. To dissect the functional relevance of NUP153-mediated CTCF and cohesin binding during transcriptional activation or silencing, we utilized epidermal growth factor (EGF)-inducible immediate early genes (IEGs). We found that NUP153 binding at the cis-regulatory elements controls CTCF and cohesin binding and subsequent POL II pausing during the transcriptionally silent state. Furthermore, efficient and timely transcription initiation of IEGs relies on NUP153 and occurs around the nuclear periphery suggesting that NUP153 acts as an activator of IEG transcription. Collectively, these results uncover a key role for NUP153 in chromatin architecture and transcription by mediating CTCF and cohesin binding in mammalian cells. We propose that NUP153 links NPCs to chromatin architecture allowing developmental genes and IEGs that are poised to respond rapidly to developmental cues to be properly modulated.

SG61
Nuclear pore complex quality control in neurodegeneration
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A G\textsubscript{4}C\textsubscript{2} hexanucleotide repeat expansion (HRE) in the \textit{C9orf72} gene is causative of ALS and FTD. Recently, we have demonstrated that pathologic repeat RNA species arising from this HRE leads to a reduction in the nuclear levels of 8 specific components (nucleoporins, Nups) of the nuclear pore complex (NPC) in \textit{C9orf72} induced pluripotent stem cell (iPSC) derived spinal neurons (iPSNs) and postmortem patient tissue. Here, we have uncovered the underlying mechanisms that lead to this Nup loss and disruption of NPCs in neurodegeneration. We provide evidence that CHMP7 and VPS4, components of an ESCRT pathway linked to NPC quality control, are dramatically increased in \textit{C9orf72} iPSN nuclei prior to the emergence of Nup alterations. Consistent with a role in regulating Nup levels within NPCs, knockdown of CHMP7 mitigates disease-associated injury and restores the nuclear levels of specific Nups in human neurons. Additionally, by specifically inhibiting the nuclear export of CHMP7, we can trigger Nup reduction. Thus, our data support a role for disruptions in CHMP7 and ESCRT-III mediated Nup homeostasis in mammalian nuclei and \textit{C9orf72}mediated disease pathogenesis.

SG62
Evidence that Hutchinson-Gilford progeria syndrome is driven by toxic progerin protein accumulation specifically within the cardiovascular system.
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Lamins are intermediate filament proteins that assemble into a meshwork termed the nuclear lamina that connects the nuclear envelope to chromatin at the periphery of the nucleus. Mutations to \textit{LMNA}, the gene that encodes the lamin A protein, cause at least 15 distinct syndromes known as “laminopathies”. Using stable isotope labeling and quantitative proteomics, we have determined that the nuclear lamins are intrinsically stable and long-lived proteins within non-dividing cells. Hutchinson-Gilford progeria syndrome (HGPS) is caused by an autosomal dominant mutation to the \textit{LMNA} gene that generates a toxic “progerin” protein variant. Progerin thickens and distorts the nuclear lamina, suggesting that progerin may accumulate within cells over time and may have an even longer half-life than normal lamin A. We first tested this hypothesis in cultured cells, and found that lamin A and
progerin had similar half-lives in wild-type and progeroid fibroblasts, respectively\textsuperscript{1}. However, interfering RNA oligonucleotides can remove progerin protein much more effectively from skin cells than from heart tissue\textsuperscript{2}, suggesting that tissue environment influences protein lifetime. We are directly addressing this question by applying \textit{in vivo} metabolic labeling and proteomics to define lamin and progerin protein half-life and abundance within healthy and progeroid tissues. We find that wild-type lamin A is more long-lived within the healthy heart than in other tissues, and quantification of progerin lifetime is ongoing. We found that the progerin:lamin A ratio is higher in the heart and vasculature of progeroid mice than in other tissues, which may explain why the cardiovascular system is especially vulnerable to progerin expression. In addition, we find that all lamin A variants accumulate to higher levels in progeroid hearts, suggesting that the progerin mutation may influence degradation of both progerin and wild type lamin A within the heart. Moreover, we observe widespread and discordant effects of progerin expression on tissue proteomes. Notably, ribosome biogenesis is dramatically upregulated in mouse progeroid skin, as we have previously reported in human patient fibroblasts\textsuperscript{1}; however, this effect was skin-specific and did not occur in the heart or in the aorta, where pathology drives mortality. This observation underscores the need to understand the tissue-specific effects of progerin. Within the aorta, we instead see evidence for rewiring of fat and carbohydrate metabolism. Progerin disrupts heterochromatin and induces DNA damage, eventually causing cell senescence and cell death; however, our observations indicate that the consequences of these cellular changes are tissue-specific.\textsuperscript{1}Buchwalter & Hetzer, \textit{Nat Comms} 2017. \textsuperscript{2}Osorio et al., \textit{Sci Transl Med} 2011.

SG63
\textbf{LINC complex disruption suppresses laminopathies}
\textbf{C. L. Stewart}; Instute of Medical Biology, Singapore, SINGAPORE.

Mutations in the lamin A/C (\textit{LMNA}) gene are the 2\textsuperscript{nd} most common genetic cause of Dilated Cardiomyopathy (DCM). It affects as many as 1/12,500 individuals and is curable only by a heart transplant. We find that deletion of the nuclear envelope LINC complex protein SUN1 extends the lifespan of mouse models that develop \textit{Lmna} DCM from 1 month to >1 year. Similarly, displacement of another LINC complex protein, Nesprin-1, by disrupting the KASH domain, also ameliorates and extends the lifespan of \textit{Lmna} mutant mice. Together, these findings reveal that LINC complex disruption significantly suppresses pathologies caused by \textit{LMNA} mutations. Consequently, we developed an adeno-associated virus (AAV)-based gene therapy approach to introduce and express in diseased hearts, a modified version of SUN1 that disrupts the LINC complex, that acts as a dominant negative suppressor preventing DCM progression. Our findings show that the DN-SUN1 can extend the lifespan of \textit{LMNA} DCM mice from ~30 days to at least 4-6 months, identifying a key role the LINC complex has in mediating the laminopathies. It also identifies the DN-SUN1 as a potential therapeutic for some of the laminopathies.

SG64
\textbf{Emerin expression rescues nuclear structure in invasive breast cancer cells to inhibit cell migration and metastasis}
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Nuclear envelope proteins play an important role in regulating nuclear size and structure in cancer. Altered expression of nuclear lamins are found in many cancers and its expression is correlated with better clinical outcomes. The nucleus is the largest organelle in the cell with a diameter between 10 and 20 µm. Nuclear size significantly impacts cell migration. Nuclear structural changes are predicted to impact cancer metastasis by regulating cancer cell migration. Here we show emerin regulates nuclear structure in invasive breast cancer cells to impact cancer metastasis. Invasive breast cancer cells had 40-50% less emerin than control cells, which resulted in decreased nuclear size. Overexpression of GFP-emerin in invasive breast cancer cells rescued nuclear structure and inhibited migration and invasion through 3.0 µm pores. Standard, unimpeded migration assays were done to confirm the migration defects were due to changes in nuclear structure. Mutational analysis showed emerin binding to nucleoskeletal proteins was important for its regulation of nuclear structure. Importantly, emerin expression inhibited lung metastasis by 91% in orthotopic mouse models of breast cancer. Emerin nucleoskeleton-binding mutants failed to inhibit confined cell migration, invasion and lung metastasis. Patients with high-grade breast cancer also showed dramatically reduced emerin protein. Collectively, these results support a model whereby emerin binding to the nucleoskeleton regulates nuclear structure to impact metastasis. In this model, emerin plays a central role in metastatic transformation, since decreased emerin expression during transformation causes the nuclear structural defects required for increased cell migration, intravasation and extravasation.

SG65
Modulating nuclear pore complex numbers for cancer therapies
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Nuclear pore complexes (NPCs) are multiprotein channels that penetrate the nuclear envelope connecting the nucleus with the cytoplasm. NPCs work together with nuclear transport receptors to ferry molecules in and out of the nucleus. Because nucleocytoplasmic transport is essential for cell proliferation and survival, it is not surprising that alterations in the nuclear transport machinery have long been observed in cancer cells. Many transformed cells have increased expression of NPC components and nuclear transport receptors, higher numbers of NPCs and elevated nucleocytoplasmic transport activity. Even more, recent evidence has exposed that many cancer cells become addicted to the nucleocytoplasmic transport and points to the nuclear transport machinery as promising target for cancer therapies. Because NPCs are essential cellular structures, directly targeting these channels has historically been viewed as an unfeasible therapeutic strategy. In this work we provide evidence that modulating the number of NPCs differentially affects normal and cancer cell survival and discuss the potential causes of this selective response. We also show that reducing NPC numbers causes selective cancer cell death, prevents tumor growth and results in tumor regression. Our findings expose a novel vulnerability of many cancer cells and indicate that modulating the number of nuclear pores is a promising therapeutic strategy for cancer.
When Branches of the Tree of Life Meet: Cell Interactions Between Organisms

SG66
Cooperation and conflict in multicellular bacterial aggregates

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Cooperation of individuals in multicellular collectives allows microbes to survive stress and access nutrients. However, a group of individuals need not cooperate: competition for space and nutrients is a more likely outcome especially among cells of the same genotype. How do microbes mitigate conflict and allocate nutrients to achieve cooperation, and what are the genetically encoded mechanisms underlying cooperative behavior? I will discuss recent work combining experimental studies and simulations to address these questions in the context of ‘public goods’ cooperation. A common form of public goods cooperation arises when individuals secrete enzymes to access a limiting nutrient from a large polymer. Cooperation is sustained when the combined enzymatic activity of the collective provides individuals with more nutrients than they can acquire on their own. We investigated the emergence of cooperation during growth of marine bacterium Vibrio splendidus on the polymer alginate. When grown on alginate polymer, V. splendidus forms aggregates which grow in size to more than 20 µm in diameter. At this size our simulations predict that diffusion should limit the delivery of nutrients to the center of aggregates. However, we observe that V. splendidus aggregates develop complex spatial structuring sufficient to overcome diffusion limitation and sustain cooperation within large populations. To understand the origins of this complex multicellular behavior we performed transcriptional profiling (RNASeq) and secondary ion mass spectrometry (nanoSIMS) of aggregates. These studies revealed that aggregate structures are formed by physiologically distinct populations of individuals with distinct catabolic and anabolic activity. Moreover, populations within aggregates express different surface adhesins, suggesting that the spatial structuring of physiologically distinct populations may arise from differential growth and adhesion. In ongoing work, we are genetically perturbing adhesins to understand how physiologically distinct populations of cells form the complex structures that sustain cooperation within multicellular collectives.

SG67
EEEnteroendocrine cells sense bacterial tryptophan catabolites to activate enteric and vagal neuronal pathways

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The intestine harbors complex and dynamic microbial communities that contribute significantly to host health and disease. However, mechanisms by which the intestine perceives distinct microbial species and relays that information to the rest of the body remain unresolved. Enteroendocrine cells (EECs) are specialized sensory epithelial cells in the intestine that detect nutrients and other chemicals in the intestinal lumen and respond by releasing hormones via transient increases in intracellular calcium. Recent studies in mice show EECs also synapse directly with vagal neurons and that nutritional stimuli in the intestine can activate this neuroepithelial circuit. Despite these central roles of EECs in intestinal nutrient sensing, it remained unknown if EECs sense bacteria and what the downstream effects may be.

To test the impact of bacteria on EECs in vivo, we developed a novel genetic system to record EEC activity in free swimming zebrafish following different bacterial stimuli. In a small screen of zebrafish-
associated bacteria, the only strain that significantly elicited EEC activity was the fish pathogen, *Edwardsiella tarda* (*E. tarda*). *E. tarda* stimulation of EEC activity required the transient receptor potential ankyrin 1b (*trpa1b*) gene, which was highly expressed in a subset of zebrafish EECs. Trpa1 is a cation channel that can be activated by a range of noxious chemicals. Optogenetic activation of Trpa1+ EECs led to increased peristalsis via signaling to enteric neurons and *trpa1b* mutant zebrafish were unable to clear *E. tarda* from the intestinal lumen. These data suggest *E. tarda* or chemical stimulation of Trpa1 promotes clearance of those noxious luminal stimuli via increased intestinal motility. In addition to these intestinal responses, we found that intraluminal delivery of Trpa1 agonist or *E. tarda* also activated neurons in the vagal ganglia within the central nervous system (CNS). Transgene-mediated targeted ablation of EECs revealed that EECs are required for this vagal response to intraluminal Trpa1 agonist and *E. tarda* stimulation. Using bacterial chemistry and HPLC-MS analysis, we identified a distinct subset of indole derivatives of tryptophan catabolites produced by *E. tarda* that potently activate zebrafish EEC Trpa1 signaling and also directly activate human and mouse Trpa1. Collectively, these results establish a molecular pathway by which EECs regulate enteric and vagal neuronal pathways in response to specific microbial signals. This microbe-gut-brain axis could be manipulated to promote specific CNS activities and behaviors or to treat neurologic disorders which are associated with alterations in the intestinal microbiome.

**SG68**

*The Stimulation of Tubeworm Metamorphosis by a Bacterial Injection System*

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To find a location on the sea floor for adult life, the swimming larvae of animals like tubeworms and corals are stimulated to undergo metamorphosis by surface-bound bacteria—the same developmental transition that turns a caterpillar into a butterfly. A current grand challenge in the field is to identify the bacterial factors and their mechanisms of action that stimulate this developmental transition. We recently discovered a unique bacterial secretion system called MACs (Metamorphosis Associated Contractile structures) that stimulate the metamorphosis of a model marine tubeworm called *Hydroides elegans*. MACs are evolutionarily related to the contractile tails of bacteriophage and are specialized to puncture membranes, often delivering effectors to target cells. While we identified MACs as the structures stimulating tubeworm metamorphosis, it remained unclear how MACs influenced *Hydroides* metamorphic transition. In this talk, I will present our latest research where we directly observe of a proteinaceous effector loaded within the inner tube of the MACs syringe-like needle by Electron Cryo-Tomography. We further identify a gene and show the gene product, termed Mif1, is sufficient for stimulating tubeworm metamorphosis by promoting lipid second messenger signaling—an ancient and conserved animal signaling pathway. Our finding that bacteria inject stimulating factors prompting animal development has far reaching implications for how we think of and study bacterial interactions with other organisms promoting both pathogenic and beneficial relationships.

**SG69**

*A bacterial protein promotes membrane fusion during Chlamydia infection*

**F. Paumet, J. Wesolowski;** Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

We have recently discovered that the intracellular bacterium *Chlamydia* has developed its own unique bacterial fusion machinery. *Chlamydia* replicates within a “parasitic niche” called the inclusion. At a high
multiplicity of infection, host cells contain several inclusions that ultimately fuse into a single compartment through the process of homotypic fusion. We demonstrate that this process is mediated by the bacterial fusion protein, IncA. While eukaryotic and viral fusion machinery are well-documented, membrane fusion mediated by a bacterial protein is a unique and striking feature of *Chlamydia*, which is poorly understood. In this context, we have now established that IncA is necessary and sufficient to drive fusion. Furthermore, this event depends on the specific lipid environment of a synapse-like structure formed at the interface of apposing inclusions, in which IncA concentrates. Strikingly, in cells that are unable to synthesize Sphingomyelin, IncA is displaced outside of the synapse and the fusion on the inclusions is blocked, demonstrating the major influence of lipids in controlling this bacterial fusion protein. We also show that IncA promotes liposome fusion and that the lipid composition drastically influences the fusogenic activity of IncA. Finally, we have identified the bioactive domain of IncA that is responsible for its fusogenic activity. We will use the sequence of this domain to screen bacteria proteomes and identify whether IncA belongs to a novel family of bacterial fusion proteins. Overall, this study constitutes the first characterization of a bacterial fusion machinery.

SG70
**Host factors induce a rapid developmental transition in the parasitic chytrid fungus Batrachochytrium dendrobatis**
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Successful morphological transitions rely on the careful integration of internal and external cues to coordinate the necessary genetic and mechanical changes of the transition. The chytrid fungus *Batrachochytrium dendrobatis* (Bd) has a biphasic life cycle with distinct morphological states. During its juvenile stage, Bd uses a single posterior flagellum to swim through water and actin-filled pseudopods and an actin cortex to crawl across surfaces, resembling amoeboid animal cells. As the chytrid matures, *Bd* switches to a yeast-like sessile stage by retracting its flagellum, building a cell wall, and assembling actin patches to facilitate the growth of root-like rhizoids and an exit tube for the release of new juveniles. We discovered that mucin, a signature of *Bd*'s host environment, acts as an external switch to trigger the transition between the two life stages. We find that nearly 100% of mucin exposed cells encyst within ten minutes, while control cells take hours. We have identified two requirements for this response: mucin and physical adhesion to a surface. We have used a panel of small molecule inhibitors to probe the mechanisms that control this transition and found that protein translation is not required for this striking developmental transition. We are currently determining the molecular mechanisms controlling *Bd*'s reaction to mucin, as well as assessing the species specificity of this response. By understanding the cell biology of *Bd*, we can develop better management strategies for combating chytridiomycosis - skin infections causing a dramatic decline in amphibian populations worldwide.

SG71
**From endosymbionts to organelles: genetic, biochemical, and cell biological integration of bacteria into host cells**
J. McCutcheon; Arizona State University, Tempe, AZ.

Mitochondria and chloroplasts are now called organelles, but they used to be bacteria. While a great deal is known about how organelles work in extant cells, understanding the genetic, biochemical, and
cell biological events that happened during the transition from 'bacterial endosymbiont' to 'organelle' is obscured by both time and by a lack of comparative examples. Surprisingly, some of the best comparative models for organelle formation to emerge in the last ten years are the bacterial endosymbionts of sap-feeding insects. These bacterial endosymbionts synthesize key nutrients and thus, like their mitochondrial and plastid counterparts, are strictly required and vertically transmitted through eggs by their insect hosts. These bacteria have tiny genomes that overlap mitochondrial and plastid genomes in terms of size and coding capacity. My lab has been trying to understand how these insect endosymbionts function with such small gene sets, and how they have become integrated into the cell biology of their host cells in ways that might be similar to the classic organelles. Here, using combination of light and electron microscopy, genomics, and various other biophysical and biochemical techniques, I will show how two bacterial endosymbionts work together with their insect host to build a peptidoglycan layer specifically at one of the two bacterial cell peripheries. Peptidoglycan (PG) is a defining feature of bacteria, involved in cell division, shape, and integrity, and host control of PG synthesis is known to be important for plastid replication and control in some photosynthetic eukaryotes. We have shown that several genes related to PG biosynthesis were horizontally transferred from bacteria to the nuclear genome of an insect in a remarkably convergent pattern to plastids. I will show that these horizontally transferred genes on the insect genome work together with genes retained one bacterial symbiont genome to produce a bona fide PG layer. Furthermore, I will show that an insect protein encoded by a horizontally transferred gene of bacterial origin is transported into a bacterial endosymbiont cytoplasm. Overall, these results provide a striking parallel to the genetic, biochemical, and cell biological mosaicism found in mitochondria and plastids, and show that these insect systems are likely good models for understanding the endosymbiont to organelle transition.

SG72

Cooperation and Conflict in a Social Fungus

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Social cooperation is important for development and survival of many eukaryotic species. In syncytial filamentous fungi, the acquisition of multicellularity is associated with cooperative behavior that regulates chemotropic interactions and somatic cell fusion within a colony and also between colonies, which has fitness benefits. However, intraspecific cooperation via somatic cell fusion entails risks, as cell fusion can transmit mycoviruses, deleterious genotypes and senescence plasmids that reduce fitness. Allorecognition mechanisms have evolved in syncytial fungi to regulate somatic cell fusion by operating pre-contact during chemotropic interactions, during cell adherence, and post-fusion by triggering programmed cell death reactions. Alleles at these fungal allorecognition loci are highly polymorphic, fall into distinct haplogroups in populations samples, and show evolutionary signatures of balancing selection, similar to allorecognition loci across the tree of life. Molecular characterization of allorecognition loci that induce programmed cell death revealed that a number of them have a similar architecture to NOD-like receptors, which are involved in innate immunity in both animals and plants. One fungal allorecognition locus, rcd-1, identified in the filamentous fungus Neurospora crassa, triggers a rapid cell death response when cells containing incompatible alleles at rcd-1 undergo somatic cell fusion. By in silico analyses, RCD-1 was identified as a remote homolog of the N-terminal membrane pore-forming domain of gasdermin, the executioner protein of a highly inflammatory cell death reaction termed pyroptosis, and which plays a key role in mammalian innate immunity. The RCD-1 incompatibility system was reconstituted in human 293T cells, where co-expression of incompatible rcd-
ORAL ABSTRACTS – SUBGROUPS

1-1/rcd-1-2 alleles was necessary and sufficient to trigger a pyroptotic-like cell death. Oligomers of RCD-1 were associated with the cell death reaction, further supporting the evolutionary relationship between gasdermin and rcd-1, representing an ancient trans-kingdom relationship of cell death execution modules. The emergence of allorecognition systems in filamentous fungi may result from the co-option of genes that evolved primarily for heterospecific non-self recognition and organismal defense. These studies have revealed a rich repertoire of mechanisms that regulate the outcome of social interactions in filamentous fungi.

SG73

**Choanoflagellates and the origin of animal innate immunity**

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While all animals must sense and respond to pathogens, little is understood about how animal innate immunity first evolved. As the closest living relatives of animals, choanoflagellates can provide unique insights into mechanisms of immunity that preceded animal origins. However, studying innate immunity in choanoflagellates has been hindered by the dearth of known choanoflagellate pathogens. Here, we establish *Monosiga brevicollis* as the first choanoflagellate model for the study of pathogen recognition and response. First, we determined that the bacterium *Pseudomonas aeruginosa* has pathogenic effects on *M. brevicollis*, and used bioactivity-guided fractionation to identify *P. aeruginosa* effectors that induce *M. brevicollis* cell death. We then profiled the transcriptional response of *M. brevicollis* to *P. aeruginosa* to identify candidate immune genes, and found that the *M. brevicollis* homolog of *STING* (stimulator of interferon genes), a key facilitator of cytosolic DNA-induced innate immune signaling in animals, is upregulated in response to *P. aeruginosa* bacteria. Phylogenetic alignment of mbSTING and human STING (hSTING) revealed that key residues implicated in hSTING cyclic dinucleotide (CDN) binding are conserved in *M. brevicollis*. Therefore, we performed in vitro and in vivo assays to identify the stimuli responsible for activating mbSTING, and found that mbSTING signaling is activated by the CDN 2’3’cGAMP. To further characterize the role of mbSTING in choanoflagellate immune responses, we developed transgenics and CRISPR/Cas-9-mediated genome editing for *M. brevicollis*. Fluorescently tagged STING protein localized to the endoplasmic reticulum and perinuclear regions of *M. brevicollis*. Targeted disruption of the STING gene revealed that mbSTING deficient cells fail to respond to 2’3’cGAMP, and have an altered response to other immune agonists, including lipopolysaccharide. Finally, by comparing wild-type and mbSTING deficient cells respond to *P. aeruginosa* bacteria and purified immune agonists, we identified choanoflagellate-specific and conserved genes involved in *M. brevicollis* STING-mediated immune responses. Together, these data begin to elucidate mechanisms by which STING signaling contributes to antimicrobial immunity in *M. brevicollis*, and provide functional insights into the role of STING in pre-animal immunity.

SG74

**Dinoflagellate symbionts escape vomocytosis by suppressing immunity in coral host cells**

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Establishing residency inside a cell of another organism may seem like an oddity but many microorganisms take advantage of this intracellular lifestyle. Alveolata comprises diverse taxa of single-celled eukaryotes; many of which are renowned for their ability to live inside animal cells. Notable
examples are the apicomplexan parasites that cause malaria and toxoplasmosis and the dinoflagellate symbionts of corals, which power whole reef ecosystems. Despite their functional diversity, they all evolved from a common, free-living photosynthetic ancestor, and all must evade the innate immune response of their respective host for persistence. While some mechanisms employed by apicomplexan parasites for intracellular persistence are known, the role of host immune modulation in coral-dinoflagellate endosymbiosis is unclear. Likewise, the initial cellular events that occurred during the emergence of this intracellular lifestyle are poorly understood. Here, we use a comparative approach in the cnidarian endosymbiosis model *Exaiptasia pallida* (commonly Aiptasia), which re-establishes endosymbiosis every generation by acquiring free-living, photosynthetic dinoflagellates from the environment. We find that initial uptake of microalgae is indiscriminate, yet only symbionts induce host cell-specific suppression of innate immunity and form a LAMP1-positive niche. Unexpectedly, non-symbiotic microalgae are cleared by ‘vomocytosis’ (non-lytic expulsion) and not by phagolysosomal digestion. Stimulation of the host immune system enhances vomocytosis of dinoflagellate symbionts. In contrast, inhibiting canonical toll-like receptor (TLR) signaling by targeting MyD88 results in increased infection of host animals. Our study suggests that in cnidarian-dinoflagellate symbiosis the suppression of the host cell innate immune response to escape vomocytosis is a key step in initial symbiont selection and symbiosis establishment. This work expands our knowledge about the evolution of the cellular immune response and the remarkable alveolate-host interaction.

**Tuesday, December 8, 2020**

**Building the Cell - 20 Years After**

SG75

**Building the cytoskeleton: How do the building blocks of biological polymers combine to produce the spectrum of behaviors displayed by actin, tubulin, and their relatives?**

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What makes actin filaments different from microtubules? Obvious physical differences include the number of protofilaments, the type of nucleotide, and the stiffness of the resulting polymers. However, there are also major behavioral differences that have an enormous impact on the biological functions of these polymers: actin filaments display treadmilling (a phenomenon where polymers exhibit growth at one end and shortening at the other), while microtubules undergo dynamic instability (a behavior with stochastic transitions between length growth and shortening at one or both ends). The treadmilling of actin enables it to be the engine of amoeboid motility, while dynamic instability allows microtubules to explore space and self-organize into structures such as the mitotic spindle. What causes these differences in behavior? One might expect that they result from fundamental physical differences in polymer structure and/or biochemistry, but the observation that tubulin relatives can treadmill while actin relatives can display dynamic instability indicates that these behaviors are part of a spectrum. Using a combination of computational modeling, mathematical modeling, and experimental biochemistry, we are working to establish a basic predictive understanding of how subunit-scale
biochemical parameters relate to the filament-scale behaviors of treadmilling and dynamic instability, with the goal of being able to use this information to engineer polymers with particular behaviors.

SG76

**Turnover, mechanics, and structural plasticity of lamellipodial actin networks**  
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The dendritic network of actin filaments that provides the force for lamellipodial protrusions has been a powerhouse system for quantitative cell biology. We developed filament-level kinetic and mechanical models to study key questions in lamellipodial dendritic actin structure, turnover, and mechanics that remain poorly understood. Electron microscopy studies revealed a dense brushwork composed of short filaments near the leading edge followed by longer and more linear filaments near the center and rear. Single molecule imaging experiments have indicated frequent actin assembly and disassembly throughout the lamellipodium. Our three-dimensional kinetic model includes mechanisms for polymerization, depolymerization, branching, capping, uncapping, severing, oligomer diffusion, annealing, and debranching. We show that frequent severing and annealing can account for the structural change in the actin network, as well as the measured single molecule lifetimes. To understand how force transmits through the actin network, we studied a 3D Brownian dynamics model with retrograde flow maintained by pushing forces from the leading edge due to actin polymerization, pulling forces due to molecular motors, and frictional forces at nascent focal adhesions. We simulate the tension and bending distributions of actin filaments and the change from compressive stress close to the leading edge versus extensile stress at the back. Filament bending close to the severing threshold (as revealed in prior experiments) is seen around simulated nascent focal adhesions. Network connectivity requires a sufficient number of permanent and temporary crosslinkers, which can also bias the formation of filopodial- or arc-like bundles.

SG77

**How immune cells respond to physical cues - the role of cytoskeletal dynamics**  
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The activation of lymphocytes, an essential step in the adaptive immune response, involves the binding of specialized receptors with antigens. This results in large-scale dynamics and re-structuring of the cytoskeleton, and movement of receptors into sub-micron clusters, which are critical for immune cell activation. Antigen presenting surfaces possess a wide variety of physical attributes, which influence cytoskeletal organization and receptor mobility, but how cells respond to these physical cues is not well understood. We have examined how immune cells respond to physical cues such as stiffness, topography and surface mobility. Regulation of membrane receptor mobility is important in tuning cellular response to external signals, such as during B cell signaling following the binding of B cell receptors (BCR) to antigen. We have used single molecule imaging to examine BCR movement and machine learning techniques to relate receptor trajectories to their signaling states. We find that the dynamic actin network fine-tunes receptor mobility and receptor-ligand interactions, thereby modulating B cell signaling. In vivo, B cells encounter surfaces of antigen presenting cells that are highly convoluted with a wide range of curvatures. We have used nanotopographic surfaces that allow systematic variation of geometric parameters to show that surface features on a subcellular scale influence B cell signaling and actin dynamics. Nanotopography-induced actin dynamics requires BCR
signaling, actin polymerization, and myosin contractility. The topography of the stimulatory surface also modulates the distribution of BCR clusters and calcium signaling in activated B cells. Active cytoskeletal control of receptor diffusion may be a general feature that directs how diverse cell types respond to physical stimuli and transduce external signals into internal chemical signals.

SG78

**Chromosome clustering excludes cytoplasm during nuclear assembly**

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Cells of higher eukaryotes disassemble their nuclei during each mitosis to enable proper chromosome segregation between daughter cells. This temporary loss of compartmentalization leads to intermixing of nuclear and cytoplasmic components. At the end of mitosis, nuclear and cytoplasmic localization is thus reestablished, mainly through the action of nuclear pores, which transport components up to ~40 nm in diameter to their proper compartments. However, how cytoplasmic components that exceed the size limit of nuclear pores are excluded from the nucleus after mitosis remains unknown. We developed high resolution confocal imaging assays to determine the localization of large cytoplasmic particles during mitotic exit. By imaging genetically encoded multimeric nanoparticles (GEMs) of 40 nm diameter we found that bulk cytoplasm is excluded from regions in between anaphase chromosomes before the nuclear envelope reforms. The exclusion of GEMs during mitotic exit also occurred in the absence of a spindle and is regulated by the chromosome surface protein Ki-67. Chromosome clustering during mitotic exit also contributed to the exclusion of mature ribosomes from the reassembling nucleus, suggesting that it is relevant for physiological substrates. Our study reveals how chromosome mechanics contribute to the unmixing of nuclear and cytoplasmic components after open mitosis.

SG79

**Life in a crowded environment**

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The cytoplasm is a crowded subcellular environment packed with organelles, proteins, nucleic acids and other large macromolecules, as well as water and small molecules. The density of molecules inside cells - the concentration of cellular components - is a critical but unappreciated factor that potentially impacts on all cellular processes. Density affects numerous parameters, for instance the concentration of molecules and the effects of macromolecular that can impact cellular mechanics and phase transitions. We are interested in how cellular density is maintained and regulated, and how the biophysical properties of the cytoplasm impact cellular processes. To measure cytoplasmic density in living S. pombe cells, we have developed a method of quantitative phase imaging (QPI) that involves computational processing of a Z-stack of bright field images. We show that cytoplasmic density is not constant, but varies in the course of the normal fission yeast cell cycle: it decreases during G2-phase during tip growth, and increases progressively in mitosis and cytokinesis. The density accumulation during mitosis and cytokinesis may arise as consequence of persistent biosynthesis when volume growth ceases. Cells...
arrested in mitosis or cytokinesis exhibit increasing density. Other means of slowing or halting volume growth such as actin inhibition also lead to increased density. Our findings demonstrate that biosynthesis and volume growth can be uncoupled, suggesting that general biosynthesis is not subject to feedback control and that density can be changed through modulation of volume growth rate. These and other studies highlight cellular density as a variable parameter that has global effects on cellular functions and physiology.

SG80
Robustness and universal scaling in endomembrane organelle size control
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One of the grand challenges in quantitative cell biology is understanding the precision with which cells assemble and maintain subcellular organelles despite being buffeted by stochastic fluctuations. A critical property that governs organelle function is its size. Organelle sizes must be flexible enough to allow cells to grow or shrink them as intra and extracellular environments demand, yet maintained within homeostatic limits to ensure proper functioning. Dysfunctional organelle size control, often manifest by increased variability in organelle sizes, has been linked to a wide variety of metabolic, developmental, and neurodegenerative disorders. Random subcellular fluctuations in organelle size can lead to severe phenotypic defects, such as impaired Chlamydomonas reinhardtii motility in uncoordinated flagellar length control, inappropriately sized secretory vesicles due to variability in Golgi size, and impaired metabolism due to defects in mitochondrial and peroxisomal fission among others. Despite identification of numerous molecular factors that regulate organelle sizes we lack quantitative insight into the principles underlying organelle size control. We therefore set out to develop a quantitative framework to understand the ability of single cells to control the sizes of their organelles. Here we combine single cell measurements of organelle sizes from the eukaryote Saccharomyces cerevisiae and human induced pluripotent stem cells with mathematical theory to show that cells can robustly control average fluctuations in organelle size. By demonstrating that organelle sizes obey a universal scaling relationship we predict theoretically, our framework suggests that organelle size increases in random bursts from a limited pool of building blocks. Burst‐like growth provides a general, potentially evolutionarily conserved biophysical mechanism by which the eukaryotic cell can maintain on average reliable yet plastic organelle sizes.

SG81
Decoding the variance in integrated intracellular organization of the undifferentiated hiPS cell
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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 subcellular structures, and how they transition among distinct states during differentiation in the context of development or disease. One initial step toward this goal is to determine the full range of natural variation in intracellular organization in undifferentiated hiPSCs under normal, unperturbed conditions. To do this we take advantage of ~25 of the endogenous fluorescently tagged hiPSC lines in the Allen Cell Collection (www.allencell.org), each expressing a monoallelic EGFP-tagged protein labeling a particular
organelle or structure. We collected thousands of replicate high resolution 3D images of single cells for each structure (over 150,000 in total) and developed image-based assays and segmentation algorithms for quantitative analyses. To measure variation in cell and nuclear shapes, we fit 3D segmented masks using spherical harmonic functions, and then performed a principal component analysis of the spherical harmonic coefficients. We found that the largest overall variation among individual cells in this data set corresponds to how flat (vs. round) cells and nuclei are along the apical-basal axis (Z-direction). This represents variability in how cells pack together within hiPSC colonies, which depends on experimental factors such as colony size, plating confluency, and passage number. The second principal shape mode closely corresponds to overall volume, representing cell growth through the cell cycle. We took advantage of the wide range of cell and nuclear sizes and large numbers of structures to perform a survey analysis of intracellular structure size scaling. We found that many key cellular structures scale with cell size, however structures vary in the strength of their scaling relation and whether they scale more strongly with cell size, nuclear size, or the size of other structures. To explore variation in subcellular organization of our EGFP-tagged structures, we parameterized the cytoplasm and nucleoplasm via spherical harmonics to generate spherical maps for each structure in each cell in 3D and then averaged these maps over groups of cells with similar shapes. These maps reveal similarities among subcellular distributions of some subsets of organelles; for example, lysosomes and the Golgi apparatus both occupy a particular region toward the apical surface of the cell, and systematically co-vary in their location across the full range of cell shape variation. This systematic approach has enabled us to determine how subcellular organelle organization varies with changes in cell size and shape in an integrated fashion across ~25 EGFP-tagged subcellular structures.

SG82

Mechanical constraints play a critical role in protein segregation and morphogenesis in the early C. elegans embryo

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During development, cells take on specific fates to properly build tissues and organs. These cell fates are regulated by a number of factors, including biochemical signaling, mechanical constraints, and cell geometry. However, it is not yet understood how these factors work together to regulate patterning during development, and how the same interactions can lead to different responses to perturbations. Exploiting the high conservation of developmental pathways and processes, we theoretically and experimentally explore mechanisms of protein patterning and cell fate specification during development of the nematode worm C. elegans. Biologically based mathematical models identify key components and interactions required for wild type patterning, which can differ when the model is subject to mechanical perturbations. Testable hypotheses based on model results are used to investigate protein patterning and cell fate specification in both wild type and perturbed conditions, revealing how the same underlying interaction network can give rise to different behaviors under certain conditions. The rich data sets produced by these models form the basis for further analysis and increase our understanding of cell fate regulation in development.
Entropy and mechanics drive tissue formation and breakdown


During tissue development, homeostasis, and disease, many structural changes are driven by cell mechanics, but emerge heterogeneously in time and space. Predicting when and where these events occur is challenging because no theoretical framework is available for understanding them. We use a human organoid model of the mammary gland to identify factors that determine the distributions in tissue structure observed across time and space, with a focus on the positioning of luminal and myoepithelial cells along the luminal to basal axis. Using biophysical measurement, quantitative imaging, and molecular perturbations, we establish that cell positioning follows Boltzmann statistics — dependent on cell-type specific interfacial energies, tissue configurational entropy, and activity (analogous to fluctuations in local mechanical energy around a mean). We provide further evidence that these parameters must change in order for transformed luminal cells to access the basement membrane and invade during breast cancer progression. Together, these findings provide a quantitative and general framework for understanding tissue structural heterogeneity during development, homeostasis, and disease.

Cell Polarity Signaling in Neurons

Oxygen-tension and the VHL-Hif1 pathway determine onset of neuronal polarization and cerebellar germinal zone exit

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Postnatal brain circuit assembly is driven by temporally regulated intrinsic and cell-extrinsic cues that organize neurogenesis, migration, and axo-dendritic specification in post-mitotic neurons. While cell polarity is an intrinsic organizer of morphogenic events, environmental cues in the germinal zone (GZ) instructing neuron polarization and their coupling during postnatal development are unclear. We report that oxygen tension, which rises at birth, and the von Hippel-Lindau (VHL)-hypoxia-inducible factor 1α (Hif1α) pathway, regulate polarization and maturation of post-mitotic cerebellar granule neurons (CGNs). At early postnatal stages with low GZ vascularization, Hif1α restrains CGN-progenitor cell-cycle exit. Unexpectedly, cell-intrinsic VHL-Hif1αpathway activation also delays the timing of CGN differentiation, germinal zone exit, and migration initiation through transcriptional repression of the partitioning-defective (Pard) complex. As vascularization proceeds, these inhibitory mechanisms are downregulated, implicating increasing oxygen tension as critical switch for neuronal polarization and cerebellar GZ exit.

A multi-compartment neuron reveals differences between apical-basal and axon-dendrite sorting signals

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Cells are highly organized machines with functionally specialized compartments, such as the dendrites and axon of a neuron or the apical and basolateral surfaces of an epithelial cell. To carry out their
functions, each compartment requires different proteins. A fundamental question is how proteins are sorted to the correct compartment. We recently showed that C. elegans amphid sensory neurons have a multi-compartment organization. Like other neurons, they have axons and dendrites but, like epithelial cells, they also have apical and basolateral surfaces. The apical surface consists of the distal ~5 µm of the dendrite and sensory cilium, is exposed to the environment, and is delimited by tight junctions with a glial cell. The basolateral surface consists of the proximal ~95 µm of the dendrite, cell body, and axon, and is not exposed to the environment. We reasoned that these neurons could thus be used to identify differences between apical-basal and axon-dendrite sorting signals. To this end, we focused on the neuronal adhesion molecule SAX-7/L1CAM as a model single-pass transmembrane protein. We expressed sfGFP-tagged SAX-7 in a single amphid neuron (ASER) and found that it localizes exclusively basolaterally. Constructs in which we replaced the extracellular region with sfGFP also localized basolaterally; in contrast, constructs replacing the cytoplasmic region with sfGFP localized exclusively apically. Thus, the SAX-7 cytoplasmic region is necessary and sufficient for basolateral localization. To further define the relevant sorting signals, we generated a series of constructs consisting solely of extracellular sfGFP, a transmembrane segment, and fragments of the SAX-7 cytoplasmic region. Using this approach, we identified two sorting signals that are sufficient for basolateral localization. One sequence, EPIL, resembles a dileucine sorting signal (ExxxLL) which has previously been characterized in epithelia as a basolateral motif. Interestingly, we show that the SAX-7 signal is distinct from that used in epithelia, as replacing it with a canonical ExLL or ExxxLL motif changes its localization from basolateral to "axon-only." The second sequence has not been described before, but is conserved across SAX-7/L1CAM family members. In summary, we identified signals that distinguish apical-basal from axon-dendrite sorting, thus enabling these cells to maintain their complex multi-compartment organization.

SG86

Neuronal polarity requires dendritic protein endocytosis in the axon initial segment

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Neurons are highly polarized cells that must compartmentalize a vast repertoire of proteins to either the axon or dendrites, a neuron’s morphologically, biochemically, and functionally distinct domains. The axon initial segment (AIS) is a critical region between the axon and dendrites that acts as a selective filter for intracellular vesicles and a diffusion barrier to establish and maintain neuronal polarity. How the AIS functions to establish and maintain strict compartmentalization of proteins remains unresolved.

We uncovered a novel, conserved mechanism of neuronal polarity in the AIS in which dendritic transmembrane proteins are prevented from entering the axon through their endocytosis and degradation in the AIS. We identified this mechanism by studying the dendritic morphology guidance receptor, DMA-1, which is required for the C. elegans PVD neuron’s elaborate dendritic branching. Endogenously labeled DMA-1 is highly polarized to the dendrite and excluded from the axon. Using an in vivo endocytosis reporter, we unexpectedly find that DMA-1 is highly endocytosed in the AIS, a region previously thought to lack endocytosis due to its dense cytoskeletal network. However, we find that endogenously labeled endocytic proteins, clathrin light chain and AP-2, localize to puncta in the AIS and display characteristic features of endocytic structures. Endocytic inhibition through manipulating AP-2 or dynamin causes mislocalization of DMA-1 to the axon within hours and aberrant axonal branching due to DMA-1’s function in neurite branching, thus demonstrating a functional role for AIS endocytosis in neuronal polarity. We identify two DMA-1 endocytic interaction mechanisms that together select and
ensure efficient DMA-1 endocytosis in the AIS: a cytoplasmic interaction with AP-2 and an extracellular domain interaction with two LRP (low density lipoprotein receptor-related) proteins. After its endocytosis, DMA-1 localizes to late endosomes in the AIS, suggesting that DMA-1 is endocytosed and degraded to prevent its further entry into the axon. Therefore, endocytosis is an active mechanism to remove dendritic proteins from the AIS and maintain neuronal polarity. We further demonstrate that this mechanism is broadly used by diverse dendritic proteins in additional C. elegans neurons and is evolutionarily conserved. Using induced human neurons, we find that the dendritically polarized transferrin receptor is endocytosed in the AIS and endocytosis is essential for its polarity. These results define a framework for understanding AIS endocytosis and reveal a novel mechanism of endocytosis in the AIS that is critical for neuronal polarity.

SG87

Degradative regulation of dendritic cargos
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Neuronal endosomes are essential for membrane receptor trafficking to and within dendrites and axons. Endosomes participate in a variety of signaling events, as well as regulating the rates of recycling and degradation. In neurons, endosomal trafficking is involved in receptor trafficking and various neuronal functions, such as synaptic plasticity and axon outgrowth. In addition, proper protein homeostasis depends on proper functioning of the endolysosomal system. Dysfunctions of the endo-lysosomal system have thus been implicated in a number of neurodegenerative conditions. As in other cell types, neuronal endosomes are regulated by a variety of endosomal regulators, which localize to different endosomal compartments. We previously found that distinct subtypes of late endosomes and lysosomes are present in a gradient: degradative lysosomes are highly accumulated in the soma and proximal dendrites and largely absent from more distal regions of dendrites. Furthermore, we showed that degradation of the short-lived dendritic receptor Nsg1/2 takes place in somatic/proximal lysosomes and requires retrograde transport. We find that transport and degradation require functional Rab7, a critical regulator of late endosomes. Since microtubules in dendrites have mixed polarity, it is unclear how directional retrograde trafficking is regulated. We now explore known Rab7 effectors which can recruit microtubule motors. In particular, we are determining if FYCO (which is an adaptor for kinesin) or RILP (which is an adaptor for dynein) are important for retrograde transport of pre-degradative late endosomes in dendrites.

SG88

A quality control system couples microtubule nucleation and polarity at dendrite branch points
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Neuronal polarity relies in part on selective trafficking from the cell body into axons and dendrites. One determinant of directional transport into dendrites is the presence of minus-end-out microtubules in dendrites, but not axons. Drosophila dendritic arborization neurons have branched dendrite arbors that contain almost exclusively minus-end-out microtubules. Microtubule nucleation sites are localized to
dendrite branch points and dysregulated nucleation can alter minus-end-out microtubule polarity. However, it is not clear how microtubule nucleation is linked to maintenance of minus-end-out polarity. Nucleation sites are positioned on endosomes at dendrite branch points by Wnt signaling proteins, and new microtubules generated at these sites are not uniformly initiated in the “right” orientation. We therefore tracked the fate of newly nucleated microtubules in dendrites in vivo, and found that the “right” orientation ones had a much higher probability than “wrong” plus-end-out microtubules of successfully growing out of dendrite branch points. This selective success of correctly oriented microtubules relied on pre-existing parallel microtubules. We conducted a candidate screen to identify regulators of microtubule polymerization that could promote growth of new microtubules along parallel ones. We found that kinesin-5 and Trim9 are both required to promote polymerization of newly generated correctly oriented microtubules. Using S2 cells, we found that Trim9 could be recruited to microtubule plus ends by EB1. In addition, in vitro reconstitution demonstrated that kinesin-5 promotes plus end polymerization in vitro. We propose a model in which kinesin-5 and Trim9 help newly nucleated microtubules overcome an inhibitory growth environment only when oriented in parallel with existing microtubules. This quality control system helps to ensure that local nucleation in dendrites does not disrupt overall microtubule polarity.

SG89
**Semaphorin3A and PlexinA3 association with a scaffold for cGMP increase is required for apical dendrite development**
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The development of the apical dendrite from the leading process of the bipolar pyramidal neuron might be directed by spatially organized extrinsic cues acting on localized intrinsic determinants. The extracellular cues that regulate apical dendrite polarization have remained elusive. We find that leading process and apical dendrite development is directed by class III Semaphorin signaling and mediated by a localized cGMP-synthesizing complex. We show that the scaffolding protein Scribble, which associates with cGMP-synthesizing enzyme soluble-Guanylate-Cyclase (sGC), also associates with the PlexinA3 co-receptor for Semaphorin3A (Sema3A). Deletion or knockdown of PlexinA3, Sema3A, or disruption of PlexinA3 - Scribble complex associations prevented Sema3A-mediated cGMP increase and caused severe defects in apical dendrite development in the embryonic CA1. These manipulations also caused defects in bipolar polarity and leading process establishment. Together, we show that during neuronal polarization, leading process formation and subsequent apical dendrite development is directed by a scaffold that links extracellular Semaphorin cue to cGMP increase.

SG90
**Par polarity proteins in dendritic spine morphogenesis and plasticity**
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Neurons are probably the most polarized/compartmentalized cell type in the human body. Their polarity establishment starts with axon/dendrite specification. Further compartmentalization occurs during the formation of dendritic spines, which receive most of the excitatory synaptic inputs in the brain. This raises the exciting possibility that proteins regulating global cell polarization are involved in the localized polarity during dendritic spine morphogenesis and plasticity. Indeed our studies show that the Par (Partitioning-defective) proteins, which are conserved polarity regulators originally identified in C.
elegans, play a key role in spine morphogenesis and plasticity. We show that the polarity protein Par1, a Ser/Thr kinase, is involved in dendritic spine morphogenesis downstream of NMDA receptors. Par1 functions through phosphorylating the synaptic scaffolding protein PSD-95 on Ser561. We further found that phosphorylation of this site regulates a conformational switch that is important for bidirectional spine structural plasticity. Using a forebrain-specific Par1c conditional knockout mouse model, we found that Par1c is required for proper spine morphogenesis and cognitive functions in vivo. Together, our studies reveal important roles for Par1 in dendritic spine morphogenesis and plasticity, as well as cognitive functions in vivo.

SG91
Planar cell polarity signaling in growth cone guidance and synapse formation
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Directional and positional information is essential for the diverse neuronal morphology and connectivity during development. The direction of axon growth helps build the correct networks among neurons sometimes from far away. Neuronal synapses are asymmetric cell-cell junctions with distinct pre- and postsynaptic structures to convey neural activity in a directional fashion. Recent studies show that some of the key asymmetry is mediated by highly converged cell polarity signaling pathways. These pathways, planar cell polarity and apical-basal polarity, are not required for the global axon-dendrite polarity. Therefore, the apparent distinct types of morphological asymmetry in the nervous system, growth cone turning and synaptic junctions, are mediated by similar cell polarity signaling mechanisms widely used in cellular and tissue morphogenesis. New findings on how planar cell polarity signaling regulates the community effect in axon guidance and the maintenance of glutamatergic synapses in adulthood will be presented.

SG92
Planar Cell Polarity Signaling Directs Growth Cone Guidance Through Non-Autonomous Mechanisms Acting in Target Tissues
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Type II spiral ganglion neurons innervate the sensory receptor hair cells of the mammalian cochlea and have nociceptive functions important for auditory function and homeostasis. These neurons are anatomically distinct from other classes of spiral ganglion neurons because they extend a peripheral axon that makes a distinct 90 degree turn towards the cochlear base. As a result, the Type II spiral ganglion neuron innervation of the three rows of outer hair cells is coordinated with the frequency-based tonotopic organization of the cochlea. Previously we demonstrated that peripheral axon turning is directed by a non-autonomous function of the planar cell polarity (PCP) protein VANGL2. Here we demonstrate that the Frizzled (FZD) receptors FZD3 and FZD6 similarly regulate axon turning through a non-autonomous mechanism, are functionally redundant with each other, and genetically interact with Vangl2 to guide growth cone turning. The peripheral axon of the Type II spiral ganglion neuron navigates a target environment containing distinct populations of supporting cells which surround hair cells and provide trophic and structural support. FZD3 and FZD6 proteins are asymmetrically distributed along the basolateral wall of cochlear supporting cells, and are required to promote or maintain the asymmetric distribution of VANGL2 and CELSR1 at this location. These data indicate that intact PCP complexes formed between cochlear supporting cells in the target tissue contribute to the non-autonomous
regulation of axon pathfinding during cochlear innervation. Consistent with this hypothesis, in the absence of PCP signaling type II SGN peripheral axons turn randomly and often project towards the cochlear apex. Currently unknown is whether growth cone turning is mediated by a PCP signal conveyed between the supporting cells and the growth cone or whether PCP signaling between supporting cells regulates the distribution of conventional axon guidance cues. A deeper understanding of these mechanisms is necessary for repairing auditory circuits following acoustic trauma or promoting cochlear re-innervation for the success of regeneration-based deafness therapies.

SG93
Investigating circuit mechanisms for CELSR3 and the pathophysiology of Tourette Disorder in genetic mouse models
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Tourette Disorder (TD) is a heterogeneous neurodevelopmental disorder characterized by involuntary motor and vocal tics that are precipitated by premonitory urges. It shows high comorbidity with attention-deficit/hyperactivity and obsessive-compulsive disorders, and is frequently associated with Autism Spectrum Disorder. TD is hypothesized to result from cortico-striatal-thalamo-cortical network dysrhythmia, but efforts to address this hypothesis and the underlying circuit mechanisms have been impeded by a lack of animal models. Our lab is addressing this need by developing animal models for TD based on exome sequencing results from over 800 TD simplex trios that identified multiple \textit{de novo} mutations in \textit{CELSR3}. \textit{CELSR3}, currently one of the most highly associated genes with TD, is an adhesion protocadherin G protein-coupled receptor that shows homology to \textit{NEUREXIN}, a known ASD and TD susceptibility gene. \textit{CELSR3} is also part of the core planar cell polarity family of genes, but circuit mechanisms pertaining to \textit{CELSR3} dysregulation in TD are unknown. We have now generated three different models that express human point mutations in \textit{Celsr3} that complement loss-of-function models, and we are applying electrophysiology, 3D neuron reconstructions, biochemistry, and mouse behavior to identify novel functions for \textit{Celsr3} in striatal interneurons and inhibitory neurons in the thalamus. Preliminary findings indicate that \textit{Celsr3} is required for the firing properties of these neurons, and work is underway to identify changes to neuronal morphology, dendritic patterning, and synapse formation that may underlie TD-like behavioral phenotypes. We expect these combined approaches in animal models will build a novel mechanistic framework to elucidate circuit mechanisms that underlie TD, and guide the development of specifically targeted therapies.

Centromere Structure and Function

SG94
Elucidating the Dynamics and Regulation of Kinetochore Assembly
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Accurate chromosome segregation requires a conserved megadalton-sized protein network called the kinetochore to assemble on centromeric DNA. The kinetochore carries out an array of critical functions, from mediating microtubule-attachment to controlling the cell cycle via the spindle checkpoint. Its composition and functions dynamically change throughout the cell cycle, but the fundamental
mechanics of this process are not well understood. To address the underlying regulation and dynamics of kinetochore assembly, we developed a real-time assay to monitor budding yeast kinetochore assembly via colocalization spectroscopy. Using this assay, we have monitored in real-time the first steps of kinetochore assembly on single centromeric DNA molecules at single-molecule resolution and confirmed a hierarchical and chaperone-dependent deposition of the centromeric histone H3 variant. To identify key regulatory events, we assayed the role of various mitotic kinases in kinetochore assembly and stability. We found that the Aurora B kinase is required to maintain stable binding of the centromeric nucleosome to DNA. Using this single molecule assay, we are currently identifying the key Aurora B substrate as well as additional phosphorylation and associated regulatory mechanisms in centromeric nucleosome deposition and kinetochore assembly initiation.

SG95

Epigenetic, genetic, and sex-specific facets of centromere identity and strength

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Centromeres direct chromosome inheritance by nucleating the kinetochore at cell division. The chromosomal location of the centromere is defined by the presence of an array of nucleosomes in which CENP-A replaces histone H3. I will highlight two projects, both pursuing our longstanding interest in the epigenetic contributions to centromere identity, specifically in the self-directed propagation mechanisms that maintain centromere identity through somatic cell divisions and through specialized chromosomal processes in the germline. First, we find that in the zygote, paternal chromosomes contain reduced levels of CENP-A nucleosomes. By the eight-cell stage of the embryo, however, levels on paternal chromosomes have risen to match those on maternal chromosomes. Experimentally lowering CENP-A gene dosage leads to corresponding lower numbers of CENP-A nucleosomes at centromeres. This weakened centromere state is epigenetically remembered through the two generations following restoration of full CENP-A gene dosage, but only if inherited through the male germline. Centromere weakness is completely forgotten after a single generation through the female germline, as oocytes fill all available CENP-A nucleosome assembly sites regardless of the initial epigenetic strength of the centromere. Thus, epigenetic memory through the mammalian germline depends on sex. A related project builds on our prior findings that CENP-A nucleosomes strongly prefer a primary assembly site within mouse minor satellite repeats, indicating an intimate relationship between epigenetic and genetic features at mouse centromeres (PMC5567862). Further, a mouse strain that naturally houses only minimal minor satellite repeats limit CENP-A nucleosome assembly and cause their chromosomes to be preferentially lost to the polar body in female meiosis I when their paired homolog contains larger arrays of minor satellite DNA. We are now interrogating the relationship of epigenetic and genetic features in diverged mouse species in which the satellite arrangements have radically diverged or where minor satellite is completely absent. Some of the questions we are answering include: What role does DNA repeat sequence play in delineating CENP-A chromatin from adjacent pericentromeric heterochromatin? What sequences provide primary assembly sites for CENP-A nucleosomes in mouse species that completely lack minor satellite DNA? How do epigenetic and genetic features impact centromere strength in female meiosis?
SG96

Holocentromere formation without centromeric histone variants in Lepidoptera avoids active chromatin
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Despite their essentiality for faithful chromosome segregation, centromere architectures are diverse among eukaryotes and embody two main configurations: mono- and holocentromeres, referring respectively to a localized or unrestricted distribution of centromeric activity. Previous studies revealed that holocentricity in many insects strongly coincides with the loss of the otherwise essential centromeric marker CenH3 (CENP-A), suggesting a molecular link between the two events. Here, we aim to characterize this unique CenH3-deficient chromosome segregation pathway. Using proteomic and genomic approaches in Bombyx mori (silk moth) cell lines, we aim to determine the mechanism of CenH3-independent kinetochore assembly that led to the establishment of their holocentric architecture. In this context, we have recently identified additional inner kinetochore components including CENP-T and determined their contribution to kinetochore assembly independent of CenH3. In addition, we also leveraged the identification of these kinetochore components against which we also generated antibodies to map and characterize the centromeres of B. mori. This uncovered a robust correlation between centromere profiles and regions of low chromatin dynamics found anywhere along the chromosome. Transcriptional perturbation experiments showed that centromeres become excluded from regions of active chromatin but can form de novo in regions where chromatin activity is low. The identified link to chromatin dynamics helps to discuss the plasticity of centromere identity. In this context, our study points to a novel mechanism of centromere formation that occurs in a manner recessive to the chromosome-wide chromatin landscape rather than being defined by the presence of CenH3. Based on similar profiles observed in additional Lepidoptera, we propose an evolutionarily conserved mechanism that underlies the establishment of holocentromeres through loss of a specified centromere.

SG97

Roles of novel kinesin-14s in maize meiotic drive
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A maize chromosome variant called Abnormal chromosome 10 (Ab10) converts knobs on chromosome arms into neocentromeres, causing their preferential segregation to egg cells in a process known as meiotic drive. In prior work we demonstrated that the gene Kinesin driver (Kindr) encodes a kinesin-14 that mobilizes neocentromeres composed of the major tandem repeat knob180. We have now identified a second kinesin-14 gene, TR-1 kinesin (Trkin), that activates neocentromeres containing the minor knob repeat TR-1. Trkin shows extraordinary sequence divergence from Kindr and other kinesins in plants. Despite its unusual structure, Trkin encodes a functional minus end-directed kinesin that specifically colocalizes with TR-1 in meiosis, forming long drawn out neocentromeres. The fact that some Ab10 chromosomes naturally lack Trkin, and that TR-1 repeats often co-occur with knob180 repeats suggests that the current role of the TRKIN/TR-1 system is to facilitate the meiotic drive of the KINDR/knob180 system.
SG98

**Investigating the functional conservation of centromeric retroelements in *Drosophila***

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Centromeres are essential chromosomal regions that mediate kinetochore assembly and spindle attachments during cell division. Despite their essential function, centromeres are amongst the most rapidly evolving regions of the genome. In most multicellular eukaryotes, centromeres are characterized by large arrays of satellite DNA and a specialized chromatin containing the histone variant CENP-A, whose faithful deposition is required for accurate chromosome segregation. Although the role of CENP-A in centromere identity is well documented, the function of centromeric DNA sequences in this process is still elusive. A subset of centromere-derived transcripts have been shown to be important for centromere integrity in mammals and transcription at the centromere has been shown to be coupled with CENP-A deposition in *Drosophila*. However, systematically probing the role and conservation of centromeric DNA elements and transcripts in these processes has been hindered by our limited knowledge of the sequence composition of the centromeres of multicellular organisms, which are still largely missing from even the most complete genome assemblies. We recently identified the DNA sequence and organization of all *D. melanogaster* centromeres using long-read sequencing, CENP-A ChIP-seq, and OligoPaint FISH on chromatin fibers. The functional centromere core resides on islands of complex DNA enriched in retroelements and is flanked by large arrays of satellite DNA. A specific retroelement, *G2/Jockey-3*, is the most highly enriched sequence in CENP-A chromatin and the only element shared among all *D. melanogaster* centromeres. To determine if the presence of *G2/Jockey-3* at the centromere is conserved, we performed IF/FISH in three species from the *D. simulans* clade and found that *G2/Jockey-3* is enriched at least at a subset of these species’ centromeres. Given that the pericentromeric satellites in this clade are highly divergent from those of *D. melanogaster*, this conservation is surprising and is consistent with the possibility that *G2/Jockey-3* plays a functional role in centromere identity. To investigate the transcriptional state of *G2/Jockey-3* copies across the genome, we isolated nascent transcripts using PRO-seq and found that *G2/Jockey-3* is actively transcribed. To establish if at least a subset of these nascent transcripts originate from the centromeres, we used single-molecule IF/RNA-FISH and found that most of the signal for *G2/Jockey-3* RNAs is centromere-associated in both interphase and mitotic cells. We are currently investigating the centromeric expression and regulation of *G2/Jockey-3* in different *Drosophila* species and hybrids as well as generating genome editing and shRNA-mediated knock-down tools to determine the role of *G2/Jockey-3* at the centromere.

SG101

**A genetic memory initiates the epigenetic loop necessary to preserve centromere position**

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The preservation of centromere position and function is a crucial challenge for cells in order to maintain a correct chromosome karyotype following cell division. Alterations in centromere formation or function lead to incorrect chromosome attachments and to spindle pole defects. In turn, these defects lead to
numerical and structural chromosome aberrations, the latter often present at centromeric regions, and are common features of cancer cells and developmental disorders. Centromeres are built on repetitive DNA sequences and a specific chromatin enriched with the histone H3 variant CENP-A. Much progress has been made in understanding the assembly and the regulation of centromere components at the protein level, culminating with the identification of CENP-A as the epigenetic mark required to maintain centromere position and function. The focus on CENP-A has meant, however, that the role of the underlying centromeric DNA sequences remains largely unexplored. During the talk, I will highlight recent findings from my laboratory that demonstrate the importance of centromere DNA in centromere specification. By developing a system to rapidly remove and re-activate CENP-A, we define the temporal cascade of events necessary to maintain centromere position. Here, we unveil that CENP-B bound to centromere DNA provides memory for maintenance on human centromeres by promoting de novo CENP-A deposition. Indeed, lack of CENP-B favors neocentromere formation under selective pressure. Occasionally, CENP-B triggers centromere re-activation initiated by CENP-C, but not CENP-A, recruitment at both ectopic and native centromeres. This is then sufficient to initiate the CENP-A-based epigenetic loop, as observed in a population of CENP-A-negative, CENP-B/C-positive resting CD4+ T cells capable of re-expressing and reassembling CENP-A upon cell cycle entry. Further, I will discuss mechanisms of how CENP-B models centromere DNA to create a unique DNA topology.

SG100

Cell cycle control of centromere assembly
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The centromere is the assembly site for the mitotic kinetochore that binds chromosomes to spindle microtubules for chromosome segregation in mitosis. In many eukaryotes, the centromere is determined by the presence of a centromeric histone H3 variant in chromatin called Centromere Protein A (CENP-A). CENP-A disruption causes centromere and kinetochore loss and chromosome segregation failure. Thus, a central question in chromosome segregation is how do cells maintain CENP-A at centromeres? Three factors play essential roles in assembling CENP-A nucleosomes: 1) the HJURP chaperone complex (HJURP/CENP-A/histone H4/Nucleophosmin) binds soluble CENP-A, 2) the Mis18 complex (Mis18α/Mis18β/M18BP1(Mis18c)) directs the HJURP complex to centromeric chromatin and 3) a dimer of the centromere protein CENP-C binds to CENP-A nucleosomes and also binds Mis18c and the HJURP complex to target them to centromeres. We have studied the mechanisms that control the cell cycle dependent targeting of HJURP and Mis18c to centromeres. We find that mitotic kinase phosphorylation of HJURP inhibits its association with the dimerization domain of CENP-C. HJURP mutants that mimic metaphase phosphorylation prevent HJURP binding to CENP-C in interphase and block new CENP-A assembly. Phospho-null HJURP mutants constitutively associate with CENP-C in metaphase but do not assemble CENP-A indicating a second inhibitory mechanism in metaphase. An additional metaphase phosphorylation of HJURP inhibits the interaction between HJURP and the Mis18β subunit of Mis18c. These combined phosphoregulatory mechanisms control the interphase specific assembly of CENP-A after exit from metaphase.

SG99

Centromere drive and suppression by parallel pathways for recruiting microtubule destabilizers
Selfish centromere DNA sequences can bias their transmission to the egg in female meiosis. Evolutionary theory suggests that centromere proteins evolve to suppress such centromere drive. In mouse models, selfish centromere DNA hijacks a kinetochore pathway to recruit microtubule-destabilizing proteins that act as effectors for drive. We show that drive is suppressed by minimizing the kinetochore pathway relative to a second pathway for effector recruitment by heterochromatin, which equalizes centromeres. Disruption of the kinetochore pathway by introducing a divergent allele of CENP-C reduces functional differences due to selfish centromere DNA, whereas disruption of the suppressor heterochromatin pathway by CENP-B deletion amplifies the differences. Furthermore, molecular evolution analyses using newly-sequenced Murinae genomes identify adaptive evolution in multiple protein domains that lead to effector recruitment. Together, these findings suggest that the chromosome segregation machinery has recurrently evolved to suppress drive by modulating parallel pathways for recruiting microtubule destabilizers while maintaining essential functions.

SG102

**Cell cycle regulation of CENP-T in fission yeast**

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Kinetochores are large protein complexes assembled on centromeres and play a crucial role in chromosome segregation. The kinetochore links the chromosome to microtubule polymers and ensures correct microtubule-kinetochores attachment. CENP-T, an inner kinetochore protein, serves as a platform for the assembly of the Ndc80 complex, which is an essential outer kinetochore component. How CENP-T is regulated through the cell cycle to mediate the recruitment of Ndc80 remains unclear. Previously we have shown that the NAP (Nucleosome Assembly Protein) domain-containing protein, Ccp1, plays an important role in antagonizing the histone H3 variant, CENP-A in fission yeast. Ccp1 localizes to centromeres during interphase, but dissociates from the region during mitosis. Here we identified that Ccp1 interacts with CENP-T and showed that the centromere localization of Ccp1 depends on CENP-T. We further identified a peptide motif in CENP-T that mediates the interaction between Ccp1 and CENP-T. Interestingly, dephosphorylation of the motif results in tight association of Ccp1 with centromeres during mitosis and disorganization of the Ndc80 complex. In contrast, phosphorylation of the motif leads to loss of Ccp1 from centromeres throughout the cell cycle. Our results indicated that dissociation of Ccp1 by CENP-T phosphorylation at the onset of mitosis promotes the assembly of the Ndc80 complex. Our results provide new insights into how kinetochores are assembled during the cell cycle.

**Dynamic Modes of Cellular Compartmentalization**

SG103

**Protein compartmentalization enables robustness of bacterial cell cycle under metabolic shifts**

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Free-living bacteria have evolved many regulatory mechanisms to control signaling in response to changing environmental conditions. Reversible compartmentalization of biomolecules is a ubiquitous mechanism for cells to regulate signaling and development. Often, such sequestration of biomolecules is achieved through intracellular membrane-bound compartments in response to extracellular conditions.
and developmental cues. However, most bacteria are devoid of membrane-bound organelles that enable sequestration. Here, I will discuss recent developments in our understanding of how membraneless organelles formed by protein phase separation, are utilized for cell signaling in the oligotrophic bacterium, *Caulobacter crescentus*. *Caulobacter* divides asymmetrically into a sessile stalked cell and a flagellated swarmer cell by spatially controlling the synthesis, localization, and degradation of proteins as a function of the cell cycle. Much of this asymmetric division is regulated through complex signaling pathways that are localized in the cell poles that span ~100 nm. Specifically, during differentiation the polar scaffold protein PopZ recruits a transmembrane protein SpmX to the nascent stalked pole, which then localizes the kinase DivJ that is critical for cell division. Notably, SpmX and PopZ have long, intrinsically disordered regions rich in Proline and negatively charged residues. Reconstitution of the PopZ-SpmX-DivJ signaling complex on lipid bilayers and in a bacterial heterologous system revealed that SpmX and PopZ can form condensates via phase separation. *In vivo* biochemistry, protein diffusion measurements and high resolution imaging reveal that SpmX and PopZ form a unique system of two interacting polar membraneless organelles that sequester DivJ at the stalked pole. Investigation of how PopZ and SpmX membraneless compartments impact DivJ, revealed an interplay between the metabolic state of the cell (ATP rich, versus ATP poor) and the activity of DivJ. This interplay is driven by ATP’s ability to solubilize phase-separated PopZ and SpmX compartments, through ATP’s role as a hydrotrope. Low ATP levels lead to enhanced phase separation of SpmX and in turn an increased sequestration of DivJ in the SpmX compartment. The activity of this cooperative kinase was thus enhanced even under low substrate (ATP) concentrations, thus rendering robustness to the cell cycle. Our work posits that phase separated compartments tune the cooperativity of enzymes sequestered within them, in response to substrate concentrations. This work connects the regulatory role of membraneless organelles to metabolic flux through a mechanism that enables a free-living bacterium to robustly tune its biochemistry and survive unpredictable famine and feast cycles in the wild.

SG104

**Protein clustering mediated by an internal RNA-Binding Region accelerates the CTCF target search mechanism**

A. S. Hansen; Biological Engineering, Massachusetts Inst Technology, Cambridge, MA.

**Protein clustering mediated by an internal RNA-Binding Region accelerates the CTCF target search mechanism**

Presenter: Anders S. Hansen; Department of Biological Engineering, MIT, Cambridge, MA

Mammalian genomes are very large. For a DNA-binding protein, this means that the number of off-target sites vastly outnumber the number of cognate and specific sites. How mammalian DNA-binding proteins overcome this challenge to efficiently locate their target sites is not well understood. Here we study this problem using CTCF as the model protein. CTCF is an 11-zinc finger DNA-binding protein, which regulates 3D genome structure by forming loop domains. Using live-cell single-particle tracking (SPT) of endogenously Halo-tagged CTCF in live mammalian cells we show that CTCF diffusion is highly anomalous and that CTCF is repeatedly trapped in small zones and that these zones correspond to CTCF clusters inside the nucleus. Deletion of an internal RNA-Binding Region (RBRi) in CTCF reduces CTCF clustering, and also abolishes CTCF trapping in zones. We develop a biophysical model called anisotropic diffusion through transient trapping in zones that can quantitatively explain our data. Functionally, we find that trapping of CTCF by CTCF clusters accelerates its target search, such that wild-type CTCF finds cognate binding sites ~2.5-fold faster than RBRi-del-CTCF. Overall, our results suggest a model where CTCF clusters concentrate diffusing CTCF proteins near their cognate binding sites, thus guiding them

SG105

**Heterochromatin organization and dynamics**

S. Sanulli; Stanford University, Palo Alto, CA.

DNA is wrapped around nucleosomes, forming chromatin chains that are further organized in three-dimensional assemblies. The architecture of these assemblies is crucial in determining cell transcriptional programs. Yet, the principles that underlie and regulate the architecture and organization of chromatin are poorly understood. I will present hydrogen-deuterium exchange, NMR, and mass-spectrometry data illustrating how HP1 proteins drive chromatin compaction into heterochromatin. I will propose a model for heterochromatin organization in which HP1 proteins couple chromatin compaction and phase separation by increasing the accessibility and dynamics of nucleosomes. I will further discuss the biophysical and biological implications of the proposed model in chromatin assemblies beyond heterochromatin.

SG106

**Cells tune nucleolar phase behavior in response to developmental and environmental change**

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Both eukaryotic and prokaryotic cells contain membraneless compartments that assemble through liquid-liquid phase separation. Some of these biomolecular condensates, such as stress granules and clusters of RNA polymerase, form transiently in response to stimuli. Others, like the nucleolus, are constitutive. However, even constitutive condensates are sensitive to developmental and environmental conditions. Here, we image live animals to characterize how the size, shape and material properties of the nucleolus change throughout the *C. elegans* life cycle. We previously found that nucleolar size scales with cell size during early embryogenesis and throughout larval development. We now show that this size scaling breaks down in late-stage embryos and starved dauer larvae. In addition, nucleoli become less spherical and less dynamic as worms age. A preliminary RNAi screen implicates a variety of processes, including transcription, mechanotransduction and signalling, that can regulate the phase behavior of the nucleolus. These results begin to unravel the active biological mechanisms through which cells tune the physical parameters governing condensate structure and, ultimately, condensate function.

SG107

**Differential regulation of mRNA transport during acute stress through phase separation of the nuclear mRNA export factor Nab2**

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Unidirectional transport of mRNA from the nucleus to the cytoplasm via nuclear pore complexes is an essential step in eukaryotic gene expression. Although factors involved in mRNA transport have been characterized, a comprehensive, mechanistic understanding of this critical process and its regulation is lacking. Here, we combine state-of-the-art real-time single RNA imaging and an inducible degron system to demonstrate that acute depletion of the budding yeast DEAD-box ATPase Dbp5 causes rapid nuclear accumulation of single labeled mRNAs in vivo and dramatic changes in nuclear dynamics of RNA export factors. In particular, the essential export factor Nab2 ceases to shuttle between the nucleus and cytoplasm and forms a gel-like structure throughout the nucleus. This potential phase-separation phenotype can be recapitulated in vitro, with Nab2 forming RNA-dependent liquid droplets, which dissolve in the presence of Dbp5. Intriguingly, nuclear Nab2 condensation also occurs in physiologically relevant stress conditions, where bulk mRNAs are globally retained in the nucleus. Importantly, stress-induced mRNAs can overcome nuclear retention to elicit a timely cellular stress response, suggesting that cells use selective retention in or release from nuclear condensates to re-wire mRNA export during stress. These findings establish a novel layer of gene expression regulation and provide key mechanistic insights into mRNA transport.

SG108
RNA aggregation in neurodegenerative disease
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Expansion of CAG trinucleotide repeats is associated with nearly 10 degenerative diseases including Huntington disease and several spinocerebellar ataxias. Sequences flanking the disease-associated CAG repeats exhibit unusually high GC-content, but their functional significance is not known. Previously, we have shown that transcripts containing primarily CAG repeats undergo liquid-liquid phase separation and are sequestered in the nucleus of the cell. Here, we report that the presence of GC-rich sequences upstream of these CAG-repeats facilitates the export of the RNA from the nucleus to the cytoplasm. When present in the cytoplasm, these transcripts undergo repeat-associated non-ATG (RAN) translation. The RAN translation products co-aggregate with the RNA, and induce cell death. Inhibition of translation prevents aggregation of the cytoplasmic RNA as well as cytotoxicity. Our findings directly demonstrate that RAN translation products are more toxic to cells than nuclear foci and suggest a role of cis-acting flanking sequences in mediating toxicity of repeat expansion RNAs.

SG109
Differential Surface Charge on the Plasma Membrane Polarizes Cells During Migration
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In migrating cells, components and activities of the signal transduction excitable network (STEN) such as phosphoinositides, Ras and PI3K self-organize on the plasma membrane and propagate as waves. Such self-organization of network molecules leads to the generation of activated (or ‘front’) and inactivated (or ‘back’) regions on the cell cortex, thereby establishing polarity and guiding cell migration. Although
biochemical interactions amongst these network components are often studied, the biophysical principle governing their self-organization on the membrane has remained unexplored. Our studies in Dictyostelium amoeba and RAW 264.7 monocyte/macrophage-like cells showed that negatively-charged phospholipids, including PI(4,5)P2 and phosphatidylserine, organize to the back region of the cell membrane, suggesting that it is more negatively charged than the front. Consistently, a polycationic surface charge sensor organized on the cell membrane in a similar pattern. To assess the role of the surface charge, we designed actuators to abruptly alter it. First, we transiently shielded the negative charge in the back region by optogenetically recruiting a polycationic peptide to the membrane, which resulted in de novo generation of localized protrusions in Dictyostelium and HL-60 neutrophil-like cells. Furthermore, shielding the surface charge by loading the cytosol with calcium ions resulted in increased protrusive activity all over the cell perimeter. Second, we increased the negative surface charge by recruiting polyanionic peptides. In RAW 264.7 cells, exposed to uniform chemoattractant, quietening of protrusive activity at the site of actuator recruitment led to and migration in the opposite direction. In MCF10A epithelial cells uniform membrane recruitment of the polyanionic peptide caused inhibition of Ras and ERK. Our study has uncovered a biophysical mechanism by which STEN molecules are arranged within phase domains on the plasma membrane of a migrating cell by virtue of the subtle difference in negative surface charge on the inner leaflet.

SG110
Degradative tubular lysosomes link pexophagy to starvation and early aging in C. elegans
D. Dolese, M. Junot, B. Ghosh, T. Butsch, A. Johnson, A. Bohnert; LSU, Baton Rouge, LA.

Organelle-specific autophagy directs the degradation of eukaryotic organelles under certain physiological conditions. Like other organelles, peroxisomes are subject to autophagic turnover at lysosomes. However, peroxisome autophagy, or “pexophagy,” has yet to be analyzed in a live-animal system, limiting knowledge on how this form of autophagy is regulated during the life of an animal. Here, we present a tandem-fluorophore pexophagy sensor that enables real-time tracking of peroxisome turnover in live Caenorhabditis elegans. We observe that pexophagy occurs at a population of non-canonical, non-vesicular lysosomes, which are morphologically dynamic and tubular in structure. Unexpectedly, we find that pexophagy is stimulated at tubular lysosomes not only during starvation, but also during early aging. This causes an early-age clearance of peroxisomes, which are subsequently restored in each new generation. These findings reveal new facets of peroxisome homeostasis relevant to animal aging and also challenge the prevailing perception of lysosome homogeneity in autophagy.

SG111
In vivo investigation of organelle dynamics in the liver using a novel fluorescent multi-organelle transgenic mouse model
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Organ function relies on fundamental biological processes modulated by external signals and coordinated at the tissue, cellular and subcellular levels. In agreement, defects in organelle activity and impaired inter-organelle communications have been identified as hallmarks of different illnesses including neurodegenerative, cardiovascular and metabolic diseases. The development of advanced fluorescence microscopy tools for in vitro studies has expanded our basic understanding of how organelles sense and respond to extracellular cues (e.g. nutritional signals). However, the intricate
regulation of these adaptive responses in the context of the multiscale architecture of organs is poorly understood. This gap in knowledge stems from the lack of readily available tools to simultaneously study multiple organelles in intact tissues. Here, we present a novel fluorescent multi-organelle transgenic mouse model that allows the non-toxic visualization of up to 8 subcellular compartments (mitochondria, peroxisomes, lipid droplets, plasma membrane, endoplasmic reticulum, lysosomes, Golgi, and the nucleus) in anesthetized mice or fixed tissue sections. Using this tool, we investigate how different organelles within the liver parenchyma respond to changes in nutrient availability. We observe that organelle distribution and dynamics are not uniform throughout the liver and follow distinct zonated patterns in normally-fed mice. These patterns dramatically change in response to overnight fasting or nutrient overload. Particularly, nutrient overload after a diet rich in fat and carbohydrates significantly alter the morphology, numbers, and distribution of organelles, some of which are consistent with known hallmarks of liver illnesses such as non-alcoholic fatty liver disease (NAFLD). Overall, the implementation of this novel fluorescent multi-organelle mouse model has allowed, for the first time, the simultaneous visualization of multiple subcellular structures in a living mammal. This tool not only allows the detailed investigation of liver biology at the tissue, cellular, and subcellular level, but also offers a unique opportunity to implement innovative multiplexing approaches to study organelle dynamics in different organs during health and disease states.

SG112

Motion of single molecular tethers reveals dynamic subdomains at organelle contact sites

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Membrane-bound organelles provide distinct compartments where incompatible biological processes can be separated from one another. However, biochemical reactions in these disparate environments must be coordinated to facilitate homeostasis, particularly in response to environmental changes. Sites of direct contact between the organelles play an important role in this process, serving as signaling hubs and locations of direct transfer for macromolecules. Mechanisms for regulating contact site structure and function remain enigmatic, but it seems likely to involve substantial heterogeneity and plasticity within the system. Numerous specific molecular tethers have been implicated in the process, but the small size and dynamic nature of contact sites have prevented understanding of their spatiotemporal regulation. Here, we use high-speed single molecule imaging to directly observe tethering and release dynamics of putative tethers in individual contact sites between the ER and mitochondria. We demonstrate the existence of structurally regulated subdomains within single contact sites by comparative imaging with 3D electron microscopy of vitreously-frozen samples. We show that distinct tethering molecules have unique patterns of motion, respond differently to nutrient availability, and can actively exclude one another from regions of high density. Thus, changes in the recruitment efficiency of individual tethers can support a change in contact site function without necessitating the breaking of one contact site and formation of another.
How Oncogenes Rewire the Cell

SG113
How BRCA mutations rewire stromal cells in the tumor microenvironment
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Tumors initiate through genomic alterations in cancer cells, and progress through reciprocal interactions with non-malignant cells in the tumor microenvironment (TME). Cancer associated fibroblasts (CAFs) are the most abundant cell type in the TME of most carcinomas. CAFs are genomically stable, and transcriptionally rewired by cancer cells to form heterogeneous subpopulations that play diverse and sometimes opposing roles in tumor progression and metastasis. It is well known that oncogenes rewire cancer cells, yet it is not known whether different mutations in the cancer cells lead to differential rewiring of CAFs and contribute to CAF heterogeneity. Moreover, the extent to which CAF rewiring is driven by genomic alterations in the cancer cells versus environmental stresses is not clear. To address these questions, we set out to characterize the landscape of CAF heterogeneity in BRCA WT vs BRCA cancers. Hereditary mutations in the tumor suppressor genes BRCA1 and BRCA2 are found in ~10% of breast cancer patients, and ~7% of pancreatic cancer patients. Single-cell RNA-sequencing in mice followed by multiplexed immunofluorescence of breast cancer patient samples revealed two major CAF subpopulations, the ratio of which is different between BRCA WT vs BRCA mutated patients, and correlates with disease outcome in BRCA mutated patients. In pancreatic cancer, RNA-sequencing and multiplexed immunofluorescence of patient samples revealed distinct CAF transcriptional signatures and stress-response activation patterns between BRCA WT vs BRCA mutated patients. Together, our findings portray distinct stromal compositions in BRCA-mutant and BRCA-WT tumors, suggesting that the genomic mutations in the cancer cells drive different stromal landscapes that should be targeted differently in the clinical setting.

SG114
Oncogene induced senescence in hematopoietic progenitors features myeloid restricted hematopoiesis and chronic inflammation
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Constitutive activation of BRAF-MAPK signaling pathway leads to the establishment of Oncogene-Induced Senescence (OIS), a powerful tumor suppressive mechanism for a variety of cancers. Activating mutations in the BRAF-MAPK pathway have been reported in histiocytoses, hematological inflammatory neoplasms characterized by multi-organ dissemination of pro-inflammatory myeloid cells; yet, the role of OIS in disease pathogenesis and persistent inflammation remains to be fully elucidated. Here, we generated a humanized mouse model of transplantation of human hematopoietic stem and progenitor cells (HSPCs) expressing the activated form of BRAF (BRAFV600E). All mice transplanted with BRAFV600E expressing HSPCs succumbed to bone marrow failure and multi-organ dissemination of aberrant mononuclear phagocytes within few weeks post-transplantation. In addition, we observed a myeloid-restricted hematopoiesis, resulting from a strong erythroid and lymphoid impairment of hematopoietic progenitors. At the basis of this aggressive phenotype, we uncovered the activation of a senescence program, characterized by growth arrest and a profound senescence-associated secretory phenotype.
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(SASP), which involved not only BRAFV600E-expressing cells but also non-mutated bystander cells. Mechanistically, we identified TNFα as a key determinant of paracrine senescence and myeloid-restricted hematopoiesis and showed that its inhibition dampened inflammation, delayed disease onset and rescued the hematopoietic defects in bystander cells. Our work establishes that senescence in the human hematopoietic system links oncogene-activation to the systemic inflammation observed in histiocytic neoplasms and supports the contention that accumulation of senescent cells may more broadly contribute to inflammatory disorders of the blood lineage.

SG115

Migratory Transitions and Oncogenic Transformation in Epithelial Cells are Controlled by the Threshold of the Ras/PI3K/ERK Excitable Network
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The Ras/PI3K/ERK signaling network is involved in controlling cell migration and oncogenic progression. There is a lack of systems-level understanding of how the complicated interactions and feedbacks within this network are intrinsically regulated, which largely explains the poor outcome of clinical practices that target various single components of the signaling network. These considerations prompted us to investigate whether the Ras/PI3K/ERK network in human epithelial cells is excitable, and the role of excitability in migratory transition and oncogenic transformation. We demonstrated that, first, traveling waves of Ras and PI3K activation propagate on the basal surfaces of several human mammary epithelial cell lines and drive protrusion formation. These waves can be guided by global and local growth factors, which correlates well with our computational simulation predictions. Second, the Ras/PI3K/ERK network exhibits key features of biochemical excitability, including annihilation of oppositely directed waves, all-or-none responsiveness, and refractoriness to repeated stimuli. Third, abrupt perturbations of phosphoinositide levels, and the activities of Ras, ERK, AKT, and mTORC2 change the properties of waves and protrusions. The positive and negative feedback loops within the network that underlie excitability are delineated. Fourth, the threshold is lowered in the cell with higher wave activity. These observations establish a systems-level mechanism controlling eukaryotic cell migration: cellular protrusions are controlled by an excitable Ras/PI3K/ERK network and growth factors guide cells by locally altering excitability. More importantly, we discovered that Ras transformation leads to de novo or increased wave activity, which contributes to increased protrusive activity of transformed cells. We prove these transformed cells have lowered threshold by showing their higher frequency of ERK pulses and higher sensitivity to EGF stimulus. Furthermore, the increased wave activity was strongly correlated with metastatic potential across a series of increasingly aggressive breast cancer cell lines (M1~M4 MCF10A cells). These suggest that the enhanced wave and ERK activities control cancer progression. Taken together, our studies suggest a novel view of oncogenic transformation as a shift to a lower threshold of the Ras/PI3K/ERK excitable network. This change in threshold is manifested by an increase in stochastic noise driven activities such as the number and the range of propagating waves and the frequency of ERK pulses. The lowering of threshold most likely leads to the increased migration, macropinocytosis, and proliferation of cancer cells and it possibly can be used to assess cancer severity as well as a target for intervention.
SG116
**Stress-adaptation in cancer: role of stress granules in KRAS-driven tumorigenesis**

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Oncogenic mutations in the KRAS gene occur with high frequency and are key drivers of some of the deadliest cancers (pancreas, lung, colon). One of the critical mechanisms through which oncogenic KRAS promotes tumorigenesis is the hijacking of stress-coping cellular processes. Our previous work identified that oncogenic KRAS enhances cancer cell fitness and survival under adverse condition through modulating the assembly of the stress-adaptive organelles termed stress granules. Here we delve into recent work that investigates the role of stress granules in KRAS-driven tumorigenesis. Data on the molecular mechanisms that determine stress granule formation in KRAS cancers, how stress granules modify cancer cell signaling, and potential stress-granule dependent molecular targets for the treatment of KRAS-driven cancers will be discussed.

SG117
**Metabolic rewiring by NRF2 in cancer**

G. M. DeNicola; Cancer Physiology, Moffitt Cancer Center, Tampa, FL.

Redox regulators are emerging as critical mediators of lung tumorigenesis. Notably, NRF2 and its negative regulator KEAP1 are commonly mutated in human lung cancers. These mutations lead to NRF2 accumulation and constitutive expression of NRF2 target genes, many of which are at the interface between antioxidant function and anabolic processes that support cellular proliferation. One such metabolic process is the uptake and metabolism of the amino acid cysteine, which is required for maintaining cellular redox homeostasis in both normal and transformed cells. We find that in the presence of abundant cysteine, NRF2 promotes its intracellular accumulation and engages the cysteine homeostatic control mechanism mediated by cysteine dioxygenase 1 (CDO1), which metabolizes cysteine to wasteful and toxic byproducts to deplete NADPH. Notably, CDO1 is preferentially silenced by promoter methylation in human non-small cell lung cancers (NSCLC) harboring mutations in KEAP1, the negative regulator of NRF2. These results demonstrate that not all NRF2-regulated metabolic alterations are favorable and that NRF2 activation results in metabolic liabilities that must be overcome during tumorigenesis. Indeed, NRF2 has recently been shown to deplete intracellular glutamate as a consequence of increased cystine uptake and glutathione synthesis by glutamate-cysteine ligase catalytic subunit (GCLC), rendering cells sensitive to glutaminase inhibitors. We find that under cysteine-limiting conditions, GCLC is instead protective against the iron-dependent form of cell death known as ferroptosis. Surprisingly, we find this is not a consequence of glutathione synthesis but due to a non-canonical function of GCLC, which prevents glutamate accumulation through the generation of alternative γ-glutamyl peptides that sequester glutamate. These results indicate that GCLC has an additional, non-canonical role in the protection against ferroptosis to maintain glutamate homeostasis under cystine starvation. Further, the metabolic rewiring induced by NRF2 impacts cells differently depending on the environmental nutrient conditions.
The role of antioxidant enzyme GSTA2 in the nucleolus of mutant fallopian tube epithelial cells

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High grade serous carcinoma (HGSC) is the most common ovarian cancer subtype and is characterized by a highly aggressive and genomically unstable phenotype. In a previous publication, we showed higher levels of GST (glutathione-S-transferases) isoenzymes in the fimbrial end of the fallopian tube relative to the ampulla. We suggest an upregulation of detoxification genes at an area normally bathed with cytotoxic factors is protected by GST’s that decrease the level of oxidative stress by conjugating glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) to carcinogens and chemotherapeutic drugs. We used patient derived fallopian tube epithelial (FTE) cells incubated with 50µM H2O2 for 10mins at room temperature to induce ROS, which was measured by fluorescence intensity change. Total γ-H2Ax (DNA damage marker) positive and negative FTE cells were expressed as a percentage of the total cell count for treated and untreated cells. A comparison of FTE tissues showed increased GSTA2 positive cells in the fimbria compared to the ampulla. There were significantly more GSTA2+ cells in the distal end of the fallopian tube (the high risk zone) compared to the proximal end. We probed FTE tissues for other GSTs and determined that GSTA 1-4 localized to different cellular compartments. GSTA2 localized within nucleophosmin positive compartments in the nucleus (nucleolus). FTE stably expressing mutant p53, which recapitulates early lesions of ovarian cancer, had higher basal levels of γ-H2Ax. γ-H2Ax localizes with GSTA2 in both nucleolus and non-nucleolus compartments of the nucleus. p53 modulates the activity of the RNA polymerases I and III. We observed that UBF, a nucleolar transcription factor, and GSTA2 co-localize in the nucleolus at the site of rRNA synthesis suggesting a role for GSTA2 in ribosomal biogenesis. FTE mutant p53 showed decreased co-localization of UBF and GSTA2 in the nucleolus. Our results suggest a role for GSTA2 in mediating the DNA damage response in fallopian tube epithelial cells. GSTA2 functions in the nucleolus to regulate rRNA synthesis therefore modulating ribosomal biogenesis and the cell’s ability to synthesize downstream antioxidants. The differential gene expression of GSTA2 in HGSC and fallopian tube tissues suggests a role in regulating the pathogenesis of ovarian cancer through detoxification mechanisms.

The Interplay Between Amino Acid Metabolism and Ferroptosis Sensitivity

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Cancer cells accumulate high levels of reactive oxygen species (ROS) and as a consequence are particularly sensitive to perturbation of intracellular antioxidant networks compared to non-transformed cells. The tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) is a crucial intracellular antioxidant required to prevent oxidative damage and ferroptotic cell death. From a large-scale chemical screen, we identified ATP-competitive inhibitors of mechanistic target of rapamycin (mTOR) kinase as potent suppressors of ferroptosis induced by cystine deprivation or inhibition of system x_c^-mediated cystine import. The protective effects of mTOR inhibitors are mediated in part through inhibition of protein synthesis, which increase the intracellular pool of cysteine available for de novo glutathione synthesis, thereby preventing the onset of ferroptosis. We have also uncovered a role of arginine in promoting ferroptosis that is independent of mTOR activation. These studies pinpoint the amino acid-dependent mechanisms that promote ferroptosis via mTOR signaling and other pathways.
SG120
The Macropinosome: An Oncogene-driven Organelle that Supports Metabolic Stress Tolerance
C. Comisso; SBP Medical Discovery Institute, La Jolla, CA.

Macropinocytosis has emerged as an important nutrient scavenging pathway that supports tumor cell fitness in a variety of oncopathological settings. By internalizing extracellular protein and targeting it for lysosomal degradation, this endocytic pathway functions as an amino acid supply route, permitting tumor cell growth and survival despite the nutrient-poor conditions of the tumor microenvironment. Here, we will present work from our laboratory that deciphers how Ras-mutant tumors are wired to integrate contextual metabolic inputs to regulate macropinocytosis, dialing up or down this uptake pathway depending on nutrient availability. We will discuss the signaling nodes that respond to intratumoral regional depletion of glutamine to increase levels of macropinocytosis. These results provide a mechanistic understanding of how nutritional cues can control protein scavenging in PDAC tumors.

Mechanisms and Regulation of Membrane Trafficking

SG121
Golgi and next-door neighbors - a comparative view of yeast, plant and animal cells
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The Golgi apparatus represents a core compartment of membrane traffic. Its apparent architecture, however, differs much among species, from unstacked and scattered cisternae in S. cerevisiae to beautiful ministacks in plants and invertebrates and further to gigantic ribbon structures in vertebrates. Considering the well-conserved functions of the Golgi, its fundamental structure must have been optimized despite seemingly different architectures. In addition to the central set of cisternae, the Golgi is usually accompanied by next-door compartments both on the cis and trans sides. The idea that trans-Golgi network (TGN) should be deemed as a compartment independent from the Golgi stack has emerged from studies of plant cells and is getting also extended to the cases of yeast and animal cells. On the cis side, the intermediate compartment between the ER and the Golgi (ERGIC) has been long recognized in mammalian cells, and its functional equivalent is recently suggested also for yeast and plant cells. We have been studying trafficking around the Golgi by the high-speed and super-resolution live imaging microscopy (SCLIM) we developed. In this symposium, I would like to show dynamic behaviors of the Golgi and its next-door neighbors from a comparative point of view among yeast, plant and animal cells and try to propose a common model of the structure and function of the Golgi.

SG122
The Molecular Organization and Cisternal Transport of the Golgi under the Light Microscopy
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The mammalian Golgi complex comprises a network of laterally linked Golgi stacks, which are further consisting of tightly adjacent and flattened membrane sacs called cisternae. The Golgi is one of the most important processing and sorting stations along the secretory and endocytic pathways. At the moment, we don’t completely understand the organization and function of the Golgi at the cisternal level. The
fundamental question regarding how cargos are transported and sorted within Golgi cisternae is still under debate. One of the greatest challenges in this field is to spatiotemporally resolve residents and transiting cargos among individual Golgi cisternae. Using the nocodazole-induced Golgi mini-stacks as a valid and simplified model for the native Golgi, we attempted to tackle this problem by developing new quantitative imaging tools or methods. The Golgi protein localization by imaging centers of mass (GLIM) employs the conventional light microscopy to quantitatively express the sub-Golgi localization as a numerical value. The resolution of GLIM is comparable or better than the immuno-electron microscopy. By GLIM, we generated a molecular map of the Golgi and quantitatively monitored the intra-Golgi trafficking of synchronized trafficking waves. Next, to directly visualize the en face and side view of the Golgi mini-stack, we developed methods to identify different views of mini-stacks by examining Giantin-staining patterns under the Airyscan microscope. En face view images uncovered that Golgi enzymes preferentially localize to the interior of the stack, whereas components of the trafficking machinery, such as Golgins and SNAREs, reside at the periphery of stack. Especially, at the medial and trans-Golgi region, enzymes and machinery components appear as the characteristic central disk and outer-ring in en face views. Using en face and side view averaging approaches, we spatiotemporally visualized the cisternal localization and progressive transition of synchronized trafficking waves. Together with GLIM, our data suggest that constitutive secretory cargos, such as VSVG, exit the Golgi at the trans-Golgi while the secretory targeting to the trans-Golgi network (TGN) requires special signals, which are possessed by TGN resident proteins.

SG123

**ER-to-Golgi protein delivery through an interwoven, tubular network extending from ER**

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Cellular versatility depends on the accurate trafficking of diverse proteins to their organellar destinations. Secretory proteins are concentrated and sorted in ER-derived structures called ER exit sites (ERESs) before their delivery to the Golgi in transport intermediates. The physical nature of the vessel conducting this ER-to-Golgi protein portage is unclear. We provide a dynamic 3D view of early secretory compartments in mammalian cells with unprecedented isotropic resolution and precise protein localization using whole-cell, focused ion beam scanning electron microscopy with cryo-structured illumination microscopy and live-cell synchronized cargo release approaches. Rather than vesicles alone, the ERESs were comprised of an elaborate, interwoven tubular network of contiguous lipid bilayers with a diameter of 360 nm. This receptacle was capable of extending microns along microtubules while still connected to ER by a thin neck. COPII localized to the exit-site neck region and regulated cargo entry from ER, while COPI acted more distally, escorting the detached, accelerating pearled tubular entity on its way to joining the Golgi apparatus. These findings support a model of ERES activity dependent on COPII- and COPI-induced membrane shape changes, allowing dynamic control of protein sorting and transport.

SG124

**Deciphering the Golgi logic circuit**

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The Golgi apparatus is often described as a set of ordered compartments, but those compartments have not been precisely defined. We prefer instead to view the Golgi as a set of maturing cisternae, with maturation driven by a molecular logic circuit that switches various membrane traffic pathways on and off. Our goals are: (1) Identify the membrane traffic pathways that operate at the Golgi. (2) Determine the recycling routes used by individual resident Golgi transmembrane proteins. (3) Characterize how the Golgi membrane traffic pathways are mediated by GTPases, vesicles, tethers, and SNAREs. We work with *Saccharomyces cerevisiae*, in which individual non-stacked Golgi cisternae can be followed by 4D microscopy. An exciting prospect is to answer the long-standing question of how resident Golgi proteins achieve a polarized distribution. We propose that the recycling pathway of a Golgi protein determines its maturation kinetics and therefore its distribution within the organelle. For example, proteins that recycle to the ER in one type of COPI vesicles should be found in the earliest cisternae, whereas proteins that recycle within the Golgi in another type of COPI vesicles should be found in later cisternae. To test this model, we are examining the trafficking and the maturation kinetics of Golgi proteins that recycle with the aid of COPI. Recently, we showed that the AP-1 clathrin adaptor is involved in a second intra-Golgi recycling pathway that is used by late Golgi proteins. This recycling pathway also involves the epsin-related Ent5 protein. By deleting both AP-1 and Ent5, we can force certain late Golgi proteins to recycle via the plasma membrane. Other proteins that cycle between the late Golgi and prevacuolar endosomes are unaffected. This experimental setup is allowing us to identify proteins that follow the AP-1/Ent5 pathway, and to test the prediction that those proteins all have similar maturation kinetics.

Parallel efforts are exploring mechanisms that operate during the early-to-late Golgi transition. Our hypothesis is that the Ypt6 GTPase activates tethers that capture recycling AP-1/Ent5 vesicles, which deliver a protein that turns on the Arl1 GTPase system. Ypt6 and Arl1 effectors then cooperate to capture vesicles recycling from prevacuolar endosomes. This analysis is illuminating a piece of the molecular logic circuit for the Golgi.

SG125

The relationship between Rab11-endosomes and the centrosome during embryonic cell divisions

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The final stage of cell division occurs after the cell’s genome is duplicated and segregated into two daughter cells. At this point, cytokinesis begins through the formation of an actomyosin based contractile ring that constricts at the equatorial plane of the cell creating a cytokinetic bridge connecting the two daughter cells. The bridge is then cleaved in the final step in cell division, abscission, when the endosomal sorting complexes required for transport (ESCRT) is recruited. Directed membrane transport into the cytokinetic bridge by Rab11-vesicles is required for ESCRT recruitment. However, how and when Rab11 REs transport into the bridge is unknown. We have identified that upon cytokinetic furrow ingression, Rab11 REs enrich at centrosomes and then unidirectionally transport to the cytokinetic bridge. During this process, both mitotic centrosomes maintain a population of Rab11 associated REs, and then asymmetrically reorient from the polar ends of the cell toward the cytokinetic bridge. Using Rab11 null cells and optogenetic oligomerization of Rab11-associated REs we identified a requirement for Rab11 in centrosome reorientation towards the cytokinetic bridge both in *in vitro* cell culture and in the zebrafish embryo. In addition, Rab11-loss resulted in transferrin receptor positive endosomes that are unable to track with the centrosomes during abscission. We propose that Rab11-endosomes regulates centrosome placement during pre-abscission to direct RE transport into the cytokinetic bridge for abscission completion.
ApoER2, the Reelin Receptor, is a Cargo for the Adaptor Protein AP-4: Implications for Exocytic Trafficking and Polarized Sorting of ApoER2 in Neurons

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AP-4 is a heterotetrameric complex that plays a role in selective cargo export from the trans-Golgi network (TGN) in mammalian cells. Mutations in the genes encoding each of the AP-4 subunits (AP4M1, AP4B1, AP4S1, AP4E1) cause a complicated form of Hereditary Spastic Paraplegia (HSP) referred to as "AP-4 deficiency syndrome" in humans. Clinical features in this disease include progressive spasticity, intellectual disability, microcephaly, cerebellar atrophy and seizures. These features suggest an important role for AP-4 in the development and function of the central nervous system. To date, very few cargos have been shown to be sorted by AP-4; these include the amyloid precursor protein (APP), the auxiliary transmembrane AMPAR regulatory proteins (TARPs), the autophagy protein ATG9, and the serine incorporator proteins SERINC-3/5. Herein we report the identification of a new AP-4 cargo, ApoER2/LRP8, which functions as the receptor for Reelin in neuronal migration during development and synaptic plasticity in the adult brain. Using the yeast two-hybrid system and co-immunoprecipitation assays, we found that the cytosolic domain of ApoER2 interacts with the μ4 subunit of AP-4 (encoded by the AP4M1 gene). Alanine-scanning mutagenesis identified a motif within the Mu4 tail and critical residues within μ4 that are responsible for the interaction. Using a synchronized ER-exit assay in HeLa cells and hippocampal neurons, we found that AP-4 KO delayed export of ApoER2 from the Golgi complex. We also examined the polarized transport of ApoER2 splice variants in relation to AP-4 in rat hippocampal neurons. Some ApoER2 splice variants include exon 19, which determines the presence of a proline-rich sequence in the cytoplasmic domain of the protein. We found that ApoER2 lacking exon 19 preferentially distributes to the somatodendritic domain. The full-length isoform is less polarized, indicating that the presence of the proline-rich sequence favors axonal distribution. Further experiments showed that the ApoER2 isoforms containing exon 19 specifically bind to μ4, suggesting that the interaction with AP-4 promotes axonal transport. Finally, we determined that some of the documented roles of Reelin on hippocampal neurons were affected in AP-4 KO neurons. Overall, our results suggest that some of the features of AP-4 deficiency could be explained by alterations in ApoER2 trafficking and Reelin signaling. Supported by Fondecyt 1200393

The Rab40 small GTPase subfamily regulates cell migration by modulating protein ubiquitylation

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One of the most fundamental questions in cell biology is how cells migrate through three-dimensional (3D) spaces. Rearrangement of the 3D extracellular matrix requires coordinated changes in membrane traffic, the actin cytoskeleton, adhesion dynamics, and the targeted secretion of matrix metalloproteinases (MMPs). However, the molecular machinery that governs these processes are not
fully understood. Our lab has identified the small GTPase Rab40b as a regulator of actin dynamics, targeted MMP secretion, and cell migration. The Rab40 family of small GTPases (consisting of Rab40a, Rab40al, Rab40b, and Rab40c in humans) are unlike any other small GTPase because they contain a C-terminal Suppressor of Cytokine Signaling (SOCS) box, a binding motif known to act as a substrate recognition module for the E3 Cullin5-RING-ligase (CRL5). Although the function of SOCS/CRL5 complexes has been well defined in several other proteins, this is not yet the case for the Rab40 family of proteins. It is critical to identify ubiquitylation targets of the Rab40/CRL5 complex and understand how they modulate cell migration. To this end, we have generated a SOCS box mutant that disrupts Rab40 binding to Cullin5 in order to study the importance of ubiquitylation by the CRL5 complex during cell migration. We have identified a set of Rab40/CRL5 substrates that are either mono- or poly-ubiquitylated. Our data suggests that localized ubiquitylation of a specific subset of target proteins is required for efficient 2D and 3D cell migration. Based on our data, we propose that Rab40 is a dual-functioning Rab GTPase, given its co-regulation of vesicular MMP trafficking as well as ubiquitylation of protein substrates, and such co-regulation plays a key role in driving 3D cell migration and invasion. Results gained from this study will broaden our scientific understanding not only of Rab GTPase function but will also help uncover novel machinery governing cell migration.

SG128

The Retromer Complex Regulates Centriolar Satellite Recruitment and Ciliogenesis

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Primary cilia are sensory organelles that coordinate a variety of signaling pathways, and defects in ciliogenesis result in a wide range of ciliopathies. Centriolar satellites are peripheral aggregates of centrosome/cilium protein complexes, and serve as key regulators of ciliogenesis, potentially by serving as reservoirs for centrosomal proteins. Early steps of ciliogenesis require ciliary vesicle formation accompanied by the removal of a distal end protein, CP110, from the mother centriole. This process is regulated by the endocytic regulatory protein Eps15 Homology Domain protein 1 (EHD1), and its interaction partner, Molecule Interacting with CasL-Like protein 1 (MICAL-L1). It remains unclear whether additional endocytic regulatory proteins are involved in ciliogenesis. In this study, we show that the retromer complex components, Vacuolar protein sorting-associated protein 26 (Vps26) and Vps35, as well as members of the BAR (Bin/Amphiphysin/Rvs) domain containing sorting nexin (SNX-BAR) protein family, SNX1 and SNX2, are required for ciliogenesis. In the absence of these proteins, CP110 fails to leave the mother centriole. Furthermore, we demonstrate an interaction between Vps26, Vps35 and CP110 by co-immunoprecipitation. Finally, we show that retromer depletion impairs the recruitment of centriolar satellites to the centrosome, potentially explaining the defective ciliogenesis. Taken together, our work reveals a novel role for the retromer complex in centriolar satellite recruitment and ciliogenesis.

SG129

Calcium-stimulated disassembly of focal adhesions mediated by an ORP3/IQSec1 complex

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Coordinated assembly and disassembly of integrin-mediated focal adhesions (FAs) is essential for cell migration. Many studies have shown that FA disassembly requires Ca\(^{2+}\) influx, however our understanding of this process remains incomplete. Here we show that Ca\(^{2+}\) influx via STIM1/Orai1 calcium channels, which cluster near FAs, leads to activation of the GTPase Arf5 via the Ca\(^{2+}\)-activated GEF IQSec1, and that both IQSec1 and Arf5 activation are essential for adhesion disassembly. We further show that IQSec1 forms a complex with the lipid transfer protein ORP3, and that Ca\(^{2+}\) influx triggers PKC-dependent translocation of this complex to ER/plasma membrane contact sites adjacent to FAs. In addition to allosterically activating IQSec1, ORP3 also extracts PI4P from the PM, exchanging it for phosphatidylcholine. ORP3-mediated lipid exchange is also important for FA turnover. Together, these findings identify a new pathway that links calcium influx to FA turnover during cell migration.

SG130

Characterization of Rab5 isoforms in NGF signaling and cell differentiation

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NGF signal transduction, via binding to its receptor TrkA, plays an important role in neuronal cell differentiation and survival. Using the PC12 cell model system, we previously reported that inactivation of Rab5 activity via expression of a dominant negative mutant dramatically increased NGF-mediated neurite outgrowth and cell differentiation, suggesting that Rab5-dependent entry to the endocytic pathway and degradation may reduce the strength or duration of NGF signaling for cell differentiation. However, the Rab5 mutant blocks all three Rab5 isoforms and it’s unclear whether a single isoform or multiple Rab5 isoforms may contribute to NGF signaling and cell differentiation. To address this issue, we have generated CRISPR knockout (KO) PC12 cell lines for each of the Rab5 isoforms (Rab5a, Rab5b and Rab5c) and characterized their response to NGF signal transduction and consequent neurite outgrowth. Our data show that only Rab5a KO can recapitulate the dominant negative mutant phenotype in promoting NGF signaling, neurite outgrowth and cell differentiation. Our working model is that Rab5a inactivation blocks NGF/TrkA entry to the endocytic pathway and late endosomes/lysosomes for degradation, consequently the NGF/TrkA-containing endocytic vesicles may become signaling endosomes to sustain NGF signaling necessary for cell differentiation.

SG131

GEF's regulation by membrane offers ways to modulate small GTPase signaling

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Ras-like small GTPases of the Arf subfamily orchestrate many aspects of traffic and cytoskeleton remodeling. As a consequence, Arf GTPases and their regulators have been implicated in numerous pathologies such as cancer, inflammation and infections. Yet, despite their significance, they remain difficult to target with small molecules. ArfGTPases are activated by specific guanine nucleotide exchange factors (GEFs), which stimulate their GDP/GTP exchange. Activation occurs within signaling platforms, whose assembly is tightly regulated by a precise choreography of protein-protein and protein-lipid interactions at specific membrane sites. Understanding in atomic detail how protein-membrane interactions shape small GTPases signaling is a big challenge in the field. By combining
biochemical, biophysical and structural approaches, we and others have previously shown how the membrane allows GEFs to reach optimal efficiency. We also uncovered a variety of ArfGEF regulatory mechanisms thanks to the reconstitution of GEF activity on artificial membranes. More recently, we have evaluated the importance of a membrane environment for accurately characterizing the biochemical properties of small molecules. We now describe a novel mode of inhibition whereby the small molecule Bragsin efficiently and selectively inhibits the ArfGEF BRAG2/IQSEC1 by altering the geometry of the GEF-membrane interaction. Bragsin efficiently inhibits Arf activation in cells and affect tumorsphere formation efficiency of breast cancer cells. Our study highlights a promising strategy for GTPase signaling modulation by small molecules that target protein-membrane interactions.

SG132

Rab33b regulates autophagy via a noncanonical rab binding protein

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Macroautophagy/Autophagy is an evolutionarily conserved “self-eating” process mainly to recycle or eliminate dysfunctional cellular organelles or proteins and has been associated with diverse human diseases, including cancer, neurodegeneration and pathogen infection. The formation of double-membrane phagophores that expand, enfold cytosolic cargo and finally close to form autophagosomes is the key process in autophagy, which requires two ubiquitin-like conjugation systems. Atg8 (in yeast) and its mammalian homologs MAP1LC3/LC3 (microtubule associated protein 1 light chain 3) proteins are conjugated to phosphatidylethanolamine (PE) lipid in a reaction controlled by the ATG12-ATG5-ATG16L1 complex. How the ATG12-ATG5-ATG16L1 complex is recruited to membranes is not fully understood. In this talk, I will show you how the Golgi-resident Rab33B regulate autophagosome formation through a noncanonical Rab binding protein (RBP). Crystal structures of Rab33B bound to RBP revealed a novel recognition mechanism and activation mechanism without nucleotide exchange. Fluorescence lifetime imaging microscopy (FLIM)/Förster resonance energy transfer (FRET), live cell imaging, correlative light and electron microscopy (CLEM), biochemical and cell biological analyses demonstrated a new mechanism for the recruitment of the ATG12-ATG5-ATG16L1 complex to phagophores by RAB33B, which is required for autophagosome formation.

The Physical Cell: Imaging and Manipulating

SG133

The multimodular lim domain protein testin recognizes stress fiber strain and contributes to cellular force generation

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Cells are complex machines exhibiting a plethora of mechanical behaviors, including division, movement and growth, which contribute to tissue development and homeostasis. It has been shown that mechanical signals (e.g. pressure, stiffness) are just as capable as biochemical signals of affecting cellular behavior. Cells sense these mechanical signals and convert them into biochemical signals in a process collectively called mechanotransduction. However, the molecular mechanisms underlying this process remain poorly understood. The actin cytoskeleton, in conjunction with its associated proteins, is thought to play a key role in this process via both stress fibers (SF) and focal adhesions (FA). These structures enable cells to generate forces and transmit them from the cytoskeleton to the extracellular
environment or vice versa. The family of LIM domain-containing proteins (LDP) have emerged as important mechanoresponsive cytoskeletal proteins. For example, both LDPs zyxin and paxillin have been shown to localize to SF strain sites. To gain insight into LDP mechanosensitivity and their potential contribution to cellular force generation and transmission, we investigated testin, another LIM domain and cancer-associated protein. Testin consists of an N-terminal CR (cysteine rich), central PET and three C-terminal LIM domains. When truncated, the N-terminal domains (CR and PET) recognize SFs whereas the LIM domains localize to FAs. When we induce strain in SFs, only the LIM domains and not the N-terminal half, recognize these strain sites. Interestingly, unlike zyxin, an individual LIM domain is sufficient for mechanosensitivity. Only SFs that are not fully severed show LIM domain recruitment suggesting they recognize a strain-induced conformational change in the actin filaments and not free barbed ends. Full-length testin, in contrast, is distributed throughout the cytoplasm and not capable of relocating to SF strain sites. This observation is possibly explained by testin’s ability to dimerize which potentially blocks the accessibility of the LIM domains and suggests testin’s mechanosensitivity is regulated. Using point mutations of tyrosines 111 (PET) and 288 (LIM 1), regions thought to associate during dimerization, we show that testin becomes mechanosensitive, enabling it to recognize SF strain sites. In addition, two cancer-associated testin mutants (E185K and E306K) similarly relocate to SF strain sites. Using Traction Force Microscopy, we then found that these mutations promoted cellular contractility. Together, our results show that testin recognizes local SF strain and indicate its mechanosensitivity is regulated and potentially linked to its involvement in cancer.

SG134

Integrin-dependent difference in force exertion correlates with actin cytoskeleton organization

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Integrins are mechano-sensitive, cell recognition and adhesion receptors that regulate cell differentiation, migration and the formation and maintenance of tissue architecture in all metazoans. Reported values of forces exerted by the cytoskeleton on integrin cytoplasmic domains and ligands vary widely, ~1 pN to >100 pN. Using double-stranded DNA-based tension sensors, we discovered that two types of integrins co-expressed in transformed foreskin fibroblasts require markedly different tension thresholds, <12 pN for integrin α4β1 and >33 pN for RGD-binding integrins, mainly αVβ1, to support stable cell adhesion and spreading. Remarkably, binding of α4β1 and RGD-binding integrins to their specific ligands on substrates, with enough rigidity, but not in solution, triggers the assembly of distinct actin cytoskeletal architectures. Actin assembled by α4β1 forms branched network in circularly spread cells, is in rapid retrograde flow, and exerts low forces. Actin assembled by RGD-binding integrins forms stress fibers anchored by focal adhesions at the ends in irregularly and elongated shaped cells, is in slow retrograde flow, and exerts high force. Our results demonstrate that RGD-binding integrins are specialized to exert high forces within focal adhesions contracted by myosin II. Their force-sensing mechanism may aid cells in finding attachment sites and forming architectures that are highly force-resistant including at myotendinous junctions.

SG135

Cellular and molecular force regulation by receptor-mediated interactions

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Mechanical and chemical properties of the extracellular environment regulate cell adhesion and migration. Designing materials and tools which allow the local control of cell-matrix and cell-cell adhesion is of great interest to address how specific receptor-ligand interactions affect cell mechanics. I will present the development of surface functionalization strategies to control integrin clustering and the generation of molecular and cellular forces at the interface during adhesion. I will also introduce the application of opto-chemical tools to control the assembly and disassembly of E-cadherin-mediated cell-cell junctions and their impact on collective cell dynamics.

SG136
Spatiotemporal control of talin-mediated mechanotransduction by inducible dimerization
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Talin is a key constituent of integrin-based focal adhesions (FAs). Talin serves as the structural and molecular backbone of FAs, physically connecting integrin with the actin cytoskeleton, and playing crucial roles in the sensing of the cell mechanical microenvironment. Characterisation of FAs by super-resolution microscopy and proteomics have revealed a complex hierarchy of structural and compositional organization. However, further mechanistic dissection of the dynamics of talin-mediated mechanotransduction remains challenging due to such complexity as well as the lack of precise molecule-specific control. In particular, although perturbations of force-generation and cell signalling by pharmacological or mechanical intervention are well established, the ability to control intramolecular force-transmission with molecular specificity has been relatively underdeveloped. To address this, we have generated a toolbox of engineered talin constructs in which chemically or optically inducible dimerizer modules such as FRB/FKBP, iLID, and LOVTRAP are inserted at specific sites between talin rod domains. These modules separate the key integrin and actin binding sites, thus allowing talin-mediated force transmission to be turned on by rapamycin, or turned on/off by blue light illumination, respectively. Using magnetic tweezer force spectroscopy, we showed that these dimerizer modules can support 5-10 pN force, thus withstanding tension being transmitted through talin in FAs. These highly modular constructs can be combinatorially applied to probe force transmission and functions of distinct talin domains. In talin-null background cells, we demonstrated that these switchable talins support FA formation, traction force generation, cell migration, as well as recapitulate actomyosin contractility-dependent FA maturation. Finally, we showed that intra-molecular force transmission through talin is required for the nuclear translocation of the transcription factor YAP and subsequent mechanosensitive transcription. In conclusion, these tools provide a spatiotemporally precise and versatile approach for direct and molecule-specific control of mechanotransduction, compatible with live-cell imaging, and generalizable to application in other force-transmitting proteins.

SG137
Mechanical forces and the nucleus: regulation of cell fate and integrity
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Cells are constantly subjected to a spectrum of mechanical cues, such as shear stress, compression, differential tissue rigidity, and strain, to which they respond to by engaging mechanisms of mechanotransduction. These forces function as important morphogenetic cues that are transmitted to
the nucleus to alter genetic programs. On the other hand, excessive mechanical stresses have the potential to damage cells and tissues. In my presentation I will discuss our recent research on how dynamic changes in chromatin organization in response to force change the mechanical properties of the nucleus and chromatin to prevent damage, as well how cells can adapt to differential force environments by changing their structure.

SG138  
**Measuring forces in 3D cellular structures: Mechanical regulation of epithelial cyst homeostasis and morphogenesis**  
**D. E. Conway, V. Narayanan; Virginia Commonwealth University, Richmond, VA.**

*In vivo* epithelial cells exist in two-dimensional (2D) monolayers and as three-dimensional (3D) structures (ducts, acini, or tubules). A number of *in vitro* studies have shown that epithelial cells, when grown in 3D environments, exhibit striking differences in cellular physiology as compared to 2D monolayers. Although a number of approaches have been used to measure and control force in 2D cellular systems, the mechanics of 3D cellular systems are more challenging to study. Using MDCK cysts as a model system, our group has sought to study mechanical forces in 3D by focusing primarily on approaches to measure and modulate forces at cell-cell adhesions and the nuclear LINC (Linker of Nucleoskeleton and Cytoskeleton). To measure forces in epithelial cysts, we used FRET-based tension sensors for E-cadherin, desmoglein-2, and nesprin-2, as well as directly measuring cyst lumen pressure. Interestingly, we showed significantly elevated forces on these proteins in 3D, as compared to 2D. This increase in force was a direct result of CFTR-generated osmotic pressure within the cyst lumen (on average 37 Pa). This pressure regulates both cellular proliferation and epithelial to mesenchymal transition (EMT). Following on this previous work we sought to understand how osmotic pressure and cellular contractility regulate epithelial morphogenesis, by studying HGF-induced epithelial tubulogenesis. Using inhibitors of CFTR activity, we have observed that decreased osmotic pressure enhances epithelial branching during tubulogenesis. Additionally, using an E-cadherin FRET tension sensor, we observed higher E-cadherin tension during tubulogenesis. These data suggest that both mechanical buckling and increased contractility can promote epithelial branching. My group has also shown that the nuclear LINC complex, the structure which connects the cytoskeleton to the nuclear lamina, is necessary for the stability of epithelial cysts. Using dominant negatives to disrupt the LINC complex, we have observed that the LINC complex is necessary for orientation of the mitotic spindle and symmetric cell division, both in 2D and 3D. Furthermore, loss of the LINC complex results in a mechanically destabilized cyst, resulting in lumen occlusion, as well as apical-basal polarity inversion. In some instances, we have observed rapid failure in the cyst, in which there is an inside-out movement of cells that results in cyst inversion. Taken together, our data indicate that mechanical forces are a critical component of organization and stability of 3D epithelial cell structures.

SG139  
**Chromatin Mechanics Dictates Subdiffusion and Coarsening Dynamics of Embedded Condensates**  
**D. S. W. Lee, N. S. Wingreen, C. P. Brangwynne; Princeton University, Princeton, NJ.**

DNA is organized into chromatin, a complex polymeric material which stores information and controls gene expression. An emerging mechanism for biological organization, particularly within the crowded nucleus, is biomolecular phase separation into condensed droplets of protein and nucleic acids.
However, the way in which chromatin impacts the dynamics of phase separation and condensate formation is poorly understood. Here, we utilize a powerful optogenetic strategy to examine the interplay of droplet coarsening with the surrounding viscoelastic chromatin network. We demonstrate that droplet growth dynamics are directly inhibited by the chromatin-dense environment, which gives rise to an anomalously slow coarsening exponent, $\beta \sim 0.12$, contrasting with the classical prediction of $\beta \sim 1/3$. Using scaling arguments and simulations, we show how this arrested growth can arise due to subdiffusion of individual condensates, predicting $\beta \sim \alpha/3$, where $\alpha$ is the diffusion exponent. Tracking the fluctuating motion of condensates within chromatin reveals a subdiffusive exponent, $\alpha \sim 0.5$, which explains the anomalous coarsening behavior and is also consistent with Rouse-like dynamics arising from the entangled chromatin. Our findings have implications for the biophysical regulation of the size and shape of biomolecular condensates, and suggest that condensate emulsions can be used to probe the viscoelastic mechanical environment within living cells.

SG140
Dissecting the intercellular forces shaping tissues

During embryonic morphogenesis, tissue shape arises from interactions between cells. In tissues, the spatial patterning of cellular surface stresses generated by myosins interplays with intercellular adhesions to yield complex shapes. Here, I will present recent work examining interactions between cells in vivo and in vitro in small and large cell aggregates. To study the interplay between cortical tension and intercellular adhesion, we examine the early C elegans embryo, in which clear mechanical differences exist between the different cell lineages that arise from asymmetric division in the one cell embryo. We characterise the temporal evolution of cortical and junctional tension in the 2- and 4-cell embryos. We investigate how changes in embryo morphology arise from dynamic mechanical and adhesive changes occurring during development. Using simulations and experimental perturbation, we explore the aggregate morphologies that arise from different combinations of junctional and cortical tensions in the 4-cell embryo. While most epithelia must withstand mechanical stresses without rupture, some embryonic epithelia must rupture to allow emergence of mature organs. In Drosophila leg imaginal disks, the peripodial membrane breaks to release the leg. As it ruptures, the peripodial membrane curls basally, indicating the presence of spontaneous curvature within the epithelium. Similar curling is observed in in vitro epithelia devoid of substrate and we investigate the biology and physics that give rise to monolayer curling. We show that the polarized distribution of myosin molecular motors along the cellular apico-basal axis gives rise to active torques and estimate their amplitude using micromanipulation approaches.

SG141
Mechanical Compartmentalization of the Intestinal Organoid Enables Crypt Folding and Collective Cell Migration
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Intestinal organoids capture essential features of the intestinal epithelium such as folding of the crypt, spatial compartmentalization of different cell types, and cellular movements from crypt to villus-like domains. Each of these processes and their coordination in time and space requires patterned physical forces that are currently unknown. Here we map the three-dimensional cell-ECM and cell-cell forces in mouse intestinal organoids grown on soft hydrogels. We show that these organoids exhibit a non-monotonic stress distribution that defines mechanical and functional compartments. The stem cell compartment pushes the ECM and folds through apical constriction, whereas the transit amplifying zone pulls the ECM and elongates through basal constriction. Tension measurements establish that the transit amplifying zone isolates mechanically the stem cell compartment and the villus-like domain. A 3D vertex model shows that the shape and force distribution of the crypt can be largely explained by cell surface tensions following the measured apical and basal actomyosin density. Finally, we show that cells are pulled out of the crypt along a gradient of increasing tension, rather than pushed by a compressive stress downstream of mitotic pressure as previously assumed. Our study unveils how patterned forces enable folding and collective migration in the intestinal crypt.

Wednesday, December 9, 2020

Covid-19 Infection: Deciphering Cellular Responses

SG143
Building a visual consensus model of the SARS-CoV-2 life cycle

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A significant and positive outcome of the COVID-19 epidemic is the speed at which many members of the biological research community have been able to redirect and apply their specialized skills towards an increased understanding SARS-CoV-2. The rapid pace of publication and sharing of data, however,
brings new concerns: what aspects of coronavirus biology are now well documented and understood, and what areas represent "black boxes" where there is inadequate research coverage or conflicting results? How trustworthy are the currently available data, and how close are we to having a consensus model of different stages of the SARS-CoV-2 life cycle? We have recently launched a project that takes a novel approach for enabling rapid and transparent communication of the research community’s knowledge about the SARS-CoV-2 life cycle. We are developing a web-based tool that combines a detailed animated molecular working model of different stages of the coronavirus life cycle with mechanisms for the research community to annotate and discuss aspects of the model. Users will be able to step through the animated life cycle model and annotate specific molecular elements, both in space and time. The annotations from the research community, which will be visible publicly, will serve to show shifts in our understanding of the viral life cycle due to emerging research, as well help to develop updated visualizations -- including competing hypotheses -- that can be shared back with the research community. Ultimately, this project will result in a visualization of consensus model(s) of the coronavirus life cycle which can inform research communities as well as the public on our current understanding of the molecular mechanisms of SARS-CoV-2 viral infection.

SG144

**Length and flexibility enable the extended intermediate of the SARS-Cov-2 Spike protein to capture host cell membranes**

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Entry of the SARS-CoV-2 virus, the causative pathogen of Coronavirus disease 2019 (COVID-19), is accomplished by surface spike (S) glycoproteins that fuse the viral membrane envelope with the host cell membrane. Following binding to the host cell ACE2 receptor, the trimeric S protein is thought to undergo a dramatic transition to a key extended intermediate (EI) driven by a “loaded-spring” mechanism, resulting in insertion of the FP into the target membrane. This EI-mediated harpooning is thought the first step in membrane fusion. However, direct experimental evidence for the EI is lacking and the factors determining its ability to capture target membranes is unknown. A major obstacle has been that computational modeling can presently offer relatively little due to time constraints: studying the action of the EI requires following molecular dynamics over long ms-s timescales, well beyond the scope of all-atom computational approaches. Hence it is vital to coarse grain to computationally study these processes. Here we used coarse-grained (CG) MARTIN and ultra-coarse grained (UCG) computational methods to examine the feasibility of the EI and the mechanisms of EI-mediated membrane capture. We constructed representations of the putative EI and measured its interaction with target membranes including the FP penetration depth, which we find is similar to that reported from all-atom simulations of the FP of influenza HA (Baylon et al., 2015). We construct the free energy FP-membrane landscape and we measured the FP pull out force. We measured the time for the EI to capture a host cell membrane for a range of viral-host membrane separations. An interesting conclusion is that critical to the ability of the EI to capture target membranes is its length and flexibility which endows it with a remarkably large capture volume. These quantitative results describe the first stage of S protein-mediated membrane fusion, and will help to assess antiviral strategies based on targeting of the S protein and the fusion process leading to viral entry.

SG145
The ACE2 SARS-CoV2 receptor and TMPRSS2/4 coreceptors localize to motile cilia of the respiratory tract and function during viral infections

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Coronavirus disease 2019 (COVID-19) is an ongoing pandemic infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-strand RNA virus. The high transmissibility and fatality rates for the virus have raised concerns worldwide. Patients with comorbidities including hypertension, diabetes, and pulmonary disease are highly represented among hospitalized patients with COVID-19 disease, suggesting risk factors for SARS-CoV-2 susceptibility. The ACE2 angiotensin-converting enzyme, a membrane-bound protease and regulator of the renin-angiotensin system (RAS), is a key viral receptor. ACE2 processes Angiotensin II, a hormone controlling vascular constriction and blood pressure, to Ang(1-7) the ligand for the Mas receptor, which counteracts these effects. ACE2 also regulates epithelial cells. The virus enters patients via the upper respiratory tract, a columnar epithelium, rich in multiciliated cells and dotted with mucus-secreting goblet cells. We investigated the expression and subcellular localization of the SARS-CoV-2 receptor ACE2 within the upper (nasal) and lower (pulmonary) respiratory tracts of healthy human donors. We detected ACE2 protein localized dramatically within the multicilia of airway epithelial cells, and by in situ staining of viral RNA, confirmed this to be an early subcellular site of SARS-CoV-2 viral entry during respiratory transmission. We further found that the TMPRSS2 and TMPRSS4 transmembrane proteases linked to the RAS system, and components of the ciliary adenylate cyclase system are also exquisitely ciliary in these tissues. Using patient samples, we found no evidence that the use of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs) increases ACE2 protein expression. Ongoing studies are addressing the importance of multicilia in the infection of epithelia with SARS-CoV-2, the effect of viral spike protein (RBD) binding to ACE2 on ciliary signaling, ciliary beating and endocytosis, the effect of ciliary inflammatory signals on viral entry, and the requirements for ciliary trafficking of ACE2/TMPRSS2/4. SARS-CoV-2 viral infection highlights critical aspects of the cell biology of multiciliated cells and a striking example of how cilia may recognize viruses and activate cellular signals akin to their role in sensory or neuroendocrine signaling.

Spying on viruses: translation and replication dynamics of single RNA viruses

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RNA viruses are among the most prevalent pathogens and represent a major burden for society. While RNA viruses have been studied extensively, very little is known about the processes that occur during the first several hours of infection due to a lack of sensitive assays. We developed a single-molecule imaging assay, virus imaging (VIRIM), to directly observe and quantify infection, translation and replication of individual RNA viruses in live cells. VIRIM uncovered a striking heterogeneity in replication dynamics between cells, and revealed extensive coordination between translation and replication of single viral RNAs. Furthermore, using VIRIM we identify the replication step of the incoming viral RNA as
a major bottleneck of successful infection, and identify novel host genes that are responsible for inhibition of early viral replication. Single-molecule imaging of virus infection represents a powerful tool to study virus replication and virus-host interactions, and may be broadly applicable to RNA viruses.

SG147

Open and Collaborative Science in the Age of the Pandemic: COV-IRT, the COVID-19 International Research Team

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The COVID-19 International Research Team (COV-IRT; www.cov-irt.org) is a multidisciplinary open-science nonprofit. The purpose of COV-IRT is to facilitate collaboration to rapidly improve our understanding of COVID-19, expedite the development of viable vaccine candidates and therapeutics against COVID-19, and improve diagnostic tests to monitor and predict disease pathogenesis and progression in COVID-19 patients. COV-IRT consists of approximately 200 scientists who span 11 countries and 75 institutions, including universities, independent research institutions, non-profit organizations, government agencies, NGOs, and industry. COV-IRT members have expertise across a broad range of scientific domains including biological, chemical, computational, and social sciences. Members include clinicians, bioinformaticians and computational biologists, proteomics and metabolomics experts, therapeutic biomarker experts, virologists, epidemiologists, machine learning and modeling specialists, as well as medicinal chemists, structural and cell biologists, computational and evolutionary biologists, neuroscientists, pharmacologists, and psychologists. This diversity equips COV-IRT to attack the global problem of the COVID-19 pandemic with an expansive set of approaches. COV-IRT is actively collecting and analyzing patient samples for identification of biomarkers and prognostic indicators and identifying and testing potential drug targets. COV-IRT is committed to open-science foundations, and is constantly welcoming new members and collaborators. COV-IRT shares all results and findings as soon and as widely as possible through publication, websites, social media, and recurring free and public symposia. Although COV-IRT is currently focused on COVID-19 research, the group demonstrates a vibrant model of open science and cross-institutional collaboration that serves as a working example for combating emerging pathogens and infectious disease worldwide. This presentation will showcase COV-IRT and provide a brief overview of all the cutting edge research being perform at this new nonprofit.

SG148

Hidden genomic diversity of SARS-CoV-2

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The COVID-19 pandemic has sparked an urgent need to uncover the underlying biology of this devastating disease. Though RNA viruses mutate more rapidly than DNA viruses, there are a relatively small number of single nucleotide polymorphisms (SNPs) that differentiate the main SARS-CoV-2 clades that have spread throughout the world. In this study, we investigated over 7,000 SARS-CoV-2 datasets to unveil both intrahost and interhost diversity. Our intrahost and interhost diversity analyses yielded three major observations. First, the mutational profile of SARS-CoV-2 highlights iSNV and SNP similarity, albeit with high variability in C>T changes. Second, iSNV and SNP patterns in SARS-CoV-2 are more similar to MERS-CoV than SARS-CoV-1. Third, a significant fraction of small indels fuel the genetic diversity of
SARS-CoV-2. Altogether, our findings provide insight into SARS-CoV-2 genomic diversity, inform the design of detection tests, and highlight the potential of iSNVs for tracking the transmission of SARS-CoV-2.

SG149

The Bioenergetics of Covid-19

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THE BIOENERGETICS OF COVID-19 Polypeptides generated by SARS-CoV2 have been reported to interact with a variety of mitochondrial proteins involved in oxidative phosphorylation (OXPHOS), mitochondrial protein synthesis, cytosol to mitochondrial protein import, and mitochondrial intermediary metabolism. This results in impaired mitochondrial function and activation of a pseudohypoxia state (1,2). We are investigating why it is important to SARS-CoV2 to inhibit mitochondrial bioenergetics and if intervention may offer therapeutic opportunities. The mitochondrion not only provides much of the cellular energy but also regulates the innate immune system. Mitochondrial activation of innate immunity is mediated by the oxidation of the mitochondrial DNA (Ox-mtDNA). The Ox-mtDNA is released from the mitochondrion where it binds and activates the inflammasome and to cGAS to activate the cGAS-STING interferon pathway. We are testing the hypotheses that by inhibiting OXPHOS, SARS-CoV2 increases mitochondrial reactive oxygen species (mtROS) production which oxidizes the mtDNA and activates the inflammasome which enhances viral dispersion. Other SARS-CoV2 polypeptides inhibit the interferon pathway thus limiting host cell antiviral functions. We further hypothesize that inhibition of mitochondrial biogenesis impairs lymphocyte blast transformation thus limiting the adaptive immune response. Finally, we hypothesize that mitochondrial inhibition is an important factor in the predisposition to the cytokine storm. We have shown that the T regulatory cells are oxidative while the T effector cells are glycolytic (3). By inhibiting OXPHOS T regulatory cell control would be diminished resulting in enhanced cytokine production. To address this integrated set of hypotheses, we are developing a system to test the toxicity of SARS-CoV2 functions in our mouse models harboring mtDNA-coded OXPHOS gene mutations and nDNA-coded mitochondrial antioxidant defense genes. 1. Gordon et al, 2020, PMID: 32511329; 2. Stukalov et al, 2020, bioRxiv. 2020:2020.06.17.156455. doi: 10.1101/2020.06.17.156455; 3. Angelin et al, 2017, PMID: 28416194.

SG150


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The COVID-19 pandemic has affected African American populations disproportionately in regards to morbidity and mortality. A multitude of factors likely account for this discrepancy. To elucidate whether levels of expression of genes implicated in COVID-19 vary in African Americans as compared to European Americans, we examine thousands of samples of existing RNA-Seq data and metadata from normal and diseased conditions. This analysis includes expression of all annotated genes as well as novel transcripts. Multiple genes integral to endocytosis, inflammation and reactive oxygen are differentially expressed, and these are the biological functions that are the most enriched among the differentially expressed genes. Expression of one gene, F8A2, is over 41-fold greater in African Americans compared to European
Americans in non-diseased liver. The F8A2 gene, like the F8A1 gene, encodes the HAP40 protein. This little-studied protein mediates early endosome movement in Huntington’s Disease. F8A2 expression is negatively correlated with F8A1. In addition, we identify multiple novel transcripts, representing potential de novo orphan genes, that are differentially expressed. These differences in gene expression patterns can establish prognostic signatures of cellular responses, and have critical implications for precision treatment of diseases such as COVID-19.

SG151
Defining the repertoire of cellular antiviral genes against pathogenic coronaviruses
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Coronaviruses (CoV) with high pathogenic potential for humans have emerged repeatedly over the last two decades, including severe acute respiratory syndrome (SARS-CoV) in 2003, Middle Eastern respiratory syndrome (MERS-CoV) in 2012, and most recently SARS-CoV-2 in 2019. The ability of RNA viruses to escape the cellular antiviral response is generally important to establish virus replication and cause disease, but very little is known of the identity and functions of specific host proteins with direct antiviral activity against CoVs. To identify so called host restriction factors, we employed a genome-wide CRISPR transcriptional activation (CRISPRa) screen in human cells infected with SARS-CoV-2 and MERS. This screen utilizes a nuclease-dead Cas9 gene fused to cellular transcriptional machinery in combination with 10 guide RNAs per gene targeting promoter regions. Gene expression is thereby induced from the endogenous promoter (~210,000 guides in total). To identify putative viral inhibitors, cells were infected with CoVs whose replication is cytopathic, and surviving cells were subjected to RNAseq. These approaches have identified a number of putative cellular inhibitors whose mechanisms of action are being determined. The identification of pan-CoV inhibitors may reveal therapeutic targets that can be exploited in the treatment of related, newly emerging viruses.

Epithelial Stem Cells

SG152
Defining the design principles and molecular mechanisms by which epithelial stem cells drive tissue expansion at single cell resolution
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Defining the design principles and molecular mechanisms by which epithelial stem cells drive tissue expansion at single cell resolution Cédric BlanpainWELBIO, Laboratory of stem cells and cancer, Université Libre de Bruxelles (ULB), 1070 Bruxelles, Belgium Stem cells (SCs) ensure tissue development, homeostasis and repair. We have recently developed new methods to study the cellular hierarchy and clonal dynamics that govern the development and homeostasis of different epithelia. Here, I will present new studies combining single cell lineage tracing, clonal analysis, proliferation kinetics, single cell transcriptional and chromatin profiling, as well as functional experiments in vivo to investigate the cellular and molecular mechanisms regulating tissue expansion and cell fate decision during development and repair. I will present the proliferation and clonal dynamics of skin stem cells in different conditions associated with tissue expansion including wound healing, mechanical stretch, postnatal development and tumorigenesis. Defining the mechanisms regulating tissue expansion during development and adult regeneration and understanding how these mechanisms differ from...
tumorigenesis will have important implications for regenerative medicine and cancer therapy. This work is supported by the ERC, WELBIO, HFSPO and the FNRS.

**SG153**

**Corneal stem cell dynamics revealed by 2-photon live imaging**

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The cornea consists of a stratified epithelium that lines the outermost surface of the eye. A significant challenge in elucidating the cellular mechanism of corneal homeostasis and regeneration is accessibility to the live limbal niche, where the bone-fide stem cells are proposed to reside. The limbal niche represents a transition zone between the two surface epithelia of the eye, namely the cornea and conjunctiva, whose interactions are poorly understood but believed to be important for corneal physiology and disease. Slow-cycling, label-retaining epithelial cells are also known to reside in the niche but their contribution to corneal homeostasis and regeneration has not been observed or tested directly *in vivo*. To address these important outstanding questions, we used 2-photon intravitral imaging to gain direct access to the intact limbal niche at high spatiotemporal resolution. With this approach, we first characterized the cellular organization and molecular properties of the limbus in the mouse eye. We then captured the cellular dynamics within the live niche, using genetically encoded markers and *in vivo* lineage tracing strategies. We demonstrate the definitive location and activity of long-lived stem cells and their transient progeny. By longitudinal live imaging we record the lifecycle of individual stem cells and their progeny to directly analyze their fates, and show that their location within the tissue can predict their differentiation status. This study demonstrates how a compartmentalized stem cell organization coordinates the homeostatic maintenance of the corneal epithelium.

**SG154**

**Temporal and spatial dynamics of stem cells in lung regeneration at single cell resolution**

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Stem cells undergo dynamic changes in response to injury to regenerate lost cells. However, the identity of transitional states and the mechanisms that drive their trajectories remain understudied. Using lung organoids, multiple in vivo repair models, single-cell transcriptomics, and lineage tracing, we find that alveolar type-2 epithelial cells undergoing differentiation into type-1 cells acquire pre-alveolar type-1 transitional cell state (PATS) en route to terminal maturation. For these studies, we developed a chemically defined and stroma-free organoid culture system that enables the generation of functional and distinct cell states encompassing alveolar stem cell expansion, maintenance, and differentiation. Transitional cells undergo extensive stretching during differentiation, making them vulnerable to DNA damage. PATS show an enrichment of TP53, TGFβ, DNA-damage response signalling, and cellular senescence. Gain and loss of function and genomic binding assays revealed a direct transcriptional control of PATS by TP53 signalling. Notably, PATS-like cells were accumulated in human fibrotic lungs, suggesting persistence of the transitional state in fibrosis. We uncovered that senescence can also occur as part of a normal tissue maintenance program, and can be derailed in human disease. Our study thus implicates a transient state associated with senescence in normal epithelial tissue repair and its abnormal persistence in disease conditions.
SG155

Neuroglian regulates Drosophila intestinal stem cell proliferation through enhanced signaling via the Epidermal Growth Factor Receptor

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The intestine of Drosophila melanogaster is an excellent system for elucidating how stem cell behavior is regulated under homeostatic conditions and as a consequence of aging or stress. Here we show that the (SJ) protein Neuroglian (Nrg) is expressed in in 'nests' of intestinal stem cells (ISCs) and enteroblasts (EBs) within the midgut. Although Nrg localized to the plasma membrane, our previous study indicated that SJJs are not present between ISC/EBs. Therefore, we hypothesized that Nrg plays another role in the Drosophila midgut. Generation of Nrg−/− ISC reveals that Nrg is required for ISC proliferation in young flies, and depletion of Nrg from ISCS/EBs was able to suppress intestinal dysplasia with age. Conversely, overexpression of Nrg in ISC/EBs was sufficient to drive ISC proliferation, leading to an increase in cells expressing ISC/EB markers. In addition, we observed an increase in EGFR activation, and genetic epistasis experiments revealed that Nrg acts upstream of EGFR in the midgut to regulate ISC proliferation. As Nrg function is highly conserved in mammalian systems, our work to characterize the role of Nrg in the intestine will likely have implications for the treatment of intestinal disorders due to altered ISC behavior, including colon cancer.

SG156

The PAR polarity complex promotes epithelial integrity during intestinal morphogenesis

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Epithelial tissues are comprised of adherent polarized cells that line organs and create selective barriers to the outside world. To do this, epithelial cells polarize along an apicobasal axis; apical surfaces face the lumen, and neighbors adhere via junctional complexes to form a continuous surface. Yet during development and homeostasis, epithelia face assaults on their integrity such as cell division, shape change, and physical forces. The developing C. elegans intestine provides an excellent in vivo epithelial model to study how epithelia overcome these assaults while maintaining barrier function and apical continuity. The intestinal primordium consists of 16 polarized cells with apical surfaces facing a central midline, the future lumen. Four cells divide following polarization, and the resulting 20 cells elongate and build an intestinal tube. Using live imaging, tissue-specific protein depletion, and genetic screening approaches, we are investigating how this polarized epithelium maintains apical continuity through mitosis and tissue elongation in vivo. To understand what happens to polarized features during mitosis, we live imaged cytoskeletal, polarity, and junctional proteins during intestinal cell divisions and observed that ACT-5/actin, apical PAR proteins, and the junctional protein HMR-1/E-cadherin remain localized during mitosis. In contrast, apical microtubules and their associated proteins are transiently lost during mitosis and returned to the apical surface after mitosis completes. This loss of apical microtubules appears coupled to the building of the microtubule-based mitotic spindle, suggesting a functional switch between these structures. Based on our localization findings, we hypothesized that PAR proteins act as a memory mark, directing the return of apical microtubules after mitosis. Using intestine-specific depletion of PAR-6/Par6 and PKC-3/aPKC, we found that the return of microtubules to
the apical surface is defective following mitosis, supporting our hypothesis. In addition, we found that intestine-specific depletion of PKC-3 or PAR-6 causes apical surfaces and junction proteins between neighboring cells to become discontinuous during elongation. The consequence of this discontinuity is larvae that die with edematous intestines that are unable to pass food. These experiments reveal a role for PAR proteins in maintaining epithelial integrity through mitosis and elongation, two critical steps in epithelial development and function across organisms. Funding: NIH K99GM13548901

SG157
Regulation of stem cell state reversibility by the niche
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Stem cells reside in specialized niches that are critical for their function. Upon activation hair follicle stem cells (HFSC) exit their niche to generate the outer root sheath (ORS), but a subset of ORS progeny returns to the niche to resume a SC state. We have established HFSC organoids that comprise of a mixture of HFSCs and ORS progenitors to study this niche-dependent regulation of stem cell fate reversibility. Our recent studies show that the ability of ORS cells to return to the SC state requires suppressing a metabolic switch from glycolysis to glutamine metabolism that occurs during early HFSC lineage progression. HFSC fate reversibility and glutamine metabolism are regulated by the mammalian target of rapamycin complex 2 (mTORC2) -Akt signaling axis within the niche. Deletion of mTORC2 in the epidermis results in failure to re-establish the HFSC niche, defective hair follicle regeneration, and compromised long-term maintenance of HFSCs. These findings highlight the importance of spatiotemporal control of SC metabolic states in organ homeostasis.

SG158
Desmosomes Pattern Cell Mechanics to Govern Epidermal Tissue Form and Function
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The epidermis is a stratified epithelium in which structural and functional features are polarized across multiple cell layers. This type of polarity is essential for establishing the epidermal barrier, but how it is created and sustained is poorly understood. Previous work identified a role for the classical cadherin/filamentous-actin network in establishment of epidermal polarity. However, little is known about potential roles of the most prominent epidermal intercellular junction, the desmosome (DSM), in establishing epidermal polarity, even though DSM constituents are patterned across the apical to basal axis. Here, we use a combination of 2D and 3D cell culture models with biochemical- and imaged-based approaches to test the hypothesis that DSMs and their associated intermediate filaments (IF) are key regulators of mechanical polarization in epidermis. Laser ablation and whole mount imaging analyses support an epidermal mechanical gradient: cells are under compressive forces in the basal layer and high tension in the suprabasal layers. Moreover, we present data suggesting that uncoupling DSMs and IF or specific targeting of apical DSMs through depletion of the superficial DSM cadherin, desmoglein 1 (Dsg 1), affects this mechanical gradient. In the basal layer, uncoupling DSMs from IF results in decreased compressive forces (assessed via laser ablation) and impedes epidermal stratification. Surprisingly, disengaging DSMs from IF also uncouples stratification from differentiation, accelerating the expression of differentiation markers including Dsg 1, desmocollin 1, and loricrin. We use a
biochemical approach to suggest these effects on differentiation are mediated through mechanosensitive pathways centering on the transcriptional regulator serum response factor (SRF) and the Epidermal Growth Factor Receptor family member ErbB2. In the superficial layers, tension is high and Dsg 1 expression peaks. Thus, we assessed the ability of Dsg 1 to polarize tension and TJs. Dsg 1 loss disrupts the polarized distribution of the tension-sensitive TJ component ZO1. Our data suggest Dsg 1 modulates TJs through the mechanosensitive kinase ErbB2, which is enriched in the TJ-forming layer. Dsg 1 loss disrupts this localization and decreases total and phosphorylated ErbB2. Finally, uncoupling the DSM/IF linkage or inhibiting ErbB2 significantly decreases epidermal barrier function as assessed by transepidermal electrical resistance experiments. As mechanical forces regulate cell behavior, our data suggest a model in which patterning of DSM components regulates epidermal polarity by orchestrating the distribution of mechanical inputs to drive morphogenesis and development of a life-essential barrier.

SG159
Mechanochemical control of epidermal stem cell divisions by B-plexins
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The precise spatiotemporal control of cell proliferation is key to the morphogenesis of epithelial tissues. Epithelial cell divisions lead to tissue crowding and local changes in force distribution, which in turn suppress the rate of cell divisions. However, the molecular mechanisms underlying this mechanical feedback are largely unclear. Here, we identify a critical requirement of B-plexin transmembrane receptors in the response to crowding-induced mechanical forces during embryonic skin development. Epidermal stem cells lacking B-plexins fail to sense mechanical compression, resulting in disinhibition of the transcriptional coactivator YAP, hyperproliferation, and tissue overgrowth. Mechanistically, we show that B-plexins mediate mechanoresponses to crowding through stabilization of adhesive cell junctions and lowering of cortical stiffness. Finally, we provide evidence that the B-plexin-dependent mechanochemical feedback is also pathophysiologically relevant to limit tumor growth in basal cell carcinoma, the most common type of skin cancer. Our data uncover a central role of B-plexins in mechanosensation to couple cell density and cell division in development and disease.

SG160
The translational repressor Brat constrains regenerative growth to ensure proper patterning after tissue damage in Drosophila
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How regenerating tissue undergoes repatterning and ensures replacement of the correct cell types is a key open question in regeneration biology. To answer this question, we used genetic tools to induce damage in *Drosophila*, and leveraged the power of *Drosophila* genetics to identify mechanisms that control patterning and cell fate after tissue repair. We damaged and induced regeneration in third-instar wing imaginal discs, and used the resulting adult wings to screen for mutants that had enhanced or reduced regenerative growth and/or impaired patterning after regeneration. This screen identified the translational repressor Brain tumor (Brat) as a regulator of both growth and patterning during regeneration. While *brat/+* wing discs regenerated better than controls, the resulting adult wings had disrupted wing margins. The enhanced regeneration in *brat/+* mutants was due to elevated expression of the WNT-family signal Wingless and the transcription factor Myc, which promote regenerative growth, as well as elevated expression of the secreted peptide Dilp8, which delays pupariation and metamorphosis. To understand why Brat acts to constrain regeneration by limiting expression of these pro-regeneration factors, we explored a link between enhanced regeneration and aberrant patterning. Interestingly, elevated Myc levels during regeneration also caused disruption of the wing margin. We determined that loss of margin cell fates was not caused by enhanced growth itself, but rather was specific to Myc overexpression. We propose that elevated expression of Myc targets such as the transcription factor Chinmo, which negatively regulates the margin cell-fate gene *cut*, disrupts patterning and alters cell fates in the *brat/+* regenerating tissue. Thus, by constraining expression of the pro-regeneration factor Myc, Brat prevents aberrant patterning of the regenerated structure.

SG161
**Bimodal function of chromatin remodeler *Hmga1* in neural crest induction and Wnt-dependent emigration**
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The neural crest is a vertebrate stem cell population characterized by multipotency, extensive migratory ability, and broad developmental potential. During gastrulation, these cells are specified at the neural plate border, as characterized by upregulation of the neural crest marker *Pax7*. Using single-cell RNA-seq, we identified several novel transcription regulators, including the chromatin remodeler *Hmga1*, that are highly expressed in newly emigrating chick neural crest cells. High resolution *in situ* hybridization shows that *Hmga1* expression precedes neural crest specification. Through a series of temporally-controlled CRISPR-Cas9-mediated knockouts, we uncovered two distinct functions of Hmga1 in neural crest development. During gastrulation, it plays an early role in *Pax7*-dependent lineage specification at the neural plate border. Importantly, ectopic expression of *Pax7* in an *Hmga1*-knockout background rescues neural crest specification. Later, in the dorsal neural tube, loss of *Hmga1* alters Wnt signaling, resulting in defects in cranial neural crest emigration and migration. Introducing activated β-catenin at the onset of delamination is sufficient to rescue these effects, thus establishing a second role for *Hmga1* as a Wnt activator at premigratory stages. Together, our results show that *Hmga1* functions in a bimodal manner during neural crest development, regulating specification at the neural plate border, and subsequently emigration from the neural tube via canonical Wnt signaling. Given that *Hmga1* is overexpressed in many invasive tumors, we propose that its control of epithelial-to-mesenchymal transitions of neural crest cells may be inappropriately redeployed during tumorigenesis.
SG162  
Cell sorting in *Hydra vulgaris* arises from differing capacities for epithelialization between cell types  
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*Hydra vulgaris* exhibits a remarkable capacity to reassemble its body plan from a disordered aggregate of cells. Reassembly begins by sorting two epithelial cell types, endoderm and ectoderm, into inner and outer layers, respectively. The cellular features and behaviors that distinguish ectodermal and endodermal lineages to drive sorting have not been fully elucidated. To dissect this process, we use micromanipulation to position single cells of diverse lineages on the surface of defined multicellular aggregates and monitor sorting outcomes by live imaging. Although sorting has previously been attributed to intrinsic differences between the epithelial lineages, we find that single cells of all lineages sort to the interior of ectodermal aggregates, including single ectodermal cells. This reveals that cells of the same lineage can adopt opposing positions when sorting as individuals or a collective. Ectodermal cell collectives adopt their position at the aggregate exterior by rapidly reforming an epithelium that engulfs cells adhered to its surface through a collective spreading behavior. In contrast, aggregated endodermal cells persistently lose epithelial features. These non-epithelialized aggregates, like isolated cells of all lineages, are adherent passengers for engulfment by the ectodermal epithelium. We find that collective spreading of the ectoderm and persistent de-epithelialization in the endoderm also arise during local wounding in Hydra, suggesting that Hydra’s wound-healing and self-organization capabilities may employ similar mechanisms. Together, our data suggest that differing propensities for epithelialization can sort cell types into distinct compartments to build and restore complex tissue architecture.

SG163  
From spikes to intercellular waves: tuning intercellular Ca²⁺ signaling dynamics modulates organ size control  
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Calcium (Ca²⁺) signaling is a fundamental mechanism for the propagation of information within cells and is necessary for proper cell function. However, the biophysical mechanisms that govern Ca²⁺ signaling dynamics between cells remain elusive. *Drosophila* wing imaginal discs are a premier model for studying conserved cell signaling mechanisms within the full organism context. Recent experimental studies in developing wing discs demonstrate the emergence of four distinct patterns of Ca²⁺ activity occurring on a tissue level. These include single cell Ca³⁺ spikes, intercellular Ca³⁺ transients, propagating tissue-level Ca²⁺ waves, and a global “fluttering” state. Here, we used a combination of computational modeling and experimental approaches to show that there are two different populations of cells in the wing disc pouch connected through gap junctional proteins. We term these two populations “initiator cells” and “standby cells.” Initiator cells initiate calcium signaling within the developing tissue and are outnumbered by standby cells. Initiator cells are predicted to exhibit higher levels of Phospholipase C (PLC) activity and produce more inositol trisphosphate, a key molecule that triggers the release of Ca³⁺ from the endoplasmic reticulum into the cytosol under agonist stimulation. We show that the strength of hormonal stimulation and the fraction of initiator cells jointly determine the predominant class of
Ca$^{2+}$ signaling activity in a tissue. Further, we demonstrate that single-cell Ca$^{2+}$ spikes are stimulated by insulin, while intercellular Ca$^{2+}$ waves are caused by stimulation of PLC as a result of G$q$ activity. Our model suggests that the fraction of initiator cells in a developing tissue scales in a power-law fashion relative to tissue size with the fraction of initiator cells in the population decreasing as tissue size increases. Phenotypic analysis of perturbations to G$q$ and insulin signaling supports an integrative model of Ca$^{2+}$ as an dynamic reporter of tissue growth, suggesting that Ca$^{2+}$ signaling dynamics help to tune the final size of organs. In sum, this study identifies important crosstalk between biochemical growth signals and heterogeneous cell signaling states during the growth of an organ to reach a final size.

**Evolutionary Cell Biology**

SG165

**Rigorous strategies for inferring homology in eukaryotic organelles**

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Organelles are defining features of eukaryotic cells, and have traditionally been classified based on their morphology. Recent studies indicate that organelle composition varies widely across eukaryotic phyla. Understanding their diversity and evolution of organelles is therefore of primary interest for the field of evolutionary cell biology. While it has become relatively straightforward to infer homology of individual genetic sequences amongst closely related organisms, the field of evolutionary cell biology lacks a well-defined and rigorous conceptual framework for determining whether organelles are homologous. Homology at the organelle level infers that their components are, in turn, homologous due to vertical inheritance. While ribosomes are clearly composed of homologous subunits, the compositional diversity of other organelles vary wildly. For example, the kinetochores of trypanosomes are composed of proteins with no obvious sequence similarity to kinetochores of other lineages. This diversity makes it exceedingly challenging to infer homology in organelles which may have dramatically different protein inventories between distantly related organisms. Using core principles of both evolutionary theory and cell biology, we have developed an intellectual framework for determining both “necessary” and “sufficient” conditions to infer organelle homology. Comparing the proteomes of candidate homologous organelles from diverse eukaryotes reveals that organelle inventories include both “core” proteins as well as “variable” proteins that are only associated with specific lineages. We propose that sufficient conditions for determining organelle homology should include specific “signature” proteins that are shared across phylogenetically distinct species. Presence of these signature proteins, then, can be used as a standard to evaluate the homology of that particular organelle. This framework is easily extended to molecular machines and protein networks, and has already been used to predict phenotypes in understudied organisms. We anticipate that the development of a standard of evidence for determining homology of complex cellular features will lay the foundation for a holistic understanding of the origins of eukaryotic organelles, particularly those whose phenotypes defy classical definition.

SG166

**Choanoflagellate Cilia Structures Revealed by Cryo-Electron Tomography**

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Most eukaryotic organisms possess motile cilia, which perform a variety of life-sustaining roles. Unicellular organisms rely on cilia for feeding behaviors and for avoiding predators, while multicellular organisms use cilia for a variety of functions, including mucosal clearance, developmental patterning, and reproduction. To date, motile cilia from less than a dozen species have been studied in high resolution using cryo-electron microscopy. Ultrastructural information is particularly lacking for unicellular organisms in the opisthokont clade, leaving a sizeable gap in our understanding of cilia evolution between unicellular and multicellular species. Choanoflagellates are important aquatic heterotrophs, uniquely positioned as the closest living unicellular relatives to animals (metazoans) within the opisthokont clade. We performed cryo-focused ion beam milling and cryo-electron tomography to analyze the ciliary ultrastructure of the choanoflagellate species Salpingoeca rosetta in unprecedented detail. We present high-resolution subtomogram averages of the S. rosetta 96-nm axonemal repeat and central pair complex, revealing strong similarities between metazoan and choanoflagellate structures with slight variations in microtubule features. Notably, S. rosetta cilia contain two rows of outer dynein arms and reduced radial spoke head morphology, more closely resembling metazoan cilia than those from other unicellular organisms. In addition, we report detailed structures of the S. rosetta basal apparatus, collar filaments, and ciliary vane: a fine, net-like extension that has been notoriously difficult to visualize using traditional methods. Our analyses also reveal previously undescribed mangrove-like structures attached to the extracellular surface of the ciliary membrane, the functions of which are currently unknown. We conclude that the reduction in opisthokont axonemal structures predated the common ancestor of choanoflagellates and metazoans. Our data suggest that functional differences between motile cilia from unicellular and multicellular opisthokonts are likely more influenced by microtubule and accessory structures than morphological variations in axonemal architecture. Together, our findings provide new insights into choanoflagellate biology and the evolution of cilia between unicellular and multicellular species.

SG167
A rapidly evolving actin-related protein localizes to germline cytoskeletal structures for roles in Drosophila male fertility
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Cytoskeletal proteins perform many fundamental biological processes in all eukaryotes and are highly conserved among most species. However, we have found that actin-related proteins (Arps) have undergone recurrent genetic innovation via gene duplication and positive selection (accelerated amino-acid substitutions) in both Drosophila and mammals. Unlike the well-conserved members of the Arp superfamily, divergent Arps appear to be testis-specific in expression, yet the function of a male gametic Arp in any species is unknown. Based on their recurrent emergence and rapid evolution, we hypothesized that testis-specific Arps play important roles in sperm production or fitness. To test this hypothesis, we investigated the function of Arp53D, a rapidly evolving Arp in the D. melanogaster testis. Arp53D is present in all Drosophila species, suggesting it performs a crucial function. Through cytological analyses, we found that Arp53D localizes to two critical germline-specific cytoskeletal structures in the testis: fusomes and actin cones. The fusome is an actin-enriched membranous organelle that connects all germ cells in a cyst in male meiosis. Actin cones are unique structures that separate syncytial spermatids post-meiosis into individual cells and push excess cytoplasm to the end of the sperm flagellar
We found Arp53D uniquely localizes to the leading edge of actin cones, and its non-canonical N-terminal tail is both necessary and sufficient for this localization. Based on these findings, we expected that loss of Arp53D would lead to a reduction in male fertility, yet surprisingly, Arp53D knockouts (KO) exhibit increased male fertility, which is more pronounced under conditions of stress. We discovered Arp53D-KO sperm are shorter than wildtype sperm, suggesting elongation defects, and surmise that shorter sperm allow for increased sperm production or storage, and thus more progenies. To address why Drosophila species would harbor a gene that appears to lower male fertility, we examined whether a fitness cost is associated with lack of Arp53D and found that the KO allele is readily outcompeted by the wildtype Arp53D allele in a population, suggesting Arp53D has been retained in all Drosophila species for a fitness advantage. Given our findings for Arp53D’s unique localization and impact on fertility, we hypothesize that recurrent specialization of Arps among many species may serve cytoskeletal functions in males and play important roles in promoting proper sperm elongation.

SG168
Conserved actin nucleators drive motility, phagocytosis, and osmoregulation in the evolutionarily divergent amoeba Naegleria
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Much of our current understanding of actin-driven phenotypes in eukaryotes has come from the "yeast to human" opisthokont lineage, the related amoebozoa, and plants. Outside of these groups lies the genus Naegleria, which last shared an ancestor with humans over a billion years ago, and includes the deadly "brain-eating amoeba." Unlike nearly every other known eukaryotic cell, Naegleria amoebae lack cytoplasmic microtubules. This suggests that Naegleria's basic cell functions are likely driven by actin. Using microscopy and flow cytometry, we probed the contributions of Naegleria's actin nucleators—the Arp2/3 complex and formin family proteins—to motility, phagocytosis, and osmoregulation. We found that despite ~1.2 billion years of evolutionary divergence, Naegleria amoebae use systems strikingly similar to animal cells; the Arp2/3 complex builds lamellar ruffles, which enable fast motility and efficient phagocytosis. Contractile vacuole mediated osmoregulation, however, is less dependent on the Arp2/3 complex. We show that under osmotic stress, impairing formin-derived networks prevents contractile vacuole pumping, causing cells to swell until they burst. This finding is particularly exciting, as contractile vacuoles could represent a much-needed drug target for the “brain-eating amoeba.” Collectively, our work supports an evolutionarily ancient origin for actin-based motility and phagocytosis, and establishes Naegleria as a model system for studying microtubule-independent cytoskeletal phenotypes.

SG169
The evolutionary diversity of eukaryotic cells, as seen with 2020 vision
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Fifteen years ago it seemed possible that the vast bulk of the major lineages of eukaryotes (e.g. groups as phylogenetically distinct as animals, or true fungi) had already been discovered and sampled using molecular approaches. Since this time, however, protistologists have characterised many ‘novel’ major lineages, at a rate approaching one per year. Almost without exception, these new lineages are various free-living protozoa, rather than algae, parasites, or multicellular forms, and most were discovered or rediscovered through cultivation methods rather than environmental molecular techniques. The
accumulated change to our understanding of the basic structure of the Eukaryote Tree of life has been substantial. Many more specialised groups of eukaryotes (e.g. algae, parasites, multicellular forms) are now placed more securely within their actual phylogenetic context, and while there are several important unresolved relationships among major eukaryote groups, there is a clearer list of living taxa appropriate to consider when inferring deep-level eukaryote cell evolution. Some currently poorly understood microbial eukaryotes, especially among the free-living protozoa, retain potentially ancient but no longer universal cell features that would be amenable to appropriately focussed evolutionary cell biological investigation. Conversely some features that are central to the biology of many free-living protozoan cells are highly heterogeneous, and would require broadly based comparative approaches for a general understanding to emerge.

SG170

**Evolutionary repair: Changes in multiple functional modules allow meiotic cohesin to support mitosis**

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The role of proteins often changes during evolution, but we do not know how cells adapt when a protein is asked to participate in a different biological function. We forced the budding yeast, *Saccharomyces cerevisiae*, to use the meiosis-specific kleisin, recombination 8 (Rec8), during the mitotic cell cycle, instead of its paralog, Scc1. This perturbation impairs sister chromosome linkage, advances the timing of genome replication, and reduces reproductive fitness by 45%. We evolved 15 parallel populations for 1,750 generations, substantially increasing their fitness, and analyzed the genotypes and phenotypes of the evolved cells. Only one population contained a mutation in Rec8, but many populations had mutations in the transcriptional mediator complex, cohesin-related genes, and cell cycle regulators that induce S phase. These mutations improve sister chromosome cohesion and delay genome replication in Rec8-expressing cells. We conclude that changes in known and novel partners allow cells to use an existing protein to participate in new biological functions.

SG171

**Non-Mendelian chromosome segregation of selfish *R2d2* locus in mouse oocytes**

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Mendel’s Law of Segregation states that each allele has an equal chance to transmit to the gametes. However, this law can be violated by selfish genetic elements, which manipulate the production of gametes to increase their own rate of transmission. This genetic cheating in meiosis, meiotic drive, is typically associated with fitness cost to the host and has significant impacts on genetics, evolution, and reproduction. In female meiosis, selfish elements bias their transmission by preferentially segregating to the egg. We focus on selfish *R2d2*, a non-centromeric locus on mouse chromosome 2, which shows over 90% transmission ratio distortion with mild embryonic lethality. The underlying cell biological basis for both biased segregation and the embryonic lethality is unknown. Here, we developed a strategy to track the *R2d2* locus by live imaging in mouse oocytes and found that the chromosome with *R2d2* locus lags in anaphase. Since *R2d2* locus is far apart from its centromere, we propose that this locus binds to spindle
SG172

**Multiple mechanisms lead to chromosome segregation defects in inviable Xenopus hybrids**

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Although central to evolution, the cell biological mechanisms underlying the barriers that drive reproductive isolation and speciation are poorly understood. Faulty chromosome segregation that leads to embryonic lethality has been reported in a number of inviable hybrids. This can be due to conflicts between rapidly evolving centromere sequences and the centromere-specific histone CENP-A, which is required for kinetochore assembly and chromosome attachment to the mitotic spindle. *Xenopus* frog species provide an ideal system to study the basis of this phenomenon, since cross fertilization experiments are easily performed, and mechanisms underlying hybrid incompatibility can be investigated in vitro by combining the sperm chromosomes and egg extracts of different species. We showed previously that hybrids produced when *X. laevis* or *X. borealis* eggs are fertilized by *X. tropicalis* sperm are viable, while the reverse crosses are not. Both inviable hybrids show loss of specific paternal chromosomes. Using the in vitro system, we found that a subset of *X. laevis* and *X. borealis* sperm chromosomes lose CENP-A upon passing through a single cell cycle in *X. tropicalis* egg extract. *X. laevis* chromosome centromere localization was fully rescued by addition of paternally-matched *in vitro* translated CENP-A with its chaperone HJURP to the extract. However, although microinjection of these proteins into embryos reduced mis-segregation and micronuclei, viability was not rescued. Interestingly, *X. borealis* chromosomes showed ultra-fine, stretched DNA morphologies in *X. tropicalis* extracts and CENP-A localization could not be rescued with paternal factors. Ongoing experiments address how inter-species conflicts in transcription and DNA replication contribute to centromere defects and chromosome mis-segregation in inviable *Xenopus* hybrids.

SG173

**Immune factor of bacterial origin protects ticks against host skin microbes**

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Hard ticks are blood-feeding arthropods that carry and transmit microbes to their vertebrate hosts. Tick-borne disease cases have been on the rise over the last several decades, drawing much-needed attention to the molecular interplay between transmitted pathogens and their human hosts. However, far less is known about how ticks control their own microbes, which is critical for understanding how zoonotic transmission cycles persist. We previously found that ticks horizontally acquired an antimicrobial toxin gene from bacteria known as *domesticatedamidase effector 2* (dae2). Using a combination of X-ray crystallography and biochemical assays, we show that this effector from...
the tick vector Ixodes scapularis (Dae2\textsuperscript{h}) has structurally and functionally diverged from ancestral bacterial representatives, expanding its antibacterial targeting range to include host skin microbes. Disruption of dae2\textsuperscript{h} increases the burden of mammalian skin-associated staphylococci within I. scapularis and adversely affects tick fitness, suggesting tick resistance of host microbes may be important for their parasitic blood-feeding lifestyle. In contrast, Dae2\textsuperscript{h} has no intrinsic ability to kill Borrelia burgdorferi, the tick-borne bacterium of Lyme disease. Our observations suggest that ticks have evolved to tolerate their own symbionts while resisting host skin commensals, which we discover are natural opportunistic pathogens of ticks. This work moves our understanding of vector biology beyond a human-centric view: just as tick commensals are pathogenic to humans, so too do our commensals pose a threat to ticks. These observations illuminate how a complex and mirrored set of interkingdom interactions between blood-feeding vectors, their hosts, and associated microbes can ultimately lead to disease.

SG174
Hemichordate development at single-cell resolution and cell-type evolution of the chordate ancestor
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Diverse cell types are generated over the course of both developmental and evolutionary timescales by unique combinations and trajectories of gene expression. While many developmental mechanisms for producing particular cell fates are increasingly well understood, it is less clear how novel cell types are generated, or how different animals produce similar cell types through differing developmental paths. Single-cell sequencing of well-studied organisms has provided unprecedented insight into the gene expression programs of cells through development and in discrete differentiated states. A similar approach in evolutionarily informative organisms can reveal how conserved developmental processes and pathways generate unique cell types in different organisms. Using droplet-microfluidics we have determined single-cell gene expression profiles across 5 developmental time-points in the hemichordate Saccoglossus kowalevskii. Hemichordates occupy a phylogenetic position key to discovering changes in gene regulation that may have contributed to the chordate lineage. For example, hemichordates do not possess a clear morphological central nervous system, yet express vertebrate brain organizing signaling centers. This system provides a unique opportunity for understanding evolutionary-scale principles for generating unique neural organization and cellular diversity from conserved genetic programs. Using our single-cell data, we can track germ layer segregation and identify increasingly diverse populations of cells through development. Previously known gene expression patterns have validated this developmental cell atlas and we have verified co-expression of previously uncharacterized genes in discrete cells and tissues. In addition, we have identified new cell-types, including lineage-specific diversifications of the ectoderm. To further insight into deuterostome cell type evolution, we are comparing our results to a well-curated developmental single-cell dataset for the vetebrate, Xenopus tropicalis. This comparison is being leveraged to investigate the developmental origins of vertebrate-specific cells as well as deuterostome-specific genes. Our data provide a novel resource as well as a powerful tool for insight into the developmental evolution of cell-type diversity.
Mapping single-cell atlases across the animal tree of life unravels cell type evolution
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The rapid accumulation of single-cell atlases from diverse organisms provides new opportunities to
investigate the origins of cellular diversity and facilitate the transfer of cell type knowledge between
species. However, recent comparative single-cell analyses are limited to closely related species within
the same phylum. Comparisons between distantly related species are thought to be prohibitively
challenging, owing to complex gene histories, lineage-specific inventions, and cell type evolutionary
diversifications. To overcome this challenge, we developed a new method capable of mapping single-cell
transcriptomes between phylogenetically remote species. To relax the tight constraints imposed by
sequence orthology, which are often relied upon for comparative studies, we instead use expression
similarity between mapped cells to refine the relative contributions of homologous genes which in turn
improves the cell type mapping. This approach accommodates evolutionary changes that usually blur
cell type interrelationships, such as gene duplications and functional substitutions among paralogs. We
mapped seven whole-body cell atlases from species spanning animal phylogeny from sponge to mouse.
Comparing well-characterized stages of frog and fish embryonic development, we found broad
concordance between cell type-specific transcriptomic signatures and their ontogenetic relationships,
highlighting a few exceptions where developmental and evolutionary lineages differ. In parallel, we
observed surprising prevalence of paralogs exhibiting greater expression similarity than orthologs across
species, resolving previously documented discrepancies between the conservation of protein sequence
and function. We also identified a surprisingly high degree of conservation in cell type identities
between a planarian flatworm and a parasitic blood fluke, two distantly related animals with highly
divergent body plans. Lastly, across phyla, we identified ancient interconnected cell type families with
conserved transcriptomic signatures, suggesting they may emerge early in evolution. Overall, these
findings represent a step towards understanding the evolutionary origins of specialized cell types and
their associated gene expression programs in animals.

Lineage dynamics of the endosymbiotic cell type in the soft coral Xenia
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Many corals harbour symbiotic dinoflagellate algae. The algae live inside coral cells in a specialized
membrane compartment known as the symbiosome, which shares the photosynthetically fixed carbon
with coral host cells while host cells provide inorganic carbon to the algae for photosynthesis. This
endosymbiosis—which is critical for the maintenance of coral reef ecosystems—is increasingly
threatened by environmental stressors that lead to coral bleaching (that is, the disruption of
endosymbiosis), which in turn leads to coral death and the degradation of marine ecosystems. The
molecular pathways that orchestrate the recognition, uptake and maintenance of algae in coral cells
remain poorly understood. Here we report the chromosome-level genome assembly of a Xenia species
of fast-growing soft coral, and use this species as a model to investigate coral-alga endosymbiosis. Single-cell RNA sequencing identified 16 cell clusters, including gastrodermal cells and cnidocytes, in Xenia sp. We identified the endosymbiotic cell type, which expresses a distinct set of genes that are implicated in the recognition, phagocytosis and/or endocytosis, and maintenance of algae, as well as in the immune modulation of host coral cells. By coupling Xenia sp. regeneration and single-cell RNA sequencing, we observed a dynamic lineage progression of the endosymbiotic cells. The conserved genes associated with endosymbiosis that are reported here may help to reveal common principles by which different corals take up or lose their endosymbionts.

Frontiers in Cytokinesis

SG177

Bacterial division proteins in *Bacillus subtilis* display two distinct sets of dynamics with stationary FtsZ binding proteins required for the condensation of Z rings essential for cytokinesis

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How proteins in the bacterial cell division complex (the divisome) coordinate to divide bacteria remains unknown. FtsZ polymers form a Z ring at future division sites and recruit cell-wall-synthesis enzymes that build a septum between daughter cells. FtsZ filaments treadmill around this division site; this treadmilling is required for the coincident motion of the cell wall synthesis enzyme Pbp2B around the division site, as well as efficient cell division. To understand how the division machinery collectively functions in *Bacillus subtilis*, we imaged the dynamics of single molecules of remaining divisome proteins using TIRF microscopy. These dynamics define two divisome subcomplexes: 1) a group that moves around the cell at the same velocity of Pbp2B, including the cell wall synthesis protein FtsW and the conserved but largely uncharacterized DivIB-DivIC-FtsL trimeric complex and 2) FtsZ binding proteins (ZBPs) that remain immobile, associated with stationary FtsZ monomers. To understand the function of these stationary ZBPs, we assayed FtsZ velocity and lifetimes and found that ZBPs have no effect on treadmilling dynamics. Rather, by removing synthetically lethal combinations of ZBPs, we found that ZBPs condense FtsZ into Z rings. We identified a mutant on a lateral surface of FtsZ that restores condensation and viability in one such synthetic lethal condition, suggesting that condensation is due to lateral bundling of FtsZ filaments. We find that this lateral bundling into condensed Z rings increases the recruitment of cell wall synthesis enzymes to the division site. Our results show that a subset of stationary divisome proteins allow essential changes in FtsZ superstructure, while another subset of directionally moving divisome proteins move collectively dependent on FtsZ treadmilling.
SG178

**Guiding the plant cytokinetic apparatus: essential motor and non-motor functions of phragmoplast orienting kinesin 2**

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In plant cytokinesis, the cytoskeletal phragmoplast mediates biosynthesis of the cell plate that bisects daughter cells. The phragmoplast and cell plate grow by centrifugal expansion, a process demanding coordinated microtubule turnover and endomembrane trafficking. Strikingly, this cytokinetic apparatus meets the parental wall at the cell cortex at a site, pre-defined before mitosis and transiently marked by the microtubule preprophase band. The preprophase band occupies temporarily the division site, a polarized membrane region that maintains positional information of the selected division plane throughout cell division. The Kinesin-12 phragmoplast orienting kinesin 1 and 2 (POK1/2) are core components of the division site playing pivotal roles in its maintenance. POKs arrive there in a microtubule-dependent manner and, following the disassembly of the latter, they remain tethered to the plasma membrane region delineating the future division plane throughout mitosis and cytokinesis and providing guidance cues for the approaching phragmoplast. Domain analysis showed that a C-terminal region of POK2 is sufficient to identify the division site and to fine tune POK2 accumulation at the phragmoplast. The present work aims at understanding the functional aspects of the POK-mediated cell plate placement. Specifically, we are interested in molecular requirements that allow POK2 to tether to the division site and navigate the phragmoplast during cytokinesis. Thus, we generated a series of deletion constructs of the C-terminal part and performed localization studies in tobacco BY-2 culture cells and in *Arabidopsis* seedlings. Deletion of the terminal region of POK2C abolished proper membrane targeting, emphasizing its importance for the association with the division site. Hydrophobicity profiling revealed the presence of a basic amino acid stretch within that region that appears to confer specificity for division site association. Importantly, when these modifications were introduced in the full length POK2 and expressed in plant cells, they also interfered with the localization pattern suggesting that the specificity of POK2 tethering to the plasma membrane requires electrostatic interactions. Furthermore, we mapped the region that is responsible for microtubule association of POK2C that seems to rely on binding partners, such as MAP65 or the division site resident TAN in an interesting interplay that requires further clarification. Nevertheless, motility-deficient mutants failed to rescue the severe pok1pok2 mutant phenotype. Collectively, these results highlight the functional significance of POK2 non-motor regions for targeting, and demonstrate that motor-dependent motility is a prerequisite in order to fulfill POKs’ guiding role in cytokinesis.

SG179

**Identification of lipids bound to membrane-associated cytokinesis proteins**

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As a cell divides, it must rearrange and separate its membrane-bound organelles as well as its plasma membrane to create equivalent daughter cells. Cells actively maintain complex and diverse lipidomes that encompass many thousands of lipids, which reside in these membrane-bound structures. We have shown that the lipid composition of dividing cells is altered relative to non-dividing cells. However, the
roles of these lipids remain largely unexplored. It is our hypothesis that specific interactions between lipids and proteins might contribute to their functions during cytokinesis. The first step to test this hypothesis is to determine if specific lipids are bound to cytokinetic proteins. We developed a technique to systematically explore protein-lipid interactions in cytokinesis. This involves detergent free lysis to obtain small membrane fragments from cells expressing the protein of interest bound to GFP, followed by GFP trap pulldowns and identification of bound lipids by liquid chromatography-mass spectrometry (LC-MS). We validated this approach in HeLa cell lines stably expressing Sec61B, a known ER transmembrane protein, Lact-C2-GFP, a phosphatidylserine (PS) binding protein, or TOMM20-GFP, a protein expressed on the outer mitochondrial membrane. All samples resulted in pulldown of expected lipids, validating our experimental setup. Many cytokinetic proteins are associated with the plasma membrane, including RACGAP1 and CHMP4B. We have identified which lipids specifically associate with these proteins and will next conduct functional studies.

SG180

**Actomyosin ring structure and its regulation by IQGAP during cytokinesis in yeast**

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Actomyosin ring (AMR) is known to drive furrow ingression and guide membrane deposition and ECM remodeling during cytokinesis in fungal and animal cells. However, its native structure and regulation remain poorly understood. Here we use immunogold labeling platinum replica electron microscopy (PREM) to study the native structure of the AMR and its basic units in budding yeast. We found that myosin-II was assembled into bipolar filaments that were further organized into a compact ring structure. During constriction, the ring became smaller not only by losing basic units but also by changing the folding pattern of the myosin tail. We also found that the assembly of the AMR is regulated by two distinct domains in Iqg1, the sole IQGAP in budding yeast. Deletion of the newly identified PAMA (Putative Activator of Myosin Assembly) domain causes defects in AMR assembly and constriction, leading to cytokinesis failure. The PAMA domain localizes to the division site in a myosin tail-dependent manner, suggesting that it may interact with myosin tail and regulate myosin assembly. In contrast to the PAMA domain, the minimum CHD (Calponin Homology Domain) localizes to the actin cables before cytokinesis and to the actin ring during cytokinesis. Deletion of CHD leads to defects in actin ring formation and decreases myosin accumulation and constriction during cytokinesis. The architecture of the AMR in the absence of PAMA or CHD will be examined by PREM in the near future. Taken together, our study reveals the organization patterns of the myosin filaments before and during AMR constriction, and defines the distinct roles of two IQGAP domains in the regulation of AMR assembly and function.

SG181

**Exploring the Functions of Two RNA-Binding Proteins within the Mechanoresponsive Network in Dictyostelium discoideum**

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Cell shape regulation is important for many biological processes, such as migration, differentiation and cell division. One of the major biological regulators of cell shape sits at the cell cortex, where the actomyosin meshwork, along with actin crosslinkers, control cell shape by regulating the structure of the meshwork and generating forces. Some components of the cortical meshwork are mechanoresponsive,
meaning that they can relocalize in response to external mechanical stimuli and trigger downstream reactions. Examples of these mechanoresponsive components in *Dictyostelium discoideum* include myosin and cortexillin I, an actin crosslinker. This mechanoresponsive property is critical during cytokinesis, when it assists with recruiting actomyosin contractile machinery to the cleavage furrow. 

Previously, to further understand the mechanoresponsive network, we pursued a mass spectrometry approach by immunoprecipitating cortexillin I and identifying its protein interactors. Interestingly, two potential RNA-binding proteins showed up as cortexillin I interactors, and they are named RNP1A and RNP1B, respectively. We had also discovered RNP1A through an expression cDNA library selection for genetic suppressors of nocodazole and found that RNP1A helps protect microtubule structures from nocodazole. In new work, knocking down RNP1A slowed cell growth on substrates and decreased adhesion. Knocking down *rnp1A* also decreased cortexillin I cortical accumulation, decreased cortexillin I mRNA and protein levels in cells. Cortexillin I also exhibited faster diffusion upon *rnp1A* knock down. 

These results collectively suggest RNP1A regulates cortexillin I on various levels and knocking down RNP1A changes cortexillin I dynamics in cells potentially via altering the components of the mechanoresponsive contractility kits, the building blocks of the mechanoresponsive network. The other RNA-binding protein, RNP1B, contains intrinsically disordered regions. RNP1B exhibited nuclear localization and puncta localization in the cytoplasm. Knocking out *rnp1B* slightly increased cell growth in suspension. In summary, RNA-binding proteins appear to play a role in regulating the mechanoresponsive network and thus cell shape, via regulating key mechanoresponsive protein concentration, localization, and dynamics.

SG182

**Ect2 and MPGAP drive the cortical excitability circuit**

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During cytokinesis in amphibian and echinoderm embryos, the cell cortex becomes excitable and spontaneously generates propagating waves of Rho activation and F-actin polymerization. This behavior, termed “cortical excitability”, operates under a reaction-diffusion model where Rho autoactivation hinges upon the Rho Guanine Nucleotide Exchange Factor (RhoGEF) Ect2, and Rho autoinhibition is mediated by a delayed, F-actin-dependent, feedback-loop. We report that M-Phase GAP (MPGAP/RGA3-4/ArhGAP11a), a Rho GTPase Activating Protein (RhoGAP), appears to play a role in regulating cortical excitability in both frogs and starfish. MPGAP displays excitable dynamics in both model systems and participates in Rho autoinhibition. Further, immature *Xenopus laevis* oocytes—which do not normally display cortical excitability—can be shifted into a high-amplitude, highly coherent, wave regime upon co-expression of both Ect2 and MPGAP, but not either protein on its own. Together, these findings support the model that balanced GEF and GAP activity is important for robust cortical excitability, and that Ect2 and MPGAP are likely major drivers of the system.
SG183

**Writhing of cytokinetic contractile rings reveals that the contractile ring is an elastoporous cable**

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We report extraordinary writhing dynamics of isolated fission yeast contractile rings, revealing basic material properties of the actomyosin ring normally hidden under physiological circumstances. Contractile rings exhibited cable-like writhing and coiling, with repeated twisting of the rings leading to multiple coils, similar to the twisting of a telephone cable. Our results suggest the twisting torque is produced by myosin-II in the ring that twisted around anchored actin filaments, and the twisting rates we measured are very close to those previously reported for myosin II-actin twisting from in vitro gliding assays (Nishizaka et al., 1993; Beausang et al., 2008). We show the twisting is quantitatively as would be predicted for a solid cable, and from the measured writhe versus ring length we extracted the effective ratio of ring twisting and bending moduli. A mathematical model quantitatively reproduced the cable-like twisting behavior, based on a continuum treatment of the contractile ring as an elastoporous solid, similar to an emerging view of the cell cytoplasm (Moeendarbary et al., 2013). The model predicts that after a short transient the ring deforms affinely, with constant twisting rate like a solid cable whose mechanical rigidity is set by its twisted actin filaments. Thus, our results show that the contractile ring, considered as a whole, is an elastoporous cable consisting of actin filaments and an effective fluid of myosin-II. The elastoporous ring cable is highly anisotropic, with anisotropic twisting properties along and perpendicular to its length. We observed these writhing rings in fission yeast cell ghosts, obtained by cell wall digestion followed by membrane permeabilization that releases cytoplasm while leaving membrane-anchored contractile rings behind (Mishra et al., 2013). The cell ghost is a laboratory allowing study of contractile rings in extraordinary and revealing circumstances (Wang and O'Shaughnessy, 2019). Writhing of rings occurred when entire sections of contractile rings become unanchored from the weakened membrane, leaving a short anchored section. The unanchored segment shortened and writhed, due to an apparent twisting torque at the anchoring points. Repeated rotation produced multiple coils in the rings. Our analysis revealing that the ring writhes as a solid cable was enabled by image analysis, which revealed 3D ring contours from which we found that the dependence of writhe on ring length was that of a solid cable with twisting to bending moduli ratio ~4, similar to the ratio from previous measurements of single actin filaments. The ring twisting rate was ~0.3 revs per µm shortened, consistent with the 0.25-2 revs per µm measured for myosin II twisting around actin filaments (Nishizaka et al., 1993; Beausang et al., 2008).

SG184

**Contractile ring component density dynamics influences contraction kinetics**

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Cytokinesis, the physical separation of one cell into two daughter cells, is required for cell proliferation and metazoan development. Cytokinesis failures can lead to aneuploidy and cancerous transformation. Completion of cytokinesis requires the formation of an actomyosin contractile ring, which is assembled around the cell equator in close association with the plasma membrane. The contractile ring matures from a wide band to a tight chord and shrinks down to a small fraction of its starting size, involving
significant changes in component organization, density and abundance during cytokinesis. We aim to define ring dynamics on the mesoscopic and molecular scales. Recent mathematical models have provided insight into potential restructuring events of actin and myosin, but the predictive power of these has been limited by various simplifications of non-muscle myosin II filaments and/or actin dynamics. We worked to bridge the gap between the theoretical models of cytokinetic ring components and quantitative cell biology by measuring component dynamics and incorporating those into models. To this end, we developed PDMS microfluidic devices to position *C. elegans* zygotest upright such that the contractile ring forms in plane with light sheet collected by a high N.A objective. Raw image sequences were segmented with the use of deep learning, and ring component intensity was measured over the length of cytokinesis. We then incorporated these data into a new agent-based model of cytokinesis where we simulated our recently developed complex load-dependent binding dynamics realistically depicting myosin filaments in Cytosim. Here we depicted 3-dimensional contractile rings and include a deforming component representing the plasma membrane. We modeled the contractile ring first as a simplified linearized ring structure in a periodic space that shrinks as contractile force is applied. These simpler rings showed different contraction dynamics as components were modulated. We next depicted more biologically relevant 3D contractile rings in a cylinder where a ring was built just beneath, and attached to, the deformable cylinder surface. In these simulations the cylinder radius shrinks as contractile force is applied. In simulations, including ring component density dynamics resulted in contraction dynamics more similar to *in vivo* measurements. Using this approach, we thus generated a new predictive model for cytokinesis.

**SG185**  
**Opposite Surfaces of the Cdc15 F-BAR Domain Create a Membrane Platform that Coordinates Cytoskeletal and Signaling Components for Cytokinesis**  
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Many eukaryotes assemble an actin- and myosin-based cytokinetic ring (CR) on the plasma membrane (PM) for cell division, but how it is anchored there remains unclear. In *Schizosaccharomyces pombe*, the F-BAR protein Cdc15 links the PM via its F-BAR domain to proteins in the CR’s interior via its SH3 domain. However, Cdc15’s F-BAR domain also directly binds formin Cdc12, suggesting Cdc15 may impact CR stability by polymerizing a protein network on the membrane. Here, we determined that the F-BAR domain binds Cdc12 using residues on the face opposite its membrane-binding surface. These residues also bind paxillin-like Pxl1, promoting its recruitment with calcineurin to the CR. Mutation of these F-BAR domain residues and subsequent disruption of the protein interaction network resulted in a shallower CR, with components localizing closer together and ~35% closer to the PM than in wild type. Additionally, cells displayed inefficient and aberrant CR constriction. This suggests that proper nanoscale spacing of components relative to the PM and to one another is required for CR function. Together these results demonstrate that an F-BAR domain can serve as a landing pad at the membrane for establishing protein interaction networks that in turn modulate the architecture of cytoskeletal elements from a distance.

**SG186**  
**Cdc42 GTPase promotes timely Rho1 activation and septum ingression during cytokinesis**  
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During cytokinesis, key steps ensure that the cytoplasm is properly partitioned, to yield two daughter cells after mitosis. It remains an enigma how cells precisely coordinate chromosomes segregation, actomyosin ring assembly, constriction, septum synthesis and cell separation during cytokinesis. Failure to coordinate cytokinesis results in a plethora of cellular defects, including cell death and improper ploidy. Here we use the model organism *Schizosaccharomyces pombe* (fission yeast) to understand the molecular details of how multiple cytokinetic events are coordinated. We and others have shown that distinct membrane trafficking events during different cytokinetic steps are essential for successful cytokinesis. It is unclear how these membrane trafficking events are spatiotemporally coordinated with different cytokinetic steps. Normally, fission yeast cytokinesis requires cell wall (septum) synthesis which drives actomyosin ring constriction. We have previously shown that, the GTPase Cdc42 is activated at the division site, as the ring assembles in early anaphase B. Cdc42 promotes membrane trafficking and delivery of at least one septum synthesizing enzyme Bgs1. However, Bgs1 is activated only in late Anaphase B after chromosomes segregate. Bgs1 catalytic activity depends on active Rho GTPase Rho1. Using a Rho bio-probe, we find that Rho1 is activated at the division site at onset of septum synthesis in late anaphase B. However, in the absence of Cdc42 activation, Rho1 is deactivated prematurely. Genetic evidence suggests that Cdc42 prevents premature Rho1 activation via its effector kinase Pak1. Indeed, in *pak1* mutants septum synthesis initiates prematurely likely due to early Rho1 activation. Earlier reports suggest that Rho1 activation depends on the septation initiation network (SIN/HIPPO) pathway. We find that the terminal SIN pathway kinase Sid2 is essential for Rho1 activation at the division site even in the absence of Cdc42 activation. We show that Sid2 localizes to the division site earlier than normal in Cdc42 activation mutants. Substantial work has shown that the SIN pathway coordinates spindle formation to cytokinesis. Our data suggest that Cdc42 prevents premature SIN and corresponding Rho1 activation at the division site. While Cdc42 activation promotes membrane trafficking and delivery of Bgs1 to the division site, its effector kinase Pak1 prevents premature septum synthesis. Thus, we find that a cross-talk between the GTPases Cdc42 and Rho1 coordinates membrane trafficking with septum synthesis during cytokinesis. We are currently investigating the molecular details of how Cdc42 via Pak1 regulates Rho1 activation.

**SG187**

**Ring canal formation in the Drosophila testis occurs via a midbody-like intermediate**

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Cytokinesis normally proceeds to completion at the end of mitosis. However, during male and female gametogenesis in various invertebrate and vertebrate species, cytokinesis is incomplete and occurs in the absence of abscission to yield cells connected by an intercellular bridge. These bridges, ring canals (RCs) in Drosophila, are membrane-attached cytoskeletal structures that stabilize the opening between cells to allow sharing of cytoplasm. RCs contain several contractile ring (CR) components suggesting that they are derived from the CR; however, the mechanism that transforms a CR into a RC is not known. Using time lapse imaging of GFP-tagged RC components during male germline RC formation, we have obtained new insight into the mechanism of its biogenesis. In contrast to proposed models of CR arrest, we find that RCs are formed from a midbody-like intermediate. Live imaging of RCs labeled with Pavarotti-GFP (Pav/MKLP1), a subunit of the centralspindlin complex and major component of RCs, shows that Pav-labeled cleavage furrows constrict to a dense midbody-like focus that resolves into an open ring over the course of one hour. We find that known midbody ring and RC components localize in
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a ring around the Pav-labeled focus, similar to the localizations of midbody ring proteins during cytokinesis. Interestingly, the midbody ring protein Citron Kinase/Sticky localizes to all Pav-GFP germline midbodies and nascent RCs, but is absent from mature, stable RCs suggesting a potential role for Sticky in the transition from midbody-like intermediates to open ring canals. Germline midbodies do not accumulate ESCRT-III/Shrub, the key regulator of abscission, and the transition from midbody to RC is not associated with microtubule severing or depolymerization, events normally associated with somatic midbody formation and function. Interestingly, the upstream ESCRT pathway component ALIX localizes to germline midbodies and nascent RCs, but not mature RCs suggesting a potential regulatory mechanism at this step to prevent membrane abscission. Taken together, our data suggest a model wherein components or protein modifications of germline midbodies facilitate RC formation. We are employing a combination of live imaging and proteomics approaches to continue our analysis of RC formation and to identify potential targets for future investigation.

SG188
A switch in the last step of cell division regulates the exit from naive pluripotency
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Cell fate transitions are key to development and homeostasis. It is thus essential to understand how different cellular processes affect fate transitions. Cell division has been implicated in fate decisions in many stem cells, including neuronal and epithelial progenitors. In embryonic stem cell, inhibiting cell division interferes with exit from naïve pluripotency, yet the mechanism for this has remained elusive. At the end of cell division, sister cells separate from each other via abscission, the process during which the membrane connecting daughter cells is cut. After anaphase, where the spindle separates the chromosomes, an acto-myosin ring drives the ingestion of the cleavage furrow. The plasma membrane forms a narrow bridge between the two sister cells and becomes anchored to the midbody, which contains proteins present at the centre of the spindle. Further narrowing the bridge allows the recruitment of ESCRT proteins, which mediate bridge abscission. Here we demonstrate that abscission dynamics accelerate during exit from naïve pluripotency, and that faster abscission facilitates pluripotency exit. Specifically, we report that while sister cells exiting naïve pluripotency separate in ~2h, similar to what has been described in HeLa cells, naïve sister stem cells remain connected and can exchange cytoplasmic material for a long time after division. Long abscission correlates with a delay in the recruitment of the ESCRT-III protein CHMP4B. Structurally, long abscission in Embryonic Stem cells is characterised by wide bridges and slow secondary constriction of the bridge, whereas short abscission happens after rapid bridge constriction. Interfering with abscission is detrimental to, and precocious bridge severing accelerates exit form naïve pluripotency. Interestingly, incomplete cell division with delayed or lack of abscission is a feature of many cell types during development of multicellular organisms, in particular multipotent cell types. We propose that delayed abscission is a conserved mechanism to allow communication and coordination between cells with high developmental potential.
**Organelle Cross Talk and Contact Sites**

**SG189**

**Lipid droplet proteome dynamics and regulation**

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Lipid droplets are endoplasmic reticulum (ER)-derived neutral lipid storage organelles that function as central hubs of cellular lipid and energy metabolism. Lipid droplets consist of a neutral lipid core that is encircled by a phospholipid monolayer. A unique set of integral and peripheral proteins, referred to as the lipid droplet proteome, associates the bounding monolayer and regulates lipid droplet functions. Deciphering the mechanisms that govern the composition of the lipid droplet proteome is paramount to understanding how lipid droplet functions are dynamically regulated under different metabolic states. Employing an APEX2 proximity labeling proteomic approach, we defined high confidence lipid droplet proteomes in multiple human cell lines. These studies identified novel lipid droplet proteins such as FSP1, which we recently discovered is a CoQ oxidoreductase that suppresses oxidative lipid damage and ferroptosis. Coupling our proteomic methods with inhibitors of the AAA ATPase p97/VCP allowed us to trap and identify a Class I lipid droplet protein as a substrate for proteasomal clearance by ER-associated degradation (ERAD), indicating a role for ERAD in regulating the lipid droplet proteome. To further understand the mechanisms of lipid droplet protein degradation, we engineered fluorescence-based reporter cell lines and performed a series of genetic screens using genome-wide and ubiquitination-focused CRISPR knockout libraries. These screens uncovered parallel compensatory ERAD pathways as candidate regulators of PLIN2 proteasomal clearance during lipolytic lipid droplet turnover. These results suggest that lipid droplet-localized PLIN2 traffics to the ER for clearance via ERAD during lipolysis, underscoring the high degree of crosstalk between the ER and lipid droplets throughout the lipid droplet life cycle. Together, our findings highlight the utility of global discovery approaches for characterizing the mechanisms of lipid droplet proteome dynamics and protein degradation pathways.

**SG190**

**Mitochondria-lysosome contact sites in cellular homeostasis and neurodegenerative disease pathogenesis.**

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Mitochondria and lysosomes are essential for maintaining cellular homeostasis, and dysfunction of both organelles has been observed in multiple neurodegenerative diseases. We recently found that mitochondria-lysosome contact sites dynamically form and are regulated by Rab7 GTP hydrolysis which promotes contact untethering. However, the multifaceted roles of these contact sites, and their contribution to disease pathogenesis remains to be explored. We found that mitochondria-lysosome contacts allow for lysosomal regulation of mitochondrial dynamics, including mitochondrial fission events and inter-mitochondrial contact untethering events. Using super-resolution and live-cell imaging, we have identified additional roles of mitochondria-lysosome contacts in regulating both mitochondrial and lysosomal homeostasis, and further demonstrated their contribution to the pathogenesis of neurodegenerative diseases. Together, our work highlights a key role for mitochondria-lysosome contact sites in modulating cellular homeostasis, and their dysfunction in various neurological diseases.
SG191

**VPS13 family proteins in the cross-talk between ER and mitochondria.**

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The evolutionarily conserved VPS13 protein family has been implicated in several cellular processes and mutations in each of the four human VPS13 paralogues are responsible for developmental disorders or neurodegenerative diseases, including Parkinson’s disease (VPS13C). However, until recently, the molecular function of VPS13 had remained elusive. Genetic, functional and structural studies have now revealed that VPS13 is a lipid transport protein that functions at contact sites between intracellular organelles and transport lipids by a novel mechanism: direct lipid transfer between bilayers via a hydrophobic channel that spans its rod-like portion. Its similarity to the autophagy protein ATG2 has further suggested a similar role for ATG2 that has been confirmed by structural and functional studies. The talk will provide an overview of the known properties of mammalian VPS13 proteins and potential disease mechanisms resulting from their mutations.

SG192

**Interplay mitochondria-lysosome regulates neuroinflammation in aging and disease**

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Lysosomal acidification is a key feature of healthy cells. Inability to maintain lysosomal acidic pH is associated with aging and neurodegenerative diseases. However, the mechanisms elicited by impaired lysosomal acidification remain unknown. We show here, using transcriptome analysis and biochemical follow-up, that inhibition of lysosomal acidification triggers cellular iron deficiency, which results in impaired mitochondrial function and necrotic cell death. These effects are recovered by supplying iron via a lysosome-independent pathway. Notably, iron deficiency is sufficient to trigger inflammatory signaling in cultured primary neurons. Using a mouse model of impaired lysosomal acidification (alpha-glucosidase knock-out mice, GAA-KO, a model of Pompe’s disease), we observed a robust iron deficiency response in the brain, confirmed by \textit{in vivo} magnetic resonance imaging. Furthermore, the brains of the GAA-KO mice present a pervasive inflammatory signature associated with instability of mitochondrial DNA (mtDNA), both corrected by supplementation of the mice diet with iron. The motor coordination defects observed in the GAA-KO mice are also reversible by dietary iron supplementation. Our results highlight a novel mechanism linking lysosomal acidification, mitochondrial function and inflammation \textit{in vivo}.

SG193

**Lysosomal nucleotide metabolism regulates ER proteostasis via mTOR signaling**

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Compartmentalized organelles require specific mechanisms to coordinate their cellular functions. In eukaryotic cells, lysosomes carry a variety of hydrolases that break down biological materials delivered from extracellular or intracellular space through endocytosis or autophagy, respectively. At the same time, lysosomes also serve as a hub to organize signal transduction and nuclear transcription. Lysosomal metabolism and its associated signaling mechanisms play crucial roles in health, diseases and aging. On
the other hand, endoplasmic reticulum (ER) is essential for protein synthesis and utilizes different quality control mechanisms to maintain proteostasis. To date, it remains poorly understood how lysosomal metabolism actively regulates ER proteostasis. Here we discovered that a NADPH phosphatase, RSH-1 is localized to the lysosome. Its loss-of-function mutation protects animals against ER stress-induced lethality. We further discovered that this ER stress tolerance requires lysosomal v-ATPase, Rag GTPase and S6 kinase (S6K) in mTOR signaling. Active phosphorylation of S6K is increased in the rsh-1 mutant and mediates a transcriptome change through specific nuclear hormone receptors, leading to improved ER proteostasis. Together, these findings reveal a specific lysosomal nucleotide hydrolase and its key role in regulating the lysosome-ER crosstalk, and suggest that dysfunction in signal transduction and organelle coordination may underlie lysosomal diseases related to hydrolase deficiency.

SG194
Ribosome-Associated Vesicles: a dynamic sub-compartment of the endoplasmic reticulum in secretory cells

The endoplasmic reticulum (ER) is a highly dynamic network of membranes. Here, we combine live-cell microscopy with in situ cryo-electron tomography (cryo-ET) and correlative light and electron microscopy (cryo-CLEM) to directly visualize ER dynamics in several secretory cell types including pancreatic beta cells and neurons under near-native conditions. Using these imaging approaches, we identify a novel, mobile form of ER, Ribosome-Associated Vesicles (RAVs), found primarily in the cell periphery, that is conserved across different cell types and species. We show that RAVs exist as distinct, highly dynamic structures separate from the intact ER reticular architecture that interact with mitochondria via direct intermembrane contacts. These findings describe a new ER sub-compartment within cells.

SG195
Characterizing a novel tethering machinery enabling the contact site between mitochondria and the nucleus
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Mitochondrial functions are crucial for cellular homeostasis. The biogenesis, quality control and activity of mitochondria are tightly regulated by the nucleus. On the other hand, the nucleus receives essential molecules (such as acetyl-coenzyme A, ATP and iron-sulfur-clusters) from mitochondria. Thus, extensive communication between the two organelles must exist. One way by which two organelles can
communicate is through membrane contact-sites, areas of close apposition, actively held together by tethering proteins. However, whether the tight crosstalk between mitochondria and the nucleus can occur via these contact-sites was not previously described in yeast. Using fluorescent and electron microscopy in the yeast Saccharomyces Cerevisiae, we demonstrate areas of contact between the two organelles in vivo. Excitingly, we show that these metabolically-regulated contact-sites, are distinguishable from contact-sites that occur between mitochondria and the Endoplasmic reticulum (ER), as they are facilitated by different machineries. Using high throughput screening techniques, we identified multiple resident proteins. We demonstrate that one of them acts as a novel tether for this contact, increasing contact extent dramatically when overexpressed. Since mitochondrial functions rely on efficient communication with the nucleus, highlighting the extent of this communication through contact-sites is crucial for better understanding mitochondrial functions in health and disease.

SG196
Loss of mitochondria-plasma membrane tethering adversely impacts organelle function and cellular fitness
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To ensure cell survival, the processes that maintain cellular homeostasis and fitness must be coordinated and tightly regulated. To coordinate these processes, the membrane-bound organelles that compartmentalize distinct cellular activities must communicate with each other or the plasma membrane (PM). This communication is facilitated by membrane contact sites (MCSs), which position organelles in close proximity without membrane fusion and mark sites of critical activities necessary for proper organelle function, such as the exchange of molecules. Thus, the proper positioning of organelles and the contacts they make are deeply connected to the ability of these organelles to engage in their essential functions. Despite their importance to cellular fitness, the molecular mechanisms and regulation of MCSs remain unclear. Our research seeks to gain insight into the molecular mechanisms that allow MCSs to integrate critical cellular functions through the study of Num1, the core component of the Mitochondria-ER-Cortex-Anchor (MECA). Num1 has dual functions: (1) tethering mitochondria to the PM and (2) tethering the dynein motor protein to the PM during nuclear inheritance. Our research suggests that Num1 serves as more than a physical tether for mitochondria but is important for maintaining proper functioning mitochondria. Specifically, loss of Num1 results in a growth defect when cells are forced to respire, which requires functional mitochondria. Consistent with a defect in mitochondrial function basal oxygen consumption is reduced. These mitochondrial defects are not due specifically to the loss of mitochondrial morphology or defects in the formation of respiratory complexes, and the growth defect is separate from Num1’s role in nuclear migration. Using a synthetic mitochondria-PM tethering system, our data demonstrate that mitochondrial function can only be rescued by the membrane binding domains of S. cerevisiae Num1 and no other mitochondrial or PM binding domains. These data suggest Num1 does more than simply position mitochondria; instead Num1’s membrane binding domains form a unique functional interface between mitochondria and the PM that maintains mitochondrial and cellular function.

SG197
Mechanism of lipid droplet/mitochondria contacts and role of Perilipin 5 in lipid metabolism
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The lipid droplet (LD) serves as a cellular lipid store; however recent work has highlighted the ability of LDs to regulate intracellular lipid trafficking. Perilipins are LD proteins which function as lipolytic barriers and protect the cell from lipotoxicity as well as excess reactive oxygen species. A recently characterized member of the family, Perilipin 5 (Plin5), is enriched in β-oxidizing tissues where it serves as a lipolytic barrier under basal conditions. Upon protein kinase A (PKA)-stimulated phosphorylation, however, Plin5 promotes lipolysis through recruitment of the lipase ATGL and its activator CGI-58. In addition to playing a role in lipolytic control, Plin5 contains a unique C-terminal domain which has been shown to promote contacts between LDs and mitochondria (Mito). However, the mechanism by which this occurs and the physiological significance of these contacts remains to be elucidated. Utilizing predictive modeling we have identified and generated constructs containing a minimal contact domain capable of inducing LD/Mito contacts. Overexpression of Plin5 lacking this contact domain (Plin5 CΔ) leads to accumulation of lipid droplets to the same degree as full-length Plin5, indicating that LD/Mito contacts are not required for the lipolytic barrier function of Plin5. Affinity purification-mass spectrometry utilizing Plin5 and Plin5 CΔ constructs indicates a role for previously unidentified protein interactors to mediate LD/Mito tethering. To assess the physiological roles of Plin5’s functional domains in fatty acid (FA) trafficking we have utilized a fluorescent FA pulse-chase assay. Our data demonstrates that efficient FA trafficking in response to nutrient deprivation is dependent on LD/Mito contacts as well as phosphorylation of Plin5 at serine 155, a known PKA phosphorylation site. Considering the expression of Plin5 primarily in β-oxidizing tissue, we hypothesize that LD/Mito contacts are not primarily involved in FA storage but rather enhance the transfer of FAs from LD to mitochondria under physiological conditions that stimulate PKA activity, leading to increased FA oxidation.

SG198

**Competition between distinct Pex30 complexes at multiple membrane contact sites regulate organelle homeostasis**

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Lipid droplets (LD) and peroxisomes are ubiquitous organelles with central roles in cellular metabolism. These organelles form at discrete domains of the endoplasmic reticulum (ER) and once they mature often remain in close proximity of the ER at membrane contact sites (MCS). The ER domains involved in the formation and MCS with LDs and peroxisomes are enriched in the membrane protein Pex30. Consistent with a role in the process, pex30Δ cells show defects in LD and peroxisome biogenesis. However, how the activity and the targeting of Pex30 to ER MCS with different organelles are regulated remains unknown. Here, we show that the function of Pex30 at different MCS is regulated by its related proteins Pex28, Pex29 and Pex32. These proteins assemble with Pex30 in two distinct complexes: one containing Pex30-Pex28-Pex32 that targets ER-peroxisome MCS, and another containing Pex30-Pex29 enriched at the nuclear membrane-vacuole junction (NVJ), a distinct MCS where a population of highly metabolically active LDs concentrates. Thus, Pex28, Pex29 and Pex32 serve as MCS adaptors conferring specificity to Pex30. The absence of any of these adaptors results in defects in the number and spatial distribution of LDs or peroxisomes in the cell. These adaptors compete for binding to Pex30, suggesting that the coordination of LD and peroxisome biogenesis is maintained by the relative abundance of these complexes. We propose Pex30 complexes as general regulators that define organelle biogenesis sites.
and regulate organelle abundance, and ultimately are responsible for maintaining membrane and lipid homeostasis.

SG199
Actin and INF2 at the intersection of organelle morphology and motility
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The actin cytoskeleton plays important roles in a variety of cellular functions, including the regulation of organelle dynamics. In particular, the role of actin in the regulation of mitochondrial fission has been previously explored. Current evidence supports the model that actin polymerizes at ER-mitochondria contact sites, promoting mitochondrial constriction and allowing recruitment of key players in mitochondrial fission such as DRP1. Using probes we designed to specifically label mitochondria- or ER-associated actin, we were able to visualize accumulation of actin at mitochondrial fission sites, in agreement with already existing evidence. In addition, we made the novel observation that ER-associated actin also consistently accumulates at a wide range of other organelle fission sites including endosomes, lysosomes, peroxisomes, and the Golgi, suggesting a broad mechanism of organelle regulation by actin polymerization at ER-organelle contact sites. We further pursued INF2 as a potential regulator of this process. INF2 is a formin protein which promotes actin assembly. The ER-localized isoform of INF2 has been shown to regulate mitochondrial morphology and motility through its actin polymerization activity. We observed that INF2 deletion causes dramatic enlargement and tubulation of endosomes as well as lysosomal enlargement, suggesting that INF2 also plays a role in promoting endolysosomal fission, similar to its role in mitochondrial fission. Additionally, expression of a dominant active mutant of INF2 drastically reduces endosomal and lysosomal movement. This demonstrates that INF2 modulates not only organelle morphology but also organelle movement and this regulation is not limited to mitochondria.

SG200
Morphologically-discrete, ER subdomains support the translation of different types of mRNAs in response to ER-lysosome interactions
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The endoplasmic reticulum (ER) has a complex morphology comprised of stacked sheets, tubules, and three-way junctions, which together function as a platform for protein synthesis of the membrane and secretory proteins. It is believed that specific ER subdomains are spatially organized to provide protein synthesis activity, but precisely where these domains occur, especially with respect to a plethora of organelle interactions that take place on ER, remains elusive. Here, we use single-molecule tracking of ribosomes and mRNA in combination with simultaneous imaging of ER to assess the site of membrane protein synthesis on the ER. We found that ribosomes were widely distributed throughout the different ER morphologies, but the synthesis of membrane proteins (including Type I, II, and multi-spanning) and an ER luminal protein (Calreticulin) occurred primarily at three-way junctions. We found that Lunapark played a key role in stabilizing the transmembrane protein mRNA at the three-way junctions. Additionally, we found that these translating mRNAs are in the vicinity of the lysosome in an mTOR independent manner. These results support the idea that discrete ER subdomains co-exist with the
lysosome to support specific types of protein synthesis activities with ER-lysosome interaction playing an important role in this organization.

**PTMs of the Cytoskeleton**

SG201

**The Effect of Arginylation on Cellular Microtubules**

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Arginylation is the post-translational addition of arginine to glutamate and/or aspartate residues at N-terminal or internal sites of a protein by arginyl transfer enzyme 1 (ATE1). In mice, global Ate1 deletion causes defects in angiogenesis and cardiac morphogenesis, resulting in embryonic lethality starting at E12.5. Many of the observed phenotypes appear to be the result of impaired cytoskeletal function. Accordingly, cellular studies have shown that Ate1 deletion causes cytoskeletal defects, including reduced cell motility and adhesion. The role of N-terminal actin arginylation in these phenotypes has been carefully characterized, but the role of other arginylated cytoskeletal proteins has not yet been studied. Here, we focus on tubulin arginylation, using mouse embryonic fibroblasts (MEFs) with Ate1 deleted (Ate1−/−) as a model system. Ate1−/− MEFs have a seemingly normal amount and distribution of tubulin. However, they show a reduction in end binding 1 (EB1) comet velocity compared to wildtype MEFs, indicating impairments in microtubule dynamics and growth. Additionally, when treated with nocodazole Ate1−/− MEFs show an increased fraction of depolymerization-resistant microtubules compared to wildtype MEFs, suggesting that microtubules in these cells are more stable. Mass spectrometry of Taxol-purified cellular microtubules has identified arginylation of α- (E77 of TUBA1B) and β-tubulin (D74 of TUBB), which is absent in Ate1−/− cells. Analysis of microtubule associated protein (MAP) composition in these microtubule preparations shows that Ate1−/− MEFs have an increased amount of microtubule associated protein 1s (MAP1S) associated with microtubules. Previous studies have shown that decreased MAP1S levels can result in increased microtubule growth rate and decreased microtubule stability, consistent with the phenotypes observed in Ate1−/− MEFs. Together, these results demonstrate a new type of tubulin regulation by post-translational modification, in which tubulin arginylation regulates microtubule growth rate and stability.

SG202

**The tubulin code in microtubule dynamics regulation**

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Deceptively uniform ultrastructurally, microtubules are mosaic and contain multiple tubulin isoforms functionalized with abundant posttranslational modifications. Tubulin isoforms and posttranslational modifications vary widely between cell types and their patterns are stereotyped, consistent with roles in spatial and temporal control. Glutamylation, detyrosination and acetylation are tubulin posttranslational modifications associated with stable microtubule subpopulations in cells. I will present recent data from my lab that illustrate how these modifications can regulate microtubule properties in cis and in trans, by precisely controlling the activity of microtubule associated proteins.

SG203

**Regulation of the formin protein INF2 by lysine-acetylated actin: effects on cytoplasmic actin polymerization and mitochondrial dynamics**
Reversible lysine acetylation of histones and other nuclear proteins has long been appreciated as an important regulatory mechanism for chromatin remodeling and transcription. Subsequently, acetylation of a number of cytoskeletal proteins, including tubulin, cortactin, and the formin mDia2, has been shown to exert regulatory roles in the cytoplasm. Recently, we found that acetylation of actin itself regulates cytoplasmic actin polymerization through the formin INF2. Many formins are regulated through an auto-inhibitory interaction between the N-terminal diaphanous inhibitory domain (DID) and the diaphanous auto-regulatory domain (DAD). However, INF2’s DID and DAD interact with low affinity, and purified INF2 is constitutively active. We found that an inhibitory complex containing acetylated actin and the cyclase-associated protein (CAP) mediates the DID/DAD interaction to inhibit INF2. Acetylation of lysines in the actin D-loop, K50 or K61, appears sufficient for INF2 inhibition by the CAP/actin complex. Deacetylation of actin by HDAC6 activates INF2 in vitro and in cells. These findings are particularly exciting because multiple mutations to INF2’s DID link to two diseases: focal segmental glomerulosclerosis, a kidney disease; and Charcot-Marie-Tooth disease, a peripheral neuropathy. In cells, INF2 mediates rapid calcium-activated actin polymerization. We are currently elucidating the signaling pathway between calcium and actin de-acetylation, and the requirements for CAP mediation of inhibition. One question is whether the two mammalian CAP proteins, CAP1 and CAP2, have differential abilities to bind acetylated actin and/or regulate INF2. In U2OS cells, CAP1 is present at close to 20-fold higher concentration than CAP2, but both CAPs are in large excess to INF2. Another question is how actin acetylation might function with other known post-translational modifications in the D-loop. Our findings add to the growing list of functional actin PTMs, which might constitute an ‘actin code’, similar to the ‘histone code’ or ‘tubulin code’, controlling functional shifts to these central cellular proteins. Given actin’s growing importance in nuclear function, its modifications might have important roles in gene expression as well.

SG204
Towards establishing a Rosetta Stone for the Tubulin Code
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Tubulin is post-translationally altered by a suite of modifications that diversify the chemical properties of microtubules. Well known post-translational modifications (PTMs) include acetylation of lysine 40 on α-tubulin, poly-glutamylation and -glycylation of the α- and β-tubulin C-terminal tails, and detyrosination of α-tubulin. In these cases, PTMs affect the functionality of microtubules in myriad contexts ranging from mitosis to neurite formation and ciliogenesis. PTMs are expected to decorate all tubulin isotypes, creating a complex “Tubulin Code” that can be leveraged by the cell to pair sub-populations of microtubules to specific cellular processes. Historically, progress in understanding the roles of tubulin PTMs within cells has relied on antibodies that detect PTMs, enabling mapping of marked residues and the identification of writers and erasers that create and remove PTMs, respectively. Systematic analyses of PTMs and how they change as a function of the physiological state of the cell are lacking. To address this, we are using mass spectrometry coupled with commercial and custom data analysis pipelines to examine PTMs on tubulin purified from several human cell lines (HEK293, HeLa, HeLa ΔT7I, CHL-1, CHL-1 ΔVASH1/2). To probe cell cycle-dependent changes in tubulin PTMs, we have also analyzed tubulin prepared from mitotically arrested cells. First, we show that,
surprisingly, most tubulin isotypes are expressed in cultured cells. Second, we identify many novel PTMs on tubulin that appear to be conserved between cell types and vary by cell cycle state. We are using tandem mass tagging mass spectrometry to quantify cell cycle-dependent changes in PTMs. Third, using an “open search” analysis approach, we show that both α- and β-tubulins are modified to high stoichiometry on tryptophan residues by a PTM whose mass is consistent with a c-mannosyl group. Collectively, our work establishes a comprehensive survey of tubulin PTMs that will pave the way for functional studies of how the Tubulin Code impacts cell physiology.

SG205

Post-translational regulation of the Actin Cytoskeleton

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Post-translational Regulation of the Actin Cytoskeleton In recent years it has become clear that the actin cytoskeleton is regulated by post-translational modifications (PTMs). These include PTMs of actin itself, in the form of acetylation, arginylation, and methylation as well as phosphorylation of actin binding proteins. I will discuss our work on 1. developing methods to express and purify actin monomers with distinct PTMs 2. characterizing the biophysical and biochemical properties of these modified actins, and 3. phosphorylation of the actin binding protein tropomyosin, in fission yeast. A combinatorial approach using synthetic biology and biochemistry reveals that phosphorylation of tropomyosin prevents interaction with F-actin. This in turn plays a key role in the positioning of the cytokinetic apparatus in fission yeast.

SG206

Set-ing methyl marks on the cytoskeleton

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In chromatin biology, post translational modifications (PTMs) on histones and DNA determine both the structure and function of the genome, and comprise what is often referred to as a “Histone Code”. Methylation of lysine and arginine residues is a key component of this Code, and the methyltransferases that add methyl marks, demethylases that remove them, and effector proteins that act on these marks have been identified. These “readers, writers and erasers” of chromatin PTMs function in a coordinated way to convey heritable information, regulate transcription, and control chromatin conformation in the cell. Recently, it was discovered the methylation machinery for chromatin also functions on the cytoskeleton. We discovered the histone methyltransferase SETD2 is an α-tubulin methyltransferase, and identified trimethylation of microtubules on lysine 40 (α-TubK40me3) as a new PTM of the “Tubulin Code” required for function of the mitotic spindle (Park et. al. Cell 2016). Loss of SETD2 and the α-TubK40me3 mark results in chromosome segregation defects and genomic instability that can drive cancer progression (Chiang et. al. Cancer Research 2018). More recently, we identified a second cytoskeletal target for SETD2: actin filaments. SETD2 methylates lysine 68 of actin (ActK68me3) to regulate actin dynamics and cell migration (Seervai et. al. Science Advances in press). We have also identified several “readers” of cytoskeletal methyl marks, including the ATPase and chromatin remodeler SWI/SNF. PBRM1, a component of the PBAF SWI/SNF complex recognizes and binds the α-TubK40me3 mark to recruit PBAF to the mitotic spindle. This activity is required for proper chromosome sorting during mitosis, and is lost in many cancers with PBRM1 mutations (Karki et. al. BioRxiv 2020).
This points to an emerging paradigm for dual-function remodelers with ‘chromatocytoskeletal’ activity that can integrate cytoplasmic and nuclear functions. For example, the SETD2 methyl mark on chromatin is required for efficient DNA repair, and its microtubule methyl mark is required for proper chromosome segregation during mitosis. This unexpected convergence of SETD2 activity on histones and microtubules to maintain genomic stability suggests an intriguing possibility that cells utilize methyl marks and their “readers, writers and erasers” to coordinate higher level cytoskeletal and chromatin functions.

SG207

UNC-45A is novel ATP-independent MT severing protein overexpressed in cancer and neurodegenerative diseases

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For the longest time, cancer and neurodegenerative diseases have been considered at the opposite end of the spectrum; cancer is characterized by dividing cells that resist death, neurodegeneration is associated with death of post-mitotic cells. Today genetic and cell biology studies show common pathways in cancer and neurodegeneration including proteins that regulate microtubule (MT) stability. Tau for instance, a MT-associated-protein (MAP) largely expressed in brain and whose hyperphosphorylation is associated with Alzheimer’s disease (AD), is abnormally expressed in human cancers. Furthermore, drugs targeting MT stability is an established anti-cancer approach and stabilization of neuronal MTs can attenuate neurodegenerative diseases. We are one of the few laboratories in the world to study the roles of UNC-45A, a highly conserved member of the UCS (UNC-45/CRO1/She4p) protein family, in mammalian cells. We (and others) have shown that UNC-45A: is required for cytokinesis and is overexpressed in a number of human cancers (Bazzaro et al., 2007; Guo et al., 2011), plays a role in exocytosis in immune cells (Iizuka et al., 2015), and is required for axonal growth (Iizuka et al., 2017) via regulation of acto-myosin contractility. While UNC-45A is implicated in regulating the actomyosin system (Barral et al., 2002; Lehtimaki et al., 2017), it is becoming clear that it has additional functions including regulation of centrosomal positioning (Jilani et al., 2015). Importantly, we have recently shown that UNC-45A is a novel Microtubule-Associated Protein (MAP) with MT depolymerizing properties in normal and cancer cells (Habicht et al., 2019; Mooneyham et al., 2019), and that UNC-45A acts on MTs independent of its myosin II binding domain and in presence of the myosin II inhibitor blebbistatin (bioRxiv 2020.06.20.163048; doi: https://doi.org/10.1101/2020.06.20.163048). Here we present our new, unpublished data that UNC-45A destabilizes MTs by weakening the MT lattice in an ATP-independent manner. This mechanism is distinct from the ones of other known MT lattice destabilizing proteins including katanin. We also for the first time show that UNC-45A is largely expressed in human brain where it co-localizes with MTs with a predominant localization to the proximal portion of the axonal shaft, the main site of neuronal MTs severing. Furthermore, we show that in neurons UNC-45A overexpression leads to MTs bending and breaking and to loss of MT mass. Lastly, using a large cohort of human clinical specimens of healthy brains, and of individuals with Mild Cognitive Impairment (MCI) and AD, we show that UNC-45A is overexpressed in MCI and AD and associated to a loss of MT mass. The implication is that UNC-45A could be a biomarker and a therapeutic target for Alzheimer’s disease.
SG208

Spatiotemporal oxidation of L-plastin downmodulates actin-dependent cellular functions

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Tumors possess a special redox homeostasis with a shift towards a prooxidative micromilieu. Moreover, several antitumor therapies work by increasing reactive oxygen species (ROS) within the tumor micromilieu. The potential influence of such therapies on immune cells required for antitumor response complicates the ROS influence on the tumor behavior. Therefore, studying regulation of redox sensitive proteins that are present in tumor cells and in immune cells would help to predict response to ROS inducing therapies. In our study, we revealed that L-plastin (LPL), an established tumor marker, was regulated by ROS-induced thiol oxidation on Cys101 and Cys42. LPL reduction was mediated by the Thioredoxin 1. LPL oxidation diminished its actin-bundling capacity which was accompanied by a marginal decrease in tumor cell migration, invasion and extracellular matrix degradation. Importantly, focusing on spatiotemporal oxidation using ratiometric imaging of LPL-roGFP-Orp1 fusion protein and a dimedone-based proximity ligation assay revealed that LPL oxidation occurs primarily in actin-based cellular extrusions. Consequently, LPL spatial oxidation at the cell periphery strongly inhibited cell spreading and formation of filopodial extension in tumor cells. Further investigation of spatiotemporal oxidation of LPL in resting and stimulated T cells in response to several physiological stimuli pointed towards involvement of spatial LPL oxidation on physiological functions of T cells as well as their pathological suppression - depending on the amount and location of oxidized LPL. Altogether, these findings highlight a delicate balance between the antioxidant capacity of cells, oxidation levels of LPL in a spatial manner and actin-based cellular functions.

SG209

A novel mechanism that promotes mitotic spindle formation in human cells

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The mitotic spindle consists mainly of microtubules (MTs) and is essential for accurate distribution of chromosomes to the two daughter cells during cell division. Errors in spindle formation can lead to incorrect separation of chromosomes or unequal size of daughter cells, which are associated with carcinogenesis or developmental disorders. However, the molecular mechanisms of spindle formation are not fully understood. In the present study, we show for the first time that Chk1, a kinase involved in the cellular response to DNA damage, is essential for optimal density and effective polymerisation of the spindle MTs in human cells. Chk1 localises to the centrosomes (the main centers of MT organization) in mitosis and phosphorylates β-tubulin in newly identified sites in vitro. Also, reduced microtubule density in cells without functional Chk1 is associated with formation of disorganized spindles. We suggest that Chk1 phosphorylates β-tubulin to promote optimal spindle MT polymerisation. These findings describe a novel mechanism that could protect against carcinogenesis and developmental disorders, through regulating formation of the mitotic spindle.
SG210

A phospho-regulated signal motif determines subcellular localization of α-TAT1 for dynamic microtubule acetylation

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Background: Microtubule (MT) acetylation is a highly conserved post-translational modification that stabilizes MTs and mediates responses to mechanical forces at molecular, cellular and organism levels. MT acetylation underlies many physiological events ranging from cell migration to tissue stiffness, intracellular trafficking, autophagy and viral infections. MT acetylation is exclusively catalysed by α-TAT1, for which the only known substrate is α-tubulin. While the functional consequences of MT acetylation have been extensively studied, the molecular mechanisms that regulate α-tubulin N-acetyltransferase 1 (α-TAT1) action remain obscure.

Results: We have utilized computational sequence analyses and mutational analyses to identify and characterize a conserved signal motif in the intrinsically disordered α-TAT1 C-terminus that mediates its intracellular distribution. This signal motif consists of three functional modules: a nuclear export sequence (NES), a nuclear localization sequence (NLS), and phosphorylation sites. Using live cell microscopy, we observed that α-TAT1 shuttles between the nucleus and cytosol but is mainly cytosolic due to CRM1-mediated nuclear export. Nuclear localized α-TAT1 catalytic domain did not induce MT acetylation, suggesting that cytosolic localization of α-TAT1 mediates its function. We show that the α-TAT1 NLS is phospho-inhibited by cyclin dependent kinases, Protein Kinase A and Casein kinase 2, and that the phosphorylated motif binds to 14-3-3 adaptor proteins to retain α-TAT1 in the cytosol for maximal substrate access. We hypothesized that inducible nuclear transport α-TAT1 will allow control of MT acetylation. We modified the previously described Light-inducible nuclear export system (LEXY) to reduce its dark-state activity and tethered it to the α-TAT1 catalytic domain. LEXY-α-TAT1 localized to the nucleus under dark conditions and was rapidly and reversibly shuttled to the cytosol on blue light stimulation. HeLa cells expressing LEXY-α-TAT1 exposed to light showed increased MT acetylation unlike those kept under dark conditions, thus validating the functionality of the tool.

Conclusions: Our findings establish a novel role for the intrinsically disordered C-terminus of α-TAT1 in its function by regulating its intracellular localization downstream of kinase and phosphatase activities. Moreover, we have developed a powerful tool to control MT acetylation that will provide new applications that can be used to elucidate the role of MT acetylation in health and diseases.

SG211

Actin Redox Balance Regulates Filament Disassembly

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Introduction: The assembly and disassembly of actin filaments (F-actin) are tightly controlled processes with critical implications for cell physiology. While many actin binding proteins, such as the ubiquitous ADF/cofilin, have been identified to directly interact and disassemble filaments, a complementary mechanism has been recently identified: actin oxidation. We recently showed that F-actin disassembly controls the timing of cytokinetic abscission, the terminal step of cell division, and that the dysregulation of actin dynamics can have dramatic consequences such as tetraploidy (Frémont et al. Nature Comm. 2017, Bai et al. PNAS 2020). In the cytokinetic bridge, we also revealed that F-actin redox balance is...
mediated by two enzymes, MICAL1 and MrkB2, which oxidizes and reduces key methionines on actin (Met44 and Met47), respectively. **Question:** It is unclear whether MICAL1 and MrkB2 can modify and possibly compete for the same pools of actin (F-actin vs. monomers). In addition, a synergy between ADF/Cofilin and MICAL1 for F-actin severing has been previously shown (Grintsevitch et al. NCB 2016), but the molecular mechanism is unknown and the interplay between F-actin oxidation and other regulatory proteins such as Tropomyosin has not been investigated yet. **Method:** To explore these questions, we performed in vitro experiments on single actin filaments, anchored within microfluidic chambers designed in the lab, and visualized with TIRF microscopy. **Results:** We first showed that MICAL1 selectively oxidizes F-actin but not monomeric actin (G-actin). In contrast, MrkB2 only reduces G-actin, but not oxidized actin filaments. We also found that Actin oxidation accelerates filament depolymerisation 10-fold from both barbed and pointed ends. However, contrary to what was initially reported, oxidized F-actin does not spontaneously fragment. ADF/cofilin is a family of proteins at the centre of virtually all F-actin disassembly machineries. We found that F-actin oxidation boosts ADF/cofilin activity by accelerating both its recruitment to F-actin (nucleation and elongation of ADF/cofilin domains) and the severing rate at the border of ADF/cofilin domains. Furthermore, we found that oxidized F-actin gets rapidly disassembled even when they are protected by Tropomyosin or when ADF/cofilin is inhibited by phosphorylation. **Conclusion:** We conclude that F-actin oxidation is a powerful mechanism to regulate filament stability. Oxidized filaments are indeed quickly disassembled in circumstances that would otherwise be of little consequences: when ADF/cofilin is at very low concentration or inhibited by phosphorylation, and when filaments are protected by Tropomyosin.

SG212

**Microtubule Glutamylation Specifies Polarized Distribution of Endoplasmic Reticulum**

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The endoplasmic reticulum (ER) comprises functionally and morphologically distinct but interconnected structures including tubules, sheets, dense matrices and the nuclear envelope. While much progress has been made in understanding how the peripheral tubular network is formed, little is known about how the high-density perinuclear ER sheets and matrices are formed and distributed. We show that three ER membrane-bound proteins -- CLIMP63, p180 and kinectin -- preferentially bind centriolar, perinuclear and peripheral microtubules, respectively, to establish proper distribution and morphology of perinuclear ER. Mechanistically, p180 and kinectin selectively bind glutamylated and polyglutamylated microtubules, respectively. Knockout of these proteins or manipulation of microtubule populations or glutamylation status dramatically changes ER positioning, either more dispersed or more clustered, which also leads to corelative spatial rearrangements of almost all other membranous organelles. Our data show that microtubule modification specifies asymmetric, perinuclear ER distribution and this also controls broad intracellular organelle positioning.

SG213

**Fyn-mediated phosphorylation of tau differentially regulates the transport of early endosomes and lysosomes**

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Different sets of kinesin and dynein motors associate with and transport specific organelles such as early endosomes and lysosomes. Microtubule-associated proteins (MAPs) regulate transport in a motor-specific manner. MAP tau strongly inhibits kinesin-1 and kinesin-3 processivity, while kinesin-2 and dynein are less sensitive to tau. Phosphorylation tunes the binding kinetics of tau to microtubules. Over 85 putative phosphorylation sites have been identified on tau, phosphorylated by multiple kinases including GSK3β, AKT, MAPK, and Fyn. Fyn phosphorylates tau at tyrosine 18, resulting in decreased affinity to microtubules and weakened inhibition of kinesin-1. We examined the effects of tau and fyn-mediated tau phosphorylation on microtubule organization and the transport of early endosomes and lysosomes in COS-7 cells. Phosphorylation relieves the inhibition of lysosomal motility by tau.

Juxtanuclear lysosomes are not inhibited by phosphomimetic tau (Y18E), whereas peripheral lysosomes are inhibited by both WT (wild-type) and phosphomimetic tau. In contrast, early endosomal motility is inhibited by both tau Y18E and WT tau. Overall, early endosomes are more sensitive to the presence of tau compared to lysosomes, which are known to associate with different sets of kinesin motor proteins. We expect that the types of engaged kinesins govern the disparate responses of different types of cargoes to tau. Motility assays on reconstituted microtubules show that phosphomimetic tau strongly inhibits kinesin-3, in contrast to kinesin-1 motility, which is partially rescued by tau phosphorylation at Y18. Cargoes positioned in central and peripheral regions of the cell exhibit differential motility in the presence of tau, likely due to different motors driving their transport. Together, our results show that tau and its phosphorylation specifically regulate the motility of differing organelles through motor-specific regulation of kinesins on the microtubule lattice and modulating microtubule network organization.

SG214
The mechanism and impact of actin N-terminal acetylation
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Actin is the most abundant intracellular protein in eukaryotes and the actin cytoskeleton controls a range of cellular processes including cell dynamics and motility. The six different human actins are known to undergo unique N-terminal processing events involving Methionine removal and post-translational N-terminal acetylation to generate highly acidic actin N-termini. The responsible machinery has remained unidentified and the impact of such processing on actin function likewise. The objectives of our studies have been to define the enzymes involved in actin N-terminal processing and to unravel the protein and cellular impact of these events. We identified the NAA80 enzyme as actin’s dedicated N-terminal acetyltransferase. In cells lacking NAA80, actin is completely lacking N-terminal acetylation while it is close to 100% N-terminally acetylated in cells harboring NAA80. Structural determination revealed that the acidic actin N-terminus perfectly fits into the catalytic site of NAA80 and further that actin and NAA80 share a large 3D-interface. We also revealed that Profilin 2 (PFN2) has a unique role as a stable cellular partner of NAA80 to facilitate contact with actin and thereby rapid acetylation of actin molecules. So why have our cells put all this effort into modifying actin in this way? NAA80 KO cells represent a unique opportunity to study this question and we found a significant impact of actin N-terminal acetylation on the balance between globular and filamentous actin, cytoskeletal protrusions,
cell motility, adhesive properties and Golgi structural integrity. In conclusion, actin N-terminal processing has evolved to maintain the unique properties of actin in animal cells and is strictly required to keep the cytoskeleton in balance.

SG215

**Tubulin glycylation controls axonemal dyneins, mammalian sperm flagellar motility and fertility**

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Microtubules, the structural core of the axonemes of motile cilia and flagella, undergo a wide variety of posttranslational modifications (PTMs). Glycylation, a key tubulin PTM enriched exclusively on the axonemal microtubules of both motile and primary cilia, has been proposed to play an essential role in ciliary stability. Glycylation is initiated by TTLL3 and TTLL8 enzymes belonging to the tubulin tyrosine-ligase like (TTLL) family of proteins and further elongated by TTLL10. Absence of elongated glycylation in humans due to a constitutive mutant TTLL10 is tolerated, but a complete loss can be damaging. Studies exclusive to cells/tissues expressing only one of the two initiating glycylases (TTLL3) revealed a defect in ciliary stability in the absence of glycylation but these were limited in exploring the physiological role of tubulin glycylation at organism level. To obtain an in-depth molecular and physiological understanding of the role of tubulin glycylation in mammals, we established a transgenic mouse model knocked out for both the initiating glycylases (*Ttll3*/*Ttll8*). These mice entirely lack glycylation in all ciliated tissues we analysed. Strikingly, absence of glycylation does not impair ciliogenesis, and *Ttll3−/− Ttll8−/−* mice do not show any signs of ciliary dysfunctions characteristic of ciliopathies. The male mice had lower litter size indicative of subfertility, despite no defects in the sperm count and very few spermatozoa with morphological defects such as unnatural head-tail bending. 2D and 3D motility analyses of the *Ttll3−/− Ttll8−/−* sperm revealed an asymmetrical flagellar beating that resulted in a circular/helical swimming pattern that impede straight-line swimming of the sperm, resulting in concomitant reduction of progressive motility. The subfertility was confirmed by a drastic decrease in *in vitro* fertilization capacity of the *Ttll3−/− Ttll8−/−* sperm. Using cryo-electron tomography, we determined that lack of glycylation causes abnormal conformations of the dynein arms within sperm axonemes, providing the structural basis for the observed dysfunction. Overall, our work unravels the importance of microtubule glycylation for controlled flagellar beating, directional sperm swimming, and male fertility.

SG216

**N-terminal modification of actin by acetylation and arginylation determines the architecture and assembly rate of linear and branched actin networks**

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The actin cytoskeleton is an intricate polymer system that is remodeled by hundreds of actin-binding proteins to provide the correct structural and mechanical support for many essential cellular processes,
including cytokinesis, cell migration, intracellular transport, and cell-cell contact. Intriguingly, it’s been known for many years that posttranslational modification of actin also affects these processes, however in most cases, it is completely unclear how these modifications alter actin network architecture and dynamics. To begin to address this question, we used a recently developed strategy to produce pure populations of selectively modified actins, and directly compared the effect of N-terminal arginylation and acetylation of β-actin, two well-known modifications that both have been shown to regulate cell migration. Our results show that arginylated and acetylated actin have opposing effects on linear and branched actin filament assembly. What is more, copolymerization experiments demonstrated that arginylated actin tunes Arp2/3-mediated nucleation, and thus branch spacing, whereas acetylated actin regulates actin filament nucleation and network density. To get a better understanding of the contribution of the charge at the N-terminus of β-actin, we also investigated pure populations of unprocessed and non-acetylated β-actin. TIRF microscopy analysis elucidated that these actins have distinct intrinsic polymerization properties, and interact differently with key actin-binding proteins, including Profilin, mDia1, Arp2/3 and Cofilin. Altogether, this study shows that the nature of the N-terminus of actin can induce distinct actin network dynamics, which can be differentially used by cells to locally fine-tune actin dynamics at distinct cellular locations, such as at the leading edge.

**Reimagining Cell Biology Through Quantitative Tools**

**SG217**

**High-throughput cell phenotyping in cancer and aging**

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A central goal of precision medicine is to predict disease outcomes and design treatments based on multidimensional information from afflicted cells and tissues. Cell morphology is an emergent readout of the molecular underpinnings of a cell’s functions and, thus, can be used as a method to define the functional state of an individual cell. We measured >200 features derived from cell and nucleus morphology for more than 30,000 breast cancer cells. We find that single cell-derived clones (SCCs) established from the same parental cells exhibit distinct and heritable morphological traits associated with genomic (ploidy) and transcriptomic phenotypes. Using unsupervised clustering analysis, we find that the morphological classes of SCCs predict distinct tumorigenic and metastatic potentials in vivo using multiple mouse models of breast cancer. These findings lay the groundwork for using quantitative morpho-profiling in vitro as a potentially convenient and economical method for phenotyping function in cancer in vivo. A similar high-throughput single-cell approach was used to determine and validate cell and nuclear features as correlates of aging in health and disease.

**SG218**

**Defining states of pluripotency at the single cell level using statistical thermodynamics**

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The Waddington landscape is frequently invoked as an illustration of how cells in an isogenic population can explore different phenotypic states. Our theoretical work builds on this concept to describe population heterogeneity in terms of physical principles. We have previously demonstrated the dynamic nature of gene expression in individual cells and have shown that phenotype expression in individual...
cells is highly dynamic even while the population distribution of phenotypic expression is stationary. We use fluorescence microscopy to quantify the dynamics of the expression of genes in individual cells. We have developed a statistical thermodynamics model that predicts that correlations in the fluctuations in expression of genes within individual cells is a measure of the interdependence between those components within an operational network (doi.org/10.1371/journal.pone.0230076.). To support our theoretical work, we are developing induced pluripotent stem (iPS) cell lines to map the different states that individual pluripotent cells can occupy in the context of their relative expression of OCT4, SOX2 and Nanog. We are using CRISPR engineering to create dual-reporter iPS cell lines that produce a fluorescent reporter associated with each of 2 of the network factors simultaneously. The fluorescence of the reporters in individual cells will allow us to create a map, i.e., a 3-dimensional potential energy landscape, of how the relative expression of these three transcription factors influence the states of pluripotency across the population of iPS cells. This will allow us to assess how a cell’s state of pluripotency is affected by cell division, spatial location, morphology, changes in culture conditions, etc. Together, the 3-D landscape and the correlations in dynamic fluctuations between network factors will allow prediction of the stability of different microstates of pluripotency, and the kinetics with which those microstates interconvert. This approach provides a unique measurement method to characterize cell populations that is based on physical understanding of the dynamical and energetic forces that control cellular heterogeneity. This method will allow a more meaningful characterization of cellular populations for directing the manufacture and release of cell therapy products.

SG219

**Physical organization of the cytosol**

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Syncytial cells are common in the biosphere and include fungal pathogens and symbionts, embryos and germlines of plants and animals, bone, blood and muscle cells, as well as many tumor cells. This multinucleate organization can enable syncytia to achieve exceptionally large sizes and perform many distinct physiological functions within a continuous cytoplasm. As nuclei sharing a syncytium can be different genotypes or in different gene expression states, these single cells can approach the functional complexity of a multicellular tissue state. Using machine-learning based tracking tools we have probed the physical properties of syncytial cytosol. We learned that the cytosol was spatially patterned in terms of its physical properties and in turn this is associated with areas where biomolecular condensation can occur. The spatially-restricted condensates allow the nuclei to divide asynchronously despite sharing a common cytosol. As the nuclear cycle is based on a biochemical oscillator made of cyclin/CDK proteins, this means these cells have coexisting and out-of-sync oscillators in a common environment. This is surprising because it is well documented that oscillators in the same environment will tend to couple and synchronize. We have developed a mathematical model for examining the emergence and maintenance of asynchronous oscillators by expanding on the classic Kuramoto oscillator model. Integration of quantitative imaging and analysis with mathematic modeling leads us to understand the mechanistic basis for how large cells pattern their cytoplasm.

SG220

**Cell states beyond transcriptomics: integrating structural organization and gene expression in hiPSC-derived cardiomyocytes**

The Allen Institute for Cell Science is developing a state space of stem cell structural signatures to study changes in cellular organization as human induced pluripotent stem cells (hiPSCs) differentiate into cardiomyocytes. In this study we combined quantitative image-based readouts of gene expression, transcript localization, and cellular organization to evaluate the changes in hiPSC-derived cardiomyocytes during the early/intermediate stages of differentiation. One of the goals of this study was to understand the relationship between gene expression and structural organization by integrating both measurements in single cells. To investigate changes in gene expression during these stages, we first performed single-cell RNA sequencing on cardiomyocyte populations collected at D12, D24 and D90. We used a cluster independent bootstrapped sparse regression statistical approach to rank differentially expressed genes based on their ability to correctly assign cells to the transcriptionally similar D12 or D24 populations. From this ranked list we identified a subset of genes to use as probes for RNA fluorescent in situ hybridization (FISH) to measure transcript abundance. These included known developmentally regulated myosin heavy chain isoforms MYH6 and MYH7. We also developed novel, scalable machine learning methods for classifying and quantifying sarcomeric organization in single cells, which improves upon manual annotation. To determine whether the changes in gene expression found in single-cell RNA sequencing correspond to differences in sarcomere organization, we combined high resolution imaging using the mEGFP-ACTN2 cell line with multiplexed RNA-FISH. This co-analysis of quantitative single-cell measurements of gene expression and sarcomeric organization revealed that while the expression of some genes such as MYH7 were positively correlated with increased sarcomeric organization, both measurements are required to describe cell state. The image-based nature of this analysis also revealed subcellular transcript distribution patterns, such as sarcomeric localization of MYH7 transcripts. While we did not find a relationship between transcript localization and myofibril organization in these cells, further analysis of transcript localization with structure may provide insights into this relationship. These findings suggest that cells may be better characterized by a combination of spatial and quantitative measurements of transcripts and structures rather than by either one alone.

While we demonstrated this concept in cardiomyocytes during early differentiation, integrating gene expression and structural organization can be applied more broadly to create a more complete understanding of cell behavior and states.

SG221

Quantification of cancer cell morphology and signaling

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Recent progress in microscope instrumentation allows volumetric time-lapse imaging of cancer cells with isotropic resolution in three-dimensional environments (such as collagen matrices and model organisms). Here I present our work on light-sheet microscopes designed to image large volumes with high spatiotemporal resolution and their application to imaging metastatic cancer cells in different “niches” of Zebrafish xenografts. We apply computational tools to segment and quantify the morphological motifs of different cancer cell populations. We further use these microscopes and computational pipelines to unravel the relationships between intracellular signaling and cell morphology.
SG223

Defining cell based biomarkers of ageing

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The process of ageing is inevitable, and while we gain one chronological year with the passing of each birthday, the biological outlook among individuals is variable. A growing body of evidence shows that the interactions of intrinsic and extrinsic factors i.e. molecular states (e.g. epigenomic) together with environmental factors and macroscopic stressors (e.g. social determinants, disparities) contribute to the rates of ageing in individuals. However, it is unclear how the underlying molecular states of an individual relate to their clinical ageing outlook. We postulate that studying age-associated changes at the intermediate length scale of cells-between the larger length scale of organs and tissues and the smaller length scales of molecules-may provide a key link to understand the inter-relation among ageing scales. Populations of cells display dynamic and heterogeneous phenotypes in the context of health and disease. As integrators of molecular signals, cells offer a sensitive meso-scale view of ageing, with cellular dysfunctions likely occurring prior to the manifestation of age-related disorders and diseases at the clinical level, suggesting that essential ageing information may be encoded within cellular properties. Based on this, we sought to answer two key questions, 1) is ageing information encoded within biophysical properties of cells? and 2) how is this ageing information encoded by/within cells? To evaluate this, we procured a panel of primary dermal fibroblasts from healthy donors spanning a wide age range (2-96 years). These cell samples were subjected to ten classes of biophysical and biomolecular measurements, then evaluated for kinetic associations with age. Since biophysical properties represent the ensemble orchestration of many molecular inputs, we hypothesize that cellular biophysics will determine the cellular age with more accuracy relative to biomolecular features, with deviations from healthy ageing trends being indicators of disease states in older adults. We found that using a bivariate framework of combinations of biophysical properties of cells, mainly morphology and motility, we could determine the cellular age of healthy individuals with an ~error of 6-yrs. As a proof of concept, using the best prediction pair, we also show significant deviations in ageing trends for individuals with premature ageing disorders (i.e. HGPS). Taking advantage of the single cell nature of our data, we also show that ageing in humans is characterized by changes in cellular heterogeneity, with ageing information being partially encoded in the cell-to-cell variations. These findings point to a new mechanistic framework of ageing, with avenues for improved biomarkers of ageing in the context of health and disease.

SG224

4D Cell Biology

J. Schöneberg; UC San Diego, San Diego, CA.

New methods in stem cell 3D organoid tissue culture, advanced imaging, and big data image analytics now allow tissue-scale 4D cell biology but currently available analytical pipelines are inadequate for handing and analyzing the resulting gigabytes and terabytes of high-content imaging data. We expressed fluorescent protein fusions of clathrin and dynamin2 at endogenous levels in genome-edited human embryonic stem cells, which were differentiated into intestinal epithelial organoids. Lattice light-sheet imaging with adaptive optics (AO-LLSM) allowed us to image large volumes of these organoids (70 × 60 × 40 μm xyz) at 5.7 s/frame. We developed an open-source data analysis package termed pyLattice to
process the resulting large (∼60 GB) movie data sets and to track clathrin-mediated endocytosis (CME) events. We then expressed fluorescent protein fusions of actin and tubulin in genome-edited induced human pluripotent stem cells, which were differentiated into human cortical organoids. Using the AO-LLSM mode on the new MOSAIC (Multimodal Optical Scope with Adaptive Imaging Correction) allowed us to image neuronal migration deep in the organoid. We augmented pyLattice with a deep learning module and used it to process the brain organoid data.

Friday, December 11, 2020

Bacterial and Archaeal Cell Organization

SG225
A Multiscale Analysis of Bacterial Predation
T. Mignot; CNRS-Aix Marseille University, Marseille, FRANCE.

A current challenge in developmental biology is to bridge molecular and multicellular scales. This task is especially complex in animals given that the dimension gap spans several orders of magnitude. In this context, multicellular microbes can be especially powerful because their lifecycle rarely exceeds a few days and it can be captured over relatively small surfaces in devices as simple as a petri dish. In addition, these organisms allow sophisticated genetic manipulations and imaging approaches. In our laboratory, we study Myxococcus xanthus for its ability to predate and develop collectively over other microbial preys. During this presentation, I will present an interdisciplinary approach combining genetics, quantitative imaging and mathematical modelling to decipher how Myxococcus cells invade their preys collectively and form periodic cellular waves over macroscopic scales spanning entire prey colonies. We find that the patterns arise at discrete sites where the cells accumulate at high density. Genetics and modelling studies indicate that wave formation is provoked by the spatial induction of a novel type of intracellular oscillator, the properties of which will be discussed. This example provides a promising experimental and theoretical framework to link molecular mechanisms to the formation of dynamic cellular patterns at the mesoscale.

SG226
High-Resolution Imaging of Single-Cell Behaviors in 3D Bacterial Biofilms using Lattice-Light Sheet Microscopy and Machine Learning-Based Image Processing
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Myxococcus xanthus is a highly social bacterium and a model system for coordinated cellular behaviors, including swarming motility and developmental cell differentiation. Under nutrient limited conditions, M. xanthus populations activate a complex developmental program that induces cells to aggregate into 3-dimensional mounds. Some of these mounds develop further into fruiting bodies with heights and diameters of up to 100 micrometers. During fruiting body development, cells either differentiate into round myxospores or into peripheral rods (a persister-like cell-state), or they undergo developmental
cell lysis. Despite intensive efforts, it remains unclear how different biochemical signals and mechanical cues coordinate cell differentiation in 3D space and time. A critical barrier is that conventional fluorescence imaging modalities suffer from phototoxicity and photobleaching limitations that prevent high-resolution, single-cell tracking of developmental progression in biofilms over many days. To address this challenge, we adapted minimally-invasive lattice light-sheet microscopy for 3D bacterial biofilm imaging in optically accessible flow cells. To automatically identify and outline individual cells in the acquired images, we combine machine learning-based segmentation approaches with mathematical image processing. By integrating complimentary segmentation approaches, we aim to achieve segmentation accuracies that enable accurate tracking of each cell’s motion, morphology, and gene expression during multicellular development. Understanding the growth and function of bacterial biofilms in terms of the behavioral phenotypes of individual cells will help inform new strategies for controlling and harnessing the emergent functional capabilities of microbial populations.

SG227
The Type IV Pilus Motor - Mechanism and Dynamics
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Type IV pili (T4P) are ubiquitous prokaryotic filamentous surface appendages that mediate functions ranging from adherence (surface sensing) to motility. They are retractable, and retraction - through conversion of chemical to mechanical energy - can generate large forces. Recent structural studies demonstrated that T4P motor ATPases undergo coordinated conformational changes that are thought to rotate and translate membrane-bound platform proteins to insert or remove individual pilin subunits to extend or retract a pilus filament. Some species have only a single motor ATPase while others have 3 or more, for reasons that remain unclear. Cryoelectron tomography studies of intact T4P machines suggest that only a single ATPase hexamer at a time can occupy a socket at the base of the apparatus, and it is not clear how the system switches from an extension to retraction state. I will discuss our ongoing work to understand the function of these intriguing biological engines.

SG228
Studying horizontal gene transfer in bacterial systems using a cell biological approach
A. Dalia; Indiana University, Bloomington, IN.

Natural transformation (NT) is a major mechanism of horizontal gene transfer in microbial species that promotes the spread of antibiotic-resistance determinants and virulence factors. During this process, bacterial cells take up free DNA from the environment and can subsequently integrate this DNA into their genome by homologous recombination. We have recently developed a comprehensive cell biological toolkit to study NT in the model organism Vibrio cholerae. In this talk, I will discuss how employing this toolkit has helped reveal the molecular mechanisms underlying both DNA uptake and DNA integration during NT and has further uncovered how NT can promote the nongenetic inheritance of traits during this conserved mechanism of horizontal gene transfer.

SG229
Trigger mechanism for contraction of the Type VI secretion system sheath.
M. Basler; Biozentrum, University of Basel, Basel, SWITZERLAND.
Gram-negative bacteria use Type six secretion system (T6SS) to deliver a wide range of effector molecules to neighboring prokaryotic and eukaryotic cells. T6SS is a complex contractile nanomachine that translocates proteins across cellular membranes by rapid contraction of a sheath wrapped around an inner tube. Although remarkable details of T6SS structure and assembly have emerged, the trigger mechanism of T6SS sheath contraction is unknown. Here, we measured the flexural rigidity of the Vibrio cholerae T6SS sheaths and analyzed their buckling during assembly prior to contraction. This indicated that an axial compressive force of at least 3.3 pN is generated in reaction to sheath polymerization within cells. In addition, we show that a rapid cell-size reduction following hyperosmotic shock increased axial compressive force on fully assembled sheaths and triggered their immediate contraction while polymerizing sheaths were unaffected. Together, our data suggest that the force generated during T6SS assembly triggers sheath contraction. This trigger mechanism allows for fast and efficient protein delivery into target cells.

SG230
Cytoskeleton Dynamics and Compartmentalized Growth in Archaea
A. Bisson; Brandeis University, Waltham, MA.

Despite the recent advances in large-scale sequencing and the consequent explosion of microbial diversity, there is still little known about how evolution shaped the way cytoskeletal polymers control the assembly of cellular structures and partition cargo in most known organisms. Here, I am going to discuss our recent work on how haloarchaea couples cell division and shape by modulating different cytoskeletal systems localized at the same cell location. Using live-cell microscopy, we have established a correlation between the dynamics and function of different tubulin homologs. We also demonstrate that the archaeal mid-cell location is also a hub for multiple other essential processes such as phospholipid modification, protein synthesis, and secretion. We anticipate our results will unveil unprecedented knowledge about the evolution of self-assembly and the emergency of novel ways of promoting confined growth in cells.

SG231
SepF is the FtsZ anchor in Archaea: implications for cell division in the Last Universal Common Ancestor (LUCA)
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Archaea present profound differences compared to Bacteria both at the molecular and cellular level. While most Archaea divide by binary fission using an FtsZ-based system similar to Bacteria, they lack the majority of proteins that form the bacterial divisome. Moreover, how FtsZ polymerizes and interacts with other proteins to assemble the archaeal division machinery remains largely unknown. FtsZ tethering factors in Bacteria include ZipA, FtsA and/or SepF, all of which have the ability to interact with the plasma membrane and with the conserved C-terminal tail of FtsZ (FtsZ_CTD). Archaea lack ZipA and FtsA, but instead have SepF homologues. Here, we combine structural, cell biology, and evolutionary approaches to investigate the function of archaeal SepF by using the human gut-associated archaean Methanobrevibacter smithii as a model organism. We show that M. smithii divides by FtsZ-based binary fission, FtsZ and SepF co-localize at the division plane, and we demonstrate that SepF interacts with the
FtsZCTD and liposomes in vitro. The crystal structure of the SepF-FtsZCTD complex at 2.8 Å resolution reveals the archaeal binding pocket for FtsZ on SepF and a partial conservation in the binding mode of FtsZ. Notably, major differences exist in the SepF intermolecular interactions and FtsZ binding compared to bacteria. Finally, we complement these results by a thorough analysis of the distribution and phylogeny of SepF and FtsZ across both Bacteria and Archaea. We conclude that SepF and FtsZ originated prior to the divergence of these two domains and therefore likely were part of the ancestral cell division machinery in the Last Universal Common Ancestor (LUCA). Interesting, while the system was retained in most Archaea, SepF was largely replaced by FtsA during bacterial evolution. Together, these results contribute key insights into the largely understudied process of archaeal cell division, and pave the way for a better understanding of the biology of methanogens in the human microbiome.

SG232
Characterizing actin homologs in halophilic archaean
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In all domains of life, tubulin and actin homologs determine cell shape, segregate DNA, and help with cell division. In halophilic archaean, there are known tubulin homologs; however, there is a current lack of knowledge about any actin homologs. By carefully searching for actin candidates in Halobacterium salinarum and Haloferax volcanii, two putative homologs, Haloactin I and Haloactin II, respectively, have been discovered. To understand the potential function of Haloactin I and Haloactin II, we observed the dynamics and localization of HaloactinI-GFP and HaloactinII-GFP in live cells. Haloactin I’s dynamics are similar to that of dynamically unstable eukaryotic microtubules; filaments appear to nucleate from both poles of the cell, polymerize toward the center, then randomly depolymerize back to the pole. This data will be analyzed to determine the in vivo kinetics, such as polymerization and catastrophe rate based on filament length. In addition, Haloactin I has been purified and will be used to understand the underlying biochemical and kinetic basis of actin dynamic instability. Haloactin II appears to localize to the future division site, occasionally moving onto the next future division site before division occurs. To quantitatively analyze if Haloactin II helps place the future division site, angular localization of Haloactin II polymers relative to the division site will be determined.

SG233
No membrane, no problem: condensing bacterial organelles
B. S. Parmar, S. Biedzinski, J. Wall, S. C. Weber; McGill University, Montreal, QC, CANADA.

Living cells are divided into functional compartments called organelles. In eukaryotes, lipid membranes separate organelles from the cytoplasm such that each compartment maintains a distinct biochemical composition that is tailored to its function. In contrast, prokaryotes typically lack internal membranes and instead must use other mechanisms to spatially organize the cell. Using fluorescence imaging and single-molecule tracking, we show that E. coli RNA polymerase (RNAP) organizes into clusters through liquid-liquid phase separation (LLPS). RNAP clusters, or "condensates", increase cell survival during stress, and appear to regulate ribosome biogenesis in response to nutrient availability. Our results
Demonstrate that bacteria, like eukaryotic cells, use LLPS to generate membraneless organelles that spatially organize biochemical processes to optimize cell fitness in various environments.

SG234

Cryogenic correlative single-molecule fluorescence localizations and electron tomography reveals bacterial subcellular organization

P. Dahlberg, S. Saurabh, A. Sartor, J. Wang, P. Mitchell, L. Shapiro, W. Chiu, W. Moerner; Chemistry, Stanford University, Stanford, CA.

Organization of biomolecules in bacteria is critical to their survival and adaptation. However, the nanoscale observation of this organization is challenging. Super-resolution (SR) fluorescence microscopy and cryogenic electron tomography (CET) are two powerful microscopy methods for observing the subcellular organization of biomolecules, but each approach has limitations. SR fluorescence microscopy utilizes attachment of specific proteins to fluorescent labels allowing for a SR image of a specific protein in situ, but the SR reconstructions lack cellular context beyond the proteins that have been labelled. CET on the other hand, provides molecular scale resolution, but the vast majority of proteins are not discernable in the reconstructions and the method lacks specific and non-perturbative labelling methods. Here I will describe a combination of cryogenic photoactivated localization microscopy (PALM) based SR and CET that provides precise and accurate localizations from fluorescence to annotate indiscernible proteins in high-resolution CET reconstructions. We call the approach Correlative Imaging by Annotation with Single Molecules or CIASM. Specifically, CIASM achieves single-molecule localizations with an average lateral precision of 9 nm, and the SR and CET datasets can be aligned with a relative error of ~30 nm. I will demonstrate our method in the model organism Caulobacter crescentus by annotating the positions of three proteins: McpA, PopZ, and SpmX. McpA, which forms a part of the chemoreceptor array, acts as a validation structure by being visible under both imaging modalities. In contrast, PopZ and SpmX, are a part of a polar membraneless organelle and cannot be directly identified in CET. While not directly discernable, PopZ fills a region at the cell poles that is devoid of electron-dense ribosomes. We annotate the position of PopZ with single-molecule localizations and confirm its position within the ribosome excluded region. We further use the locations of PopZ to provide context for localizations of SpmX, a low-copy integral membrane protein sequestered by PopZ as part of a signaling pathway that leads to an asymmetric cell division. Our correlative approach reveals that SpmX localizes along one side of the cell pole and its extent closely matches that of the PopZ region.

SG235

Unraveling the molecular basis of chromosome organization using single-molecule and genomic scale technologies

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In all organisms, chromosomes are highly condensed by DNA supercoiling, or the winding of the DNA strand about itself. Supercoiling impacts DNA replication, transcription, protein binding to DNA, and the three-dimensional organization of chromosomes. Supercoiling can be “negative” when the DNA strand is underwound or “positive” when the DNA strand is overwound. Because there are currently no methods to directly interrogate positive supercoiling, the distribution of positive supercoiling in genomes remains unknown. Here, we describe a method based on the chromatin immunoprecipitation of GapR, a
bacterial protein that preferentially recognizes overwound DNA, for generating high-resolution maps of positive supercoiling. Applying this method to Escherichia coli and Saccharomyces cerevisiae, we find that positive supercoiling is widespread, associated with transcription, and enriched between convergently-oriented genes. These data suggest that positive supercoiling can be introduced into genomes downstream of transcription, validating the so-called “twin-domain” model of supercoiling. Our results suggest that GapR-seq is a powerful approach that can be applied in any organism to investigate aspects of chromosome structure and organization not accessible by Hi-C or other existing methods.

Bioengineering in vitro Models of Disease

SG236
Lessons Learned Modeling Human Diseases In Vitro
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The organs on chips, or microphysiological systems, field is intended to accelerate the pace of therapeutic development. These technologiestake advantage of our understanding of cellular microenvironments in diseased organs, advances in our understanding of the biotic-abiotic interface, and iPSCell technology in such a way that we can gain human data without putting a patient at risk. In our studies in the Disease Biophysics Group, we have accumulated, after over a decade of work in the field, a collection of lessons learned in the designing, building, and testing of in vitro diseasemodels. I will review our work and suggest some of the capability gaps we face in designing these systems and where they should be first applied within the pharmaceutical and biotechnology industry.

SG237
Human Engineered Heart Tissues as a Model of Duchenne Muscular Dystrophy
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Human Engineered Heart Tissues as a Model of Duchenne Muscular Dystrophy Samantha Bremner1,4,5, Nathan J Sniadecki1,2,4,5, David L Mack1,3,4 1Department of Bioengineering, 2Department of Mechanical Engineering, 3Department of Rehabilitation Medicine, 4Institute for Stem Cell and Regenerative Medicine, 5Center for Cardiovascular Biology Cardiomyopathy is currently the leading cause of death for patients with Duchenne muscular dystrophy (DMD), a severe neuromuscular disorder affecting 1 in 3,500 to 5,000 males born in the US. Animal models have provided some insight into the mechanisms by which the absence of dystrophin protein causes cardiomyopathy, but there remains a need to develop human models of DMD to validate pathogenic mechanisms and identify therapeutic targets. Here, we have developed engineered heart tissues (EHTs) from genetically edited human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) expressing a truncated dystrophin protein. The 3D environment and uniaxial force provided sufficient maturational cues to expose the DMD phenotype in vitro. Compared to isogenic controls, DMD EHTs produced less contractile force, a phenotype not yet shown in vitro. This is likely due to the cumulative effect of other DMD phenotypes exposed in EHTs, including increased cytosolic calcium content, delayed calcium reuptake, and increased beat rate variability, and delayed sarcomere development. In this study, we demonstrate that the DMD EHT
platform promotes cardiac maturation to expose a variety of DMD phenotypes, ultimately providing a powerful platform for disease modeling.

SG238
**Generation of a rat model for EDMD lacking the emerin gene**  
**G. Valdez;** Brown University, Providence, RI.

Emery-Dreifuss muscular dystrophy (EDMD) is a rare genetic condition that progressively erodes the structure and function of skeletal and cardiac muscles. In some patients, EDMD is caused by mutations and complete loss of the emerin gene. Unfortunately, mice lacking the emerin gene fail to recapitulate EDMD pathology, thus impeding progress towards developing a treatment for this disease. In this talk, I will describe the generation and initial characterization of a rat model for EDMD lacking the emerin gene. In addition, I will describe ongoing efforts to develop in vitro models to accelerate the discovery of modifiers and to develop gene therapy approaches for EDMD.

SG239
**In vitro and in vivo rescue of alpha‐dystroglycan glycosylation upon gene editing of FKRP mutant iPS cells using a universal approach**  
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Mutations in the fukutin-related protein (FKRP) gene result in a broad spectrum of muscular dystrophy (MD) phenotypes, including the severe Walker-Warburg Syndrome (WWS). Here we developed a gene editing approach that replaces the entire mutant protein coding region with the wild type sequence to universally correct all FKRP mutations. We applied this approach to correct FKRP mutations in induced pluripotent stem (iPS) cells derived from patients displaying broad clinical severity. Our findings show rescue of functional α-DG glycosylation in WWS iPS cell-derived myotubes, as indicated by IIH6 positivity and laminin binding. Analysis for off-targets confirmed the safety of this gene editing approach. Transplantation of gene corrected myogenic progenitors in the FKRPp448L-NSG mouse model resulted in myofiber and satellite cell engraftment and importantly, restoration of α-DG functional glycosylation in vivo. Taken together, these findings provide proof-of-principle for the future therapeutic application of iPS cell-based autologous cell transplantation that can permanently restore FKRP function in patients, and validate the use of this in vitro model to study disease pathogenesis and the testing of novel therapeutic strategies.

SG240
**Mechanobiology of human induced pluripotent stem cell derived cardiomyocyte models**  
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Basic life sustaining functions such as breathing, circulation, and digestion are driven autonomously by coordinated contraction of specialized muscle cells, yet how these functions incorporate active feedback
via force sensing at the cellular level is an area of active study. Meanwhile, a variety of specialized stretch activated receptors and mechanically mediated biochemical signaling pathways have been identified. Defects in proteins of these mechanically mediated pathways and receptors have been implicated in disease states spanning cardiovascular disease, cancer growth and metastasis, neuropathy, and deafness. Thus, understanding the mechanical basis of homeostasis (health) and defective cell renewal function (disease) increasingly requires us to consider the role of mechanics. To study how cells and tissues integrate mechanical signals, we and others have developed specialized cell cultures systems and micromachined tools to stimulate and measure forces and displacements at the scale of proteins and cells. Using induced pluripotent stem cell derived cardiomyocytes, we observe cell outputs such as morphological changes, protein expression, electrophysiological signaling, force generation and transcriptional activity in response to mechanical stimuli with an eye to understanding how mechanics influences progression of a genotype with known point mutations into dysfunctional phenotypes.

SG241  
**Post-Infarction Inflammation Increases Matrix Production in iPSC-Derived Cardiac Fibroblasts via NF-κB**  
A. J. Whitehead, A. J. Engler; Bioengineering, University of California, San Diego, La Jolla, CA.

Matrix remodeling post-myocardial infarction (MI) can dictate patient outcomes, either by inducing adequate repair or creating a pathological scar. Inflammatory, biophysical, and necrotic signals typically modulate the matrix production of cardiac fibroblasts (CFs), which can determine the efficacy of remodeling. In particular, recruited macrophages have been shown to impair healing by increasing fibrosis and decreasing mechanical function, though the mechanism of action is unknown. To better understand mechanisms of aberrant matrix deposition after MI in a reductionist system, we first generated iPSC-derived CFs using a modified epicardial differentiation pathway from Bao et al., differentiating from pluripotent stem cells to cardiac progenitor cells, epicardial cells, and lastly CFs. Resultant cells demonstrated morphology, marker expression (TE-7, αSMA), and matrix competency (collagen 1 and fibronectin) similar to primary cells. ATAC-seq revealed clustering of iPSC-CFs near primary CFs, with intermediate cell types increasing in distance from CFs with earlier developmental stages. Media conditioned by naive and IL-4 treated U937 macrophages suppressed fibronectin translation, while the IL-4 condition alone increased collagen 1 synthesis. A mass spectrometry screen identified mimecan as a top unique protein in the IL-4 group. When dosed with MI-associated ligands (TGF-β, low molecular weight hyaluronic acid, and angiotensin II), iPSC-CFs increased collagen, fibronectin, and phosphorylated p65 production. Conversely, inhibition of agonist receptors led to a decrease in NF-κB phosphorylation, suggesting a conserved fibrotic mechanism. To gauge physical stress response, cells were plated on substrates of a healthy and pathological cardiac stiffness (8 and 20kPa) and found to contract on stiffer substrates after 3 days. In conclusion, we have established a platform for to interrogate inflammatory crosstalk between cardiac cell populations and have demonstrated iPSC-CFs to be responsive to many MI-associated stressors in an NF-κB-dependent mechanism.

SG243  
**Engineering Microscale Models of Cardiac and Skeletal Muscle Tissues to Probe Cell-Cell Interactions in Health and Disease**  
M. L. McCain; Biomedical Engineering, University of Southern California, Los Angeles, CA.
Because the function of every organ is dependent on its hierarchical structure, any major disruptions on the molecular, cellular, and/or tissue level often progress to disease. However, establishing linkages between structure and function across multiple spatial scales is problematic with conventional *in vivo* and *in vitro* models. Although these models are beneficial in many ways, *in vivo* models offer limited experimental control and access; are expensive and time-consuming; and have limited human relevance. On the other end of the spectrum, *in vitro* models are cheap, efficient, and can be integrated with human cells. However, these models are often too reductionist, especially for modeling tissue-level phenomena that depend on features such as tissue microstructure or interactions between the same or different cell types. Our limited ability to effectively and efficiently model healthy and diseased tissues has hindered the identification of disease mechanisms and therapeutic targets. To address these challenges, my research group engineers sophisticated *in vitro* models of cardiac and skeletal muscle tissues and implements them to elucidate new insights into multi-scale structure-function relationships.

In this presentation, I will highlight three projects that each focus on engineering models of muscle tissue to probe cell-cell interactions relevant to health and disease: (1) Establishing granular relationships between cell and tissue microstructure, propagation velocity, and expression of ion channels and gap junctions with micropatterned strands of cardiac myocytes; (2) Designing and implementing a fluidic device to image in real-time the regeneration of the coronary vasculature after cardiac injury in an explanted zebrafish heart; and (3) Developing skeletal muscle and motor neuron coculture models with enhanced synaptic activity to model neuromuscular diseases, such as amyotrophic lateral sclerosis, with patient specificity. These types of model systems are powerful for unlocking new insights into the physiology and pathophysiology of human diseases across molecular, cellular, and tissue levels. These systems also have applications in the pharmaceutical industry as microphysiological systems or “organs on chips”, which are emerging medium-throughput *in vitro* systems for human-relevant disease modeling and drug screening.

**SG242**

**Structured-surface cultureware promotes myotube alignment and improves *in vitro* assay robustness**

N. Estrella; Rare Disease Research Unit, Pfizer, Inc, Cambridge, MA.

**Objective:** We assessed the utility of nanopatterned plates and high-content analysis for studying a dystrophic phenotype and defining myotube growth patterns *in vitro*. **Approach:** Healthy and dystrophic human skeletal myoblasts were differentiated on nanopatterned cultureware to promote linearly aligned myotube formation. High-content analysis was used to quantify expression of markers specific to mature myotubes. Caspase 3/7 activity was tracked using live-imaging to assess apoptosis. **Results:** In contrast to the healthy control, dystrophic myotubes displayed maturation defects evidenced by decreased expression of critical structural and functional proteins, and a sharp spike in apoptosis 24 hours post-serum withdrawal which steadily increased throughout the differentiation process. **Conclusions:** Myoblasts cultured and differentiated on structured-surface cultureware results in physiologically relevant myotube morphology. This platform enabled us to develop improved assays for investigating relevant readouts at scale and robustness needed for drug discovery.

**SG244**

**3D engineered heart tissues meet 3D chromatin organization: in vitro modelling of nuclear pathology in dilated cardiomyopathy**
Mutations in nuclear lamina genes such as LMNA (encoding lamin A/C) cause so-called laminopathies, which are most often characterized by defects in skeletal and cardiac muscles. Besides providing mechanical support to the nucleus, the lamina regulates three-dimensional (3D) chromatin organization by anchoring heterochromatic lamina-associated domains (LADs) at the nuclear periphery. Thus, the pathophysiology of laminopathies may involve functional disruption of LADs, a process that could be exacerbated in mechanically active cells. We tested this hypothesis using patient-derived human induced pluripotent stem cells (hiPSCs) carrying a haploinsufficient LMNA mutation (heterozygous R225X) that causes dilated cardiomyopathy with severe conduction disease and arrhythmia. We differentiated mutant and CRISPR/Cas9-corrected control hiPSCs into cardiomyocytes (hiPSC-CMs), and generated three-dimensional engineered heart tissues (3D-EHTs) using a platform that allows to modulate the mechanical resistance to contraction (afterload). Mutant hiPSC-CMs and 3D-EHTs show disease-associated phenotypes such as electrophysiological abnormalities (irregular beat rate and prolonged field potential duration), stronger and prolonged calcium fluxes, and dysregulated contractility (diastolic dysfunction and systolic hyperfunction). RNA sequencing analyses revealed that laminopathic cardiomyocytes inefficiently silence certain cardiac progenitor and alternative lineage genes, upregulate factors involved in MAPK and TGF beta signaling, and show impaired activation of selected cardiac regulators. Furthermore, genome wide chromosome conformation capture analysis using in situ DNase Hi-C indicated that approximately ~1% of the genome changes its active/inactive (A/B) compartmentalization status. Most notably, multiple regions containing non-cardiac genes and that normally transition from A to B during cardiac differentiation are found in the A compartment in laminopathic cells. These include chr5 q31.3, chr19 p13.13, and chr19 q13.33-q13.41, corresponding to ectopic activation of selected genes contained within, such as the neuronal P/Q-type calcium channel CACNA1A. Pharmacological inhibition of P/Q-type calcium currents partially mitigates the elongation of field potential duration during the contraction of mutant hiPSC-CMs. Interestingly, however, A/B compartment changes do not largely correlate with the most robustly differentially expressed genes in laminopathic cardiomyocytes. Collectively, our findings indicate that LMNA haploinsufficiency in mechanically active cells leads to pathogenic gene expression alterations that are both dependent and independent from changes in 3D chromatin organization.

SG245
DNA-directed patterning enables fabrication of an in vitro bone marrow niche to study prostate tumor cell dormancy
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Metastasis is responsible for the vast majority of cancer deaths, motivating the investigation of disease progression in the face of each obstacle put forth by the body. While disseminated tumor cells (DTCs) are detected in the bone marrow of many prostate cancer patients, only 1% of DTCs yield macrometastases. There is pressing need for an in vitro system to study the characteristics of the
interactions with the microenvironment that influence prostate cancer progression. The objective of this study is to utilize a novel method that enables the high-throughput fabrication of intricate tumor microenvironments to meet this need. High-throughput DNA-directed single-cell patterning was used to create an \textit{in vitro} bone-marrow microenvironment whose replicates fit within an easy-to-image conventional chamber slide. Briefly, different amine-terminated 20-nucleotide sequences were patterned on slides using photolithography. Cells were then tagged with the complimentary oligo and introduced to DNA-patterned regions, whereupon hybridization leads to the patterning of the cells. This technique was used to localize bone cells (MC3T3 and OCT454 representing osteoblasts and osteocytes, respectively), cells of hematopoietic lineage (RAW264.7 to represent macrophages and osteoclasts), and endothelial cells (HUVEC). Initially, cell types selected for inclusion were cultured with different media conditions (alpha-MEM, DMEM, F12K) to select the best media for co-culture, with alpha-MEM ultimately providing the highest viability, as determined by live-dead staining (Range: 88.6%-99.5% viability in well plates; 94.7% viability, patterned cells). Mineralization by bone cells was verified by Alizarin Red S (ARS) staining, while osteoclast differentiation was visualized with tartrate-resistant acid phosphatase (TRAP) activity staining. Cellular phenotypes and behavior were verified using RNAscope (fluorescence in situ hybridization) and immunofluorescence staining. Finally, a fluorescently-labeled prostate cancer cell line (PC-3) was patterned along with bone cell types and imaged over the course of several days to assess proliferation, thereby determining the microenvironment influence on these cells. A typically highly proliferative line, PC-3 showed low proliferation in the presence of microenvironmental cells.

\textbf{Biological Size Control and Scaling}

SG246

\textbf{Cortical Pattern Formation and Cell Sizes}

\textbf{M. Wu}; Yale University, New Haven, CT.

Periodic wave patterns are widely observed in oscillatory or excitable chemical systems and in multicellular systems such as cardiac tissue and slime molds. More recently, waves of cortical activity, linked to actin dynamics in many cases, have been documented in a variety of single-cell systems. These spatial and temporal patterns offer unprecedented opportunities to dissect non-linear signaling networks within the cell. An intriguing but largely unexplored possibility is that molecular networks favoring their generations are evolved due to specific functional advantages they confer. I will discuss our current understanding of the pattern formation mechanisms, including the molecular definitions underlying a number of wave characteristics. In addition, we hypothesize that wave propagation in space could generate spatial information and our work on the role of cortical waves in cell size sensing will be discussed.

SG247

\textbf{Mass measurements during lymphocytic leukemia cell polyploidization decouple cell cycle and cell size dependent growth}

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Cell size is believed to influence cell growth and metabolism. Consistently, several studies have revealed that large cells have lower mass accumulation rates per unit mass (i.e., growth efficiency) than intermediate sized cells in the same population. Size-dependent growth is commonly attributed to transport limitations, such as increased diffusion timescales and decreased surface-to-volume ratio. However, separating cell size and cell cycle-dependent growth is challenging. To address this, we monitored growth efficiency of pseudodiploid mouse lymphocytic leukemia cells during normal proliferation and polyploidization. This was enabled by the development of large-channel suspended microchannel resonators that allow us to monitor buoyant mass of single cells ranging from 40 pg (small pseudodiploid cell) to over 4000 pg, with a resolution ranging from ~1% to ~0.05%. We find that cell growth efficiency increases, plateaus, and then decreases as cell cycle proceeds. This growth behavior repeats with every endomitotic cycle as cells grow into polyploidy. Overall, growth efficiency changes 33% throughout the cell cycle. In contrast, increasing cell mass by over 100-fold during polyploidization did not change growth efficiency, indicating exponential growth. Consistently, growth efficiency remained constant when cell cycle was arrested in G2. Thus, cell cycle is a primary determinant of growth efficiency. As growth remains exponential over large size scales, our work finds no evidence for transport limitations that would decrease growth efficiency.

SG248

Scaling of Gene Expression with Cell Size May Explain Size Control in Yeast

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Yeast cells must grow to a critical size before committing to division. It is unknown how size is measured. We find that as cells grow, mRNAs for some cell cycle activators scale faster than size, increasing in concentration, while mRNAs for some inhibitors scale slower than size, decreasing in concentration. Size-scaled gene expression could cause an increasing ratio of activators to inhibitors with size, triggering cell cycle entry. Consistent with this, expression of the CLN2 activator from the promoter of the WHI5 inhibitor, or vice versa, interfered with cell size homeostasis, yielding a broader distribution of cell sizes. We suggest that size homeostasis comes from differential scaling of gene expression with size. Differential regulation of gene expression as a function of cell size could affect many cellular processes.

SG249

The coordination of biosynthetic scaling with cell size and growth

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Biosynthesis and cell size are intimately linked - as cells grow the global rate of total RNA synthesis is known to increase in proportion to cell size, even in cells with the same DNA content (e.g. G1 cells). This ensures RNA and protein copy numbers scale with cell size and therefore concentrations are kept constant. This biosynthetic scaling is critical for the proper functioning of many core cellular processes, likely due to the importance of enzyme and reactant concentrations for biochemical reaction rates. Despite this, the mechanism(s) responsible for the size-scaling of global transcription are unknown. Here we report genomic, proteomic and single-molecule data that show the global occupancy of RNA
polymerase (RNAPII) increases on the genome in larger cells. This increase is accompanied by a corresponding increase in chromatin-association of the General Transcription-Factors (GTFs) of the RNAPII Pre-Initiation Complex (PIC). By contrast, changes in size are not correlated with major changes in chromatin accessibility or histone modification patterns. As the number of RNAPII and GTFs molecules in the cell is tightly correlated with size, we considered whether some PIC component(s) could be rate-limiting for RNAPII loading in a dose-dependent manner, such that as their amount increased so would global transcription. To test this model, we have synthetically generated conditional local perturbations in the nuclear amounts of RNAPII or GTF subunits. This, in effect, forces large cells to experience the levels of RNAPII or GTFs of a small cell. Based on these data we propose a working model where global transcription rates are set via the combinatorial amount of RNAPII and other PIC subunits - each partially limiting for global transcription.

SG250
The role of osmotic forces in nuclear size control
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The size of the nucleus scales robustly with cell size so that the nuclear-to-cell volume ratio (N/C Ratio) is maintained during cell growth in many cell types. The mechanism responsible for this scaling is still mysterious. The N/C Ratio is not determined merely by DNA amount, but is influenced by factors such as properties of the nuclear envelope and nuclear transport. Here, we develop a physical-based model for nuclear size control based upon osmotic pressure. The nuclear envelope can be regarded as a semi-permeable barrier that allows water and small ions to pass, but is relatively impermeable to large macromolecules. These macromolecules generate colloid osmotic pressure differences between the compartments that inflate the nucleus. Nuclear size may arise as a passive outcome of the numbers of osmotically-active macromolecules in the nucleus and cytoplasm. To test this model, we analyze N/C Ratio regulation in fission yeast using tools such as microrheology with Genetically-Encoded Multimeric nanoparticles (GEMs) as a measure of macromolecular crowding. We find that the fission yeast nucleus, in contrast to the more complex mammalian one, behaves as an ideal osmometer, whose volume is determined in a linear relationship by its osmotic environment following the Van’t Hoff law. We investigate how the N/C Ratio is altered by nuclear export inhibition and application of external compressive forces as well as the effect of protein synthesis inhibition. These studies support a quantitative model that explains how nuclear size is determined by a physical mechanism that integrates molecular crowding, osmotic and compressive forces.

SG251
Coordination of Mitochondrial Homeostasis with Cell Size
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Accurate homeostasis of mitochondria and mitochondrial DNA is important for cell function. In particular, the amount of mitochondria has to be adjusted according to the size of the cell, which varies about twofold during the cell cycle. Indeed, previous studies have shown that the concentration of mitochondria is maintained roughly constant independent of cell size. However, the molecular mechanisms underlying the coordination of mitochondrial homeostasis with cell growth and size are poorly understood, and how mitochondrial DNA content depends on cell size is unclear. Here, we use
genetic manipulation of cell volume and live-cell microscopy to demonstrate that in budding yeast, mitochondrial network length and mitochondrial DNA content increase in direct proportion to cell volume. By manipulating the concentration of essential DNA replication factors and quantifying their dose-dependent impact on mtDNA homeostasis, we identify potentially rate-limiting components of the replication machinery - providing a first step towards a mechanistic understanding of how growing cells maintain constant mtDNA concentrations.

SG252
Cell size is a determinant of stem cell potential during aging
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Stem cells are remarkably small in size. Human hematopoietic stem cells (HSCs) measure a mere 7μm in diameter. Whether small size is important for stem cell function is unknown. We find that murine HSCs enlarge under conditions known to decrease stem cell function. This decreased fitness of large HSCs is due to reduced proliferative potential. Preventing HSC enlargement by inhibiting macromolecule biosynthesis or reducing large HSCs size by shortening G1 averts the loss of stem cell potential. Naturally large HSCs also exhibit decreased stem cell potential indicating that large size characterizes exhausted HSCs under physiological conditions. Finally, we show that our findings are relevant to aging. A fraction of murine and human HSCs enlarge during aging. Preventing this age-dependent enlargement improves HSC function. We conclude that small cell size is important for stem cell function and propose that stem cell enlargement contributes to their functional decline during aging.

SG253
On Mechanisms How Cell Size Regulates Zygotic Genome Activation
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During early development, cell size reduces exponentially and cell cycle elongates after a period of embryonic cleavage, accompanied with zygotic genome activation (ZGA) transcribing hundreds to thousands of zygotic genes essential for germ layers specification. Previous studies have established a correlation between cell size, cell cycle and ZGA; however, the mechanisms of how embryonic cells coordinate the triad has been poorly known. Using metabolic labelling of nascent transcriptome and wholemount embryo confocal imaging on *Xenopus* early embryos that contain a gradient of cell sizes, we recently uncovered a new spatiotemporal patterning of ZGA dictated by cell size. To study cell size-dependent zygotic gene transcription, we developed a pipeline for sequencing all nascent zygotic RNAs from cells with 2-3 orders of magnitude variation in volumes at the animal pole (AP) and vegetal pole (VP) of blastula embryos, respectively. We discovered hundreds of zygotic genes whose activation following the spatial pattern of ZGA revealed by confocal imaging, i.e., first onset at the AP then
followed by the VP, suggesting a cell size-dependent fashion of activation. Interestingly, these genes are enriched for specifying the presumptive ectoderm, suggesting a potential role of cell size in regulating ectoderm specification. Rapid cell cycles during cleavage stages prevent zygotic gene transcription and cell cycle elongation is dependent of cell size reaching a threshold. To understand how cell size and cell cycle regulates ZGA, we treated embryos at 5 hours-post fertilization (hpf), which is prior to large-scale ZGA, with cycloheximide (CHX), an inhibitor of protein translation, and assayed nascent transcriptome at 7.5 hpf when ZGA is initiated. CHX treatment halted embryo development, resulting in cell cycle arrest in interphase and increased cell volume as compared with control embryos. Intriguingly, the CHX-treated embryos exhibited active nascent transcription and most zygotic genes are actively transcribed at a similar level as in control embryos, suggesting that cell cycle elongation is sufficient to induce ZGA. This is consistent with a sizer mechanism, but not a timer as CHX inhibits protein translation. To further elucidate the cell cycle effect on ZGA, we took advantage of a \textit{slbp2} mutant of zebrafish embryos whose cell cycle progression is dramatically slowed due to reduced histones. Remarkably, zygotic gene transcription initiated significantly earlier in the \textit{slbp2} embryos with slower cell cycle and larger cell size. Together, these data from different model organisms support a model in which cell cycle regulation underlies the cell size regulated genome activation and cell size may be important for regulating early cell fate specification.

SG254

\textbf{Nuclear to cytoplasmic ratio control of cell size in developing Drosophila embryos}

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Eggs are unusually large cells. The first order of business for the early embryos of many species is to undergo a series of rapid and reductive cleavage divisions to restore more typical cell sizes. These cleavages are driven by maternal components as the early stages of development in these species are transcriptionally inactive. After a species-specific, stereotyped number of divisions, the cell cycle slows and differentiation begins in a process known at the Mid-Blastula Transition (MBT). It has long been recognized that the increasing ratio of nuclei to cytoplasm (N/C ratio) determines the timing of this transition as the reductive cleavage divisions decrease cytoplasmic volume but the genome remains the same size. However, the molecular sensor(s) of the N/C ratio have remained elusive. We have shown that the size of the maternally provided histone pool determines the number of divisions before the MBT. A natural hypothesis is that histone depletion by the exponentially increasing number of genomes results in chromatin changes that facilitate cell cycle slowing. Here, we explore the feedbacks between transcription and the cell cycle and demonstrate that the effect of histone levels on the cell cycle is independent of their chromatin incorporation. However, many genes directly sense the N/C ratio to regulate transcription. We demonstrate that free (non-DNA bound) H3 can act as a competitive inhibitor of the DNA-damage checkpoint kinase Chk1. We hypothesize that this allows the free H3 pool to prevent cell cycle slowing until the correct N/C ratio is met.

SG255

\textbf{Investigation of cellular dry mass density regulation in growth plate chondrocytes}

\textbf{S. Oh}, C. Lee, C. Tabin, M. Kirschner; Harvard Medical School, Boston, MA.
Cell size growth is usually measured by volume or mass. The ratio of mass to volume is far more tightly regulated than volume or mass. Studies in cultured mammalian cells revealed that cellular mass density can be altered transiently (during mitosis) or differ in steady state (on different mechanical substrates), which implies that cell volume and mass can be independently controlled. Analysis of mass density is essential for understanding cell mass and volume scaling and may provide novel insight to cell size control. However, current understanding of cell size regulation and mass density variation in vivo remains limited. To this end, we introduce chondrocytes in growth plates that serve a unique model system for studying cell size and mass density regulation. Chondrocytes undergo highly coordinated successive phases of resting state (stem phase), proliferative expansion, differentiation, cell size enlargement (hypertrophy), mineralization and trans-differentiation, all aligned spatially in one dimension. Our previous work showed that the hypertrophic process involves a steep decrease in dry mass density, where the final enlarged hypertrophic chondrocytes have less than third of the cellular dry mass density of the proliferative chondrocytes. Notably, the final size of the differentiated chondrocytes differs across growth plates, not only correlated with cell cycle length during mitotic phase, but also to the growth rate of bones. In this study, we utilized Normalized Raman Imaging (NoRI), which can measure the dry mass density of cells in situ, to systematically compare the cell growth rate, total volume growth, total mass growth and cellular mass density change of chondrocytes in fast or slow growing growth plates. We find that despite the variation in terminal size of chondrocytes, the process of dry mass density reduction remains invariant. Moreover, a minority fraction of cells appear to escape dry mass density reduction in its entirety, suggesting regulation of terminal size occurs before the hypertrophic phase, likely at the point of cell cycle exit. Chondrocytes with the longest cell cycle length, found both at the secondary mineralization centers and distal ribs show almost no loss in dry mass density but increase in cases of cell cycle arrest. Seizing the variation of dry mass density regulation of chondrocytes from different skeletal elements and sites, single cell sequencing analysis of chondrocytes from different skeletal elements and sites corroborate the role of cell cycle regulation in dry mass density regulation. These biophysical and molecular characterization of dry mass density regulation and change in cells specialized for size change and control will provide the groundwork for investigation of cell size regulation.

SG256
Control of osteoblast regeneration by a train of Erk activity waves
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Regeneration is a complex chain of events that restores a tissue to its original size and shape. How are cells coordinated across the large sizes of regenerating adult tissues for proper morphogenesis? Signalling gradients established by diffusible morphogens regulate cell behaviour in myriad multicellular systems and contexts and have been proposed to direct regenerative events. In addition, feedback mechanisms in biochemical pathways can provide an effective mechanism of communication across large distances, but how they might regulate growth during tissue regeneration remains largely unresolved owing to difficulties in imaging, analyzing and conceptualizing these complex systems. To overcome those barriers, we developed the regenerating scale of adult zebrafish as a quantitative platform to study tissue growth and morphogenesis. Scales are external millimeter-sized bony disks coated with a mono-layer of bone-depositing osteoblasts. After scale loss, the osteoblast tissue regenerates in just a few weeks. Scales are an ideal system to study tissue regeneration quantitatively.
owing to their simple organization and accessibility to live imaging. We discovered that traveling waves of Erk activity instruct hypertrophic growth of the regenerating osteoblast tissue. Using a combination of theoretical and experimental analyses, we find that Erk activity propagates as concentric trigger waves across the millimeter-sized scale in few days and induce patterned tissue growth, thus orchestrating scale morphogenesis. Furthermore, periodic induction of synchronous, tissue-wide, Erk activation in place of travelling waves impairs tissue growth, suggesting that wave-distributed Erk activation is key to regeneration. Our findings reveal trigger waves as a regulatory strategy to coordinate cell behavior and control tissue morphogenesis during regeneration.

SG257
Cell-to-organism scaling relationships in *Xenopus* embryos
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It has long been observed that the size of the cell and the nucleus correlate robustly with genome size across species, as well as among tissues within a single organism. Furthermore, cell and genome size are thought to have profound effects on cellular metabolism and growth rate. Genome size is also known to correlate with developmental rate in amphibians. This suggests the existence of scaling relationships from genome size to cell size, to cellular and whole-body metabolism. Yet the underlying mechanisms are poorly understood. In order to investigate cell-to-whole organism scaling phenomena, we are comparing embryogenesis of three related *Xenopus* species that display a wide range of genome sizes, from the diploid *X. tropicalis* to the dodecaploid *X. longipes*. Our preliminary analysis has revealed that the timing of early cleavage divisions is similar across species, but that the developmental rate of *X. longipes* slows down at approximately 18 hours post fertilization when the embryos undergo neurulation. Current experiments address how egg size, genome size and the distribution of maternal resources affect whole-body metabolism. In parallel, we are developing a more molecularly tractable experimental system by generating diploid and triploid *X. laevis* embryos. We have found that the triploids are suitable for studying cell-to-organism scaling relationship since they show no developmental defects despite a significant increase in nuclear volume. We are currently comparing metabolic parameters as a function of ploidy and average cell size in the embryos. To investigate underlying mechanisms, we have generated transcriptome and proteome datasets from the different ploidy embryos during and following the emergence of cell size scaling. Although total RNA levels did not scale with ploidy, we have identified differentially expressed long non-coding RNAs as well as mRNAs involved in processes including cell cycle regulation and metabolism. In the long term, we aim to establish a quantitative experimental framework that will be amenable to theoretical modelling to elucidate the link between cell-scale properties such as cell and genome size and whole-body metabolism. Understanding these relationships will shed light on the size constraints operating during vertebrate genome evolution.

SG258
Cell-matrix and Cell-Cell Interactions in 3D Environments
Matrix mechanics and dynamics during branching morphogenesis
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The morphogenetic patterning that generates three-dimensional (3D) epithelial tissues requires dynamic concerted rearrangements of individual cells with respect to each other and the extracellular matrix (ECM) within their surrounding microenvironment. We have developed microfluidic and 3D-printing approaches to investigate the dynamics of the ECM during branching morphogenesis of the mammary gland and lung. I will discuss how we combine these experimental techniques with computational models to uncover the physical forces that drive development of the branched epithelial geometries of these organs. I will also describe similarities and differences between the local and global patterning cues provided by the ECM.

SG259

**EPH/EPHRIN regulates organization by cortical actomyosin contractility effects on cell contacts and interfacial tension**


Cellular self-organization by cell segregation leads to boundary formation and is critical for the organization of morphogenetic movements and tissue organization during development. Signaling between membrane-bound EPHRINS and EPH receptor tyrosine kinases is essential in boundary formation, driving segregation between EPHRIN-expressing and EPH-expressing cells. Here we examine how EPH/EPHRIN signaling modulates interfacial tension to regulate the strength of cellular contacts and drive cellular self-organization. Using a cell culture system to model EPH/EPHRIN cell segregation, we analyzed the contact angle of cells to estimate how EPH/EPHRIN signaling impacts interfacial tension. We observe an increase in interfacial tension between EPHB2- and EPHRIN-B1-expressing cells relative to homotypic cell pairs. Inhibitors of actomyosin contractility significantly diminish this increase, suggesting that actomyosin contractility drives heterotypic interfacial tension. Cell segregation assays reveal that EPH/EPHRIN driven segregation is actomyosin contractility dependent. Further, atomic force microscopy shows that EPH/EPHRIN signaling results in increased cortical tension during cell segregation. Interestingly, actomyosin contractility also drives increased EPHB2:EPHB2 homotypic contacts through an increase in tension away from the cell contact. Using a mouse model wherein mosaicism for EPHRIN-B1 results in cell segregation between EPHRIN-B1 expressing and non-expressing cells, we demonstrate that actomyosin contractility is critical for cell segregation in vivo. Finally, we demonstrate that tissue-wide changes in cellular organization and tissue shape are driven by minimization of heterotypic contact. These data suggest a model for cell segregation and tissue organization in which EPH/EPHRIN signaling results in heterotypic cortical actomyosin contractility that prevents cells from making stable contacts and drives cell segregation, and ultimately affects tissue morphology by modulating interfacial tension.

SG260

**Cadherin-mediated force transduction underlying epithelial barrier regulation during mitosis**

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Epithelia continuously undergo self-renewal, during which loss of cells is compensated by cell divisions. How epithelial integrity is maintained during the morphological changes that cells undergo in mitosis is not fully understood. We demonstrate that mitotic entry and concurrent cell rounding increases tensile
forces on adhesions between mitotic cells and their neighbors. To withstand intercellular forces, cadherin-based adhesions trigger an adaptive response through recruitment of the actin binding protein Vinculin, which we show is essential to reinforce junctions during mitotic rounding. Unexpectedly, Vinculin that is recruited to mitotic junctions originates selectively from the neighbors of mitotic cells, resulting in an asymmetric composition of cadherin junctions. Inhibition of junctional Vinculin recruitment in neighbors of mitotic cells results in junctional breakage and weakened epithelial barrier. Conversely, the absence of Vinculin from the cadherin complex in the mitotic cells is necessary for cells to successfully undergo mitotic rounding. Our data thus identify an asymmetric mechanoresponse at cadherin adhesions during mitosis, which is essential to maintain epithelial integrity while at the same time enable the shape changes of mitotic cells.

SG261
Mechanobiology of Tissues: De novo grown microtissues as disease models
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Designing the next wave of biological inquiry requires a thorough reflection how well the most commonly used in vitro assays reflect the conditions cells see in their native tissue environment, as well as on common assumptions made and parameters ignored. Although life is happening far out of equilibrium, our knowledge in biology, pharmaceutical sciences and medicine of cells as well as of proteins, is still mostly based on static 2D cell cultures. Furthermore, central hypotheses that drive the design of experiments are typically formulated based on equilibrium protein structure-function relationships not considering that mechanical forces might alter those. Micro- and nanotech tools that physicists and engineers have brought to biology are challenging these notions as many extra- and intracellular proteins are either part of force-bearing fiber and filamentous networks, or interact with tensed protein networks. As cells pull on proteins, they can partially unfold secondary structure thus switching a proteins’ structure-function relationships, a crucial step enabling cells to sense forces and the physical properties of their environments. Frequently made implicit assumptions will be discussed and how refined model systems are able to discover new principles that are not only of major significance to advance basic sciences, but also pave the way towards new diagnostic and therapeutic applications. While major insights into mechanobiological processes have been gained at the single cell level in the last decade, it is still not known how to effectively translate these findings to the organ level. To gain further insights into underpinning mechanisms, we have set up several microtissue platforms to better control mechanical and biochemical parameters within 2D and 3D cell cultures. Important insights will be discussed.

SG262
Mechanical mapping of tumor cell extravasation in vivo
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Metastasis defines the spread of cancer cells from the primary organs to distant sites. Emergence of a lesion can occur at varying intervals from diagnosis and in some cases following successful treatment of the primary tumor. Is there a difference in strategy to facilitate outgrowth? Here, we aim to understand how cancer cells tune their biomechanical states in organ specific ECM microenvironments. We map the 3D intracellular rheological properties of cancer cells using optical tweezer based active microrheology \textit{in vitro} and \textit{in vivo}. Real time mechanical mapping revealed that human breast cancer and melanoma
cells are stiffer pre extravasation in comparison to values measured after they have left the lumenal spaces to enter the tissue. However, cells stiffen at one day post extravasation in vivo. Our technique can be applied to different organs in vivo. We also examine the physical remodeling of the stromal microenvironment in response to the extravasated cancer cells. These data contribute to our efforts of understanding the role of the organ specific biophysical properties in the earliest stage of organ colonization.

SG263
Mechanics and mechanisms of 3D mesenchymal cell migration
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During cell migration, cytoskeletal contractile forces are transmitted to the microenvironment through integrin-based focal adhesions in a process known as mechanotransduction, which provides a bidirectional pathway for mechanical information to pass to and from the extracellular matrix (ECM). This process has been classically characterized on two-dimensional, flat surfaces, yet it remains unclear how these processes occur within a three-dimensional (3D) microenvironment and how they are incorporated into 3D migration. Here we have uncovered a unique contractile mechanism used by fibroblasts and mesenchymal cancer cells (HT-1080s) to migrate efficiently through 3D collagen gels. Analysis of ECM deformations during 3D cell spreading suggests that 3D cell polarization is highly force-dependent, with cells establishing an unbalanced matrix prestress: Fibroblasts protrude, polarize and initiate migration in the direction of highest ECM deformation. This matrix prestress is maintained through a unique contraction where cells locally pinch the matrix in a uniaxial fashion immediately behind the leading edge, which is concealed by the large absolute ECM deformations but is revealed by instantaneous kinetic analysis. We find this anterior contraction (AC) occurs prior to leading edge protrusion and coordinates a distinct 3D migration cycle that varies between cell types. Local matrix severing using two-photon ablation to disrupt matrix prestress halts forward migration, suggesting it is required for mesenchymal migration and may represent a mechanosensing mechanism. Analysis of ECM deformations suggest that fibroblasts transmit large forces to the microenvironment ~3.8-fold higher than MDA-MD-231 cells. We find that epithelial cancer cells (MDA-MB-231) rarely demonstrate a sustained matrix prestress or a uniaxial contraction; however, MDA cell lines established from secondary brain tumors that demonstrate higher myosin IIB expression, but not from secondary bone tumors, demonstrate both mesenchymal phenotypes. Moreover, analysis of integrin ligation and myosin II (A and B) expression suggests higher expression of both isoforms increases a cell’s mesenchymal characteristics. Fibroblasts lacking myosin IIA, or if integrin ligation has been reduced, lack a mesenchymal phenotype, while overexpression of either myosin IIA or IIB increases the AC phenotype. We propose that mesenchymal cells can sense ECM stiffness in 3D, generating their own matrix prestress in a myosin IIA- and integrin-dependent manner to migrate. They do so through continued anterior contractions that maintain a 3D migration cycle.

SG264
Cell adhesion and mechanotransduction at filopodia tips
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The extension of membrane protrusions is a prominent morphological feature during many cellular processes and serves as an important mechanism to probe the extracellular space and ascertain the appropriate cellular response. Filopodia are adhesive cellular protrusions specialized in recognition of the surrounding extracellular matrix (ECM). To this end, filopodia contain cell-surface receptors such as integrins that can interact with, and interpret, a wide variety of cues including ECM topography or stiffness. At focal adhesions, ECM sensing involves controlled integrin-activation as well as the recruitment of hundreds of proteins (“adhesome”) to tune cellular responses. In comparison, very little is known on how cell ECM-adhesion is regulated in filopodia. To understand how filopodia adhesions sense the ECM, we performed a structured-illumination-microscopy (SIM)-based screen to map the localization of 80 target proteins, linked to cell adhesion and migration, within filopodia. Our mapping reveals that filopodia adhesions consist of a unique set of proteins, the Filopodome, that are distinct from classical nascent adhesions or focal adhesions. Using live imaging, we observe that filopodia adhesions can give rise to nascent adhesions, which, in turn, form focal adhesions. Using fluctuation-based traction force microscopy, we find that filopodia typically align to the force field generated by focal adhesions indicating that these two structures are mechanically connected. Next, we thought to elucidate how integrins are regulated in these unique adhesion complexes. Using SIM and surface electron microscopy, we observed that integrin activation is spatially controlled in filopodia, with active integrin accumulating at filopodia tips while inactive integrin can be found throughout the filopodia shaft. RNAi depletion of integrin regulators identified FERM domain-containing talin and MYO10 as critical regulators of filopodia function. Importantly, deletion of MYO10 FERM ablates the active pool of integrin from filopodia, indicating that MYO10-FERM domain is required for integrin activation but not for integrin transport to filopodia tips. Yet, remarkably, the MYO10-FERM domain binds both α and β integrin tails restricting integrin activation. Swapping MYO10-FERM with talin-FERM leads to an over-activation of integrin receptors in filopodia. Our observations demonstrate a complex regulation of integrin activity, at filopodia tips, via MYO10-FERM domain and challenge the concept of MYO10-dependent integrin transport in filopodia. We are now using these results to build a comprehensive model of how cell adhesion is regulated within filopodia to direct cell migration in complex environments.

SG265
Age against the machine: How aging drives cancer progression
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Our current research focuses heavily on the effects of age on the tumor microenvironment, and subsequent changes in metastasis and therapy resistance. Our studies encompass biophysical changes that affect the ability of both tumor and immune cells to migrate, that affect vasculature integrity thus dictating routes of metastasis, and also secreted changes that drive metastatic signaling and response to therapy. We have also undertaken a global analysis of how the aged microenvironment promotes metastasis, using a unique resource of normal skin fibroblasts from healthy donors of differing ages, proteomics analysis, and animal models. The clinical implications of these data may also result in a change in clinical practice, as we are finding age-related differences in responses to both targeted and immunotherapy. We are using these proteomics data to guide further studies on how the aging microenvironment affects tumor dormancy and cellular metabolism.
SG266
The glutamatergic synapse protein NetG1 regulates pro-tumor functions of cancer associated fibroblasts in pancreatic cancer
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Pancreatic cancer incidence is on the rise and patients with the disease have an abysmal prognosis. This is in part due to its unique tumor microenvironment that can consist of up to 90% non-tumoral cells. In particular, fibroblasts are one of the major cell types present and their role in tumor progression is controversial. Additionally, the mechanisms of fibroblastic activation into cancer associated fibroblasts (CAFs) that dictate their functions are incompletely understood. Thus, the goal of this study was to determine key genes that could potentially regulate CAF activation and function within the tumor microenvironment of the pancreas. By comparing the transcriptomes of tumor adjacent fibroblasts and CAFs, we observed an upregulation of neuronal genes, with the glutamatergic synapse protein NetrinG1 (NetG1) as the most upregulated in CAFs. Using a 3D co-culturing system, orthotopic pancreatic cancer animal models, and immune profiling, we determined a pro-tumorigenic role for NetG1 in CAFs. In the orthotopic murine models, ablation of NetG1 in CAFs resulted in decreased tumorigenesis. Functionally, NetG1 was responsible for maintaining glutamate/glutamine (Glu/Gln) production in CAFs, which sustained pancreatic cancer cell survival under metabolic stress. Moreover, ablation of NetG1 in CAFs reduced pro-tumorigenic cytokine secretion, which increased the activation and killing potential of NK cells towards pancreatic cancer cell lines. Mechanistically, a novel NetG1 signaling hub was uncovered, whereby NetG1/glutamine synthase/vesicular glutamate transporter 1 signaled downstream to AKT/4E-BP1 and p38/FRA1, largely controlling the observed metabolic and immune functions in CAFs, respectively. Therapeutically, we targeted NetG1 in vivo with a neutralizing monoclonal antibody in the orthotopic murine model and demonstrated a reduction in tumor burden, increased necrosis, and decreased tumor cytokine and Glu secretion when compared to animals receiving IgG controls. Importantly, NetG1 expression in fibroblasts was inversely correlated with patient overall survival and could serve as an independent prognostic factor. Overall, we demonstrated the feasibility of targeting fibroblasts in cancer by normalizing CAF functions and reducing tumor burden, while providing a putative novel target in pancreatic cancer.

Centrioles, Basal Bodies, and Centrosomes

SG267
Revealing the molecular assembly of the centriole architecture using a combination of cryo-tomography and expansion microscopy.
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Revealing the molecular assembly of the centriole architecture using a combination of cryo-tomography and expansion microscopy

Virginie Hamel
University of Geneva, Cell Biology Department, Switzerland, Geneva Centrioles and basal bodies are evolutionary conserved organelles characterized by a nine-fold cylindrical arrangement of microtubule triplets, typically 450 nm long and 250 nm in diameter, orchestrating fundamental biological processes ranging from cell division to cell motility and signaling. Due to its crucial roles, defects in centriole structure or function have been associated with human pathologies including cancers, sterility and ciliopathies. In order to better understand how these structural defects are formed, it is therefore crucial to elucidate the assembly and molecular architecture of this large macromolecular complex composed of several dozen proteins. However, due to its complexity and relative small size in fluorescent microscopy, it is still challenging to understand the protein organization at the level of the centriolar architecture and correlate proteins with a specific structural element of the centriole. Here, I will present our latest results that tackle these fundamental questions using cryo-tomography and high-resolution molecular mapping using Ultrastructure expansion microscopy (U-ExM).

SG268
Centrioles compartmentalize a permanent pool of building blocks to encode a temporal memory of their duplications.


During the cell cycle, organelles must undergo biogenesis to increase their amount so that they are inherited by the daughter cells in an egalitarian way. Our recent studies using fly embryos have revealed essential aspects of how centriole duplication is regulated over time. Though, how and when a mother centriole initiates the growth of its daughter, and whether this information is inherited to the next generation, remains unclear. Here we present evidence that centrioles may compartmentalize a permanent pool of building blocks to monitor sustained duplications. Live-imaging experiments showed that cartwheel proteins Sas-6 and Ana2/STIL are present in excess on the mother centriole prior to the formation of the daughter. Super resolution microscopy demonstrated that the excess Sas-6 and Ana2 form a pool that is associated with, but separate than, the structure of mother centrioles. Our results suggest that this excess pool is permanently maintained on the mother centriole, but is replenished and inherited by the daughter centriole potentially when the daughter separates from its mother. We initially hypothesised that such excess pool of building blocks might function to serve as buffer that limits rate or period of daughter centriole growth. To investigate this idea, we looked at whether the size of this excess pool is correlated with these parameters, and found that this was not the case. To more directly test this, we developed a computational script that allows looking at the same parameters distinctly on old and new mothers, but we again observed no significant difference between the two. We then sought to test whether this excess pool may help mark a permanent site on the mother centriole, monitoring for a timely duplication of daughter centrioles. We reasoned that testing this idea in wild-type embryos is not trivial, as centriole duplications are normally tightly coupled to the Cdk/Cyclin oscillator. Therefore, we investigated whether arrested embryos retain such excess pool of
building blocks, and found that this is indeed the case. We next asked whether the size of this pool might correlate with how frequently centrioles duplicate. While examining this, we unexpectedly found that the chances of centriole duplication ($P_{\text{Dup}}$) is history-dependent: if a centriole has duplicated once, the $P_{\text{Dup}}$ of its offspring is significantly higher than the $P_{\text{Dup}}$ of all the centrioles cumulatively. So, there appears to be distinct families of centrioles, duplicating with different frequencies. Remarkably, we detected a strong positive correlation between the average size of the excess pool in each family and how frequently they duplicate. These results support the idea that centrioles compartmentalise a permanent pool of building blocks that may encode a temporal memory of their duplications.

SG269
An Acentriolar Centrosome At The C. elegans Ciliary Base
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In animal cells the functions of microtubule cytoskeleton are primarily coordinated by centriole-based centrosomes via microtubule-nucleating γ-tubulin complexes embedded in the surrounding pericentriolar material or PCM. PCM assembly has been best studied in the context of mitosis, where centriolar SPD-2 recruits PLK-1, which in turn phosphorylates key scaffolding components such as SPD-5 and CNN to promote expansion of the PCM polymer. To what extent these mechanisms apply to centrosomes in interphase or in differentiated cells remains unclear. Here, we examine a novel type of centrosome found at the ciliary base of C. elegans sensory neurons, which we show plays important roles in neuronal morphogenesis, cellular trafficking and ciliogenesis. These centrosomes display similar dynamic behavior to canonical, mitotic centrosomes, with a stable PCM scaffold and dynamically localized client proteins. Unusually, however, they are not organized by centrioles, which degenerate early in terminal differentiation. Yet, PCM not only persists but continues to grow with key scaffolding proteins including SPDS expressed under control of the RFX transcription factor DAF-19. This assembly occurs in the absence of the mitotic regulators SPD-2, AIR-1 and PLK-1, but requires tethering by PCMD-1, a protein which also plays an important role in the initial, interphase recruitment of PCM in early embryos. These results argue for distinct mechanisms for interphase and mitotic PCM assembly, with only the latter requiring PLK-1 phosphorylation to drive rapid expansion of the scaffold polymer.

SG270
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Centrosomes are a key microtubule organizing center in the cell. They comprise a pair of centrioles surrounded by a matrix of proteins termed the pericentriolar material. Through microtubule nucleation they organize the mitotic spindle, cilia and flagella. To fulfill these functions, centrosomes must be motile to achieve proper positioning within the cell. Very little is understood about the different mechanisms of centrosome motility. Typically, it is thought to be governed by the activity of microtubule motors, pushing or pulling on the microtubules anchored at the centrosome. In some cell types, centrioles lack PCM and microtubules, and are referred to as inactive centrioles. Inactive centrioles must be motile as their intracellular positioning is critical for asymmetric cell division. Despite this, the mechanisms of inactive centriole movement are not well understood. We investigated how inactive centrioles move in interphase cells. High resolution live imaging in Drosophila revealed that centrioles...
are microtubule cargo, appearing to move bidirectionally along the microtubule network in a manner involving Kinesin-1 and Dynein. Importantly, super resolution imaging demonstrated that Kinesin-1 localizes to the outside of the centriole in interphase Drosophila cells. To identify centriole components which could perform as an adaptor for motor transport, we performed a targeted RNAi screen to knock down centriole components and visualize movement. Pericentrin-Like-Protein (Plp) was identified as essential for interphase centriole movement. Through yeast-2-hybrid and an in vivo interaction assay we found that Plp interacts with the C-terminal cargo binding region of the Kinesin-1 heavy chain. By random mutagenesis, we have now generated a series of mutations in Plp which ablate interaction with Kinesin-1. Furthermore, we are working to reconstitute Plp driven Kinesin motility in vitro. Our data support a model where Plp acts as a novel motor adaptor that links the centriole to the microtubule transport machinery, facilitating movement. In this work we propose the first detailed mechanism of how centrioles can move independently of their role as an MTOC, in the context of developing tissue. Further understanding of inactive centriole motility has far-reaching implications in studies of asymmetric cell division and sensory ciliogenesis.

SG271
Centriolar satellites are required for efficient cilium assembly, maintenance and disassembly
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Centriolar satellites are dynamic, membraneless granules composed of over 200 proteins. They store, modify, and traffic centrosome and primary cilium proteins, and help to regulate both the biogenesis and some functions of centrosomes and cilium. In most cell types, satellites cluster around the perinuclear centrosome, but their integrity and cellular distribution are dynamically remodeled in response to different stimuli, such as cell cycle cues. Dissecting the specific and temporal functions and mechanisms of satellites and how these are influenced by their cellular positioning and dynamics has been challenging using genetic approaches, particularly in ciliated and proliferating cells. To address this, we developed a chemical-based trafficking assay to rapidly and efficiently redistribute satellites to either the cell periphery or center, and fuse them into stable clusters in a temporally controlled way. Induced satellite clustering at either the periphery or center resulted in antagonistic changes in the pericentrosomal levels of a subset of proteins, revealing a direct and selective role for their positioning in protein targeting and sequestration. Systematic analysis of the interactome of peripheral satellite clusters revealed enrichment of proteins implicated in cilium biogenesis and mitosis. Importantly, induction of peripheral satellite targeting in ciliated cells revealed a function for satellites not just for efficient cilium assembly but also in the maintenance of steady-state cilia and in cilia disassembly by regulating the structural integrity of the ciliary axoneme. Finally, perturbing satellite distribution and dynamics inhibited their mitotic dissolution, and mitotic progression was perturbed only in cells with centrosomal satellite clustering. Collectively, our results for the first time showed a direct link between satellite functions and their pericentrosomal clustering, suggested new mechanisms underlying satellite functions during cilium assembly, and provided a new tool for probing temporal satellite functions in different contexts.

SG272
A genetic screen for mechanisms that counter extra centrosomes
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Metazoan cells carefully regulate centrosome number. With the exception of multiciliated cells, the presence of more than two centrosomes per cell can be detrimental, causing a host of disease phenotypes including cellular aneuploidy and aberrant cell motility. Understanding the cellular mechanisms that counter extra centrosomes and associated phenotypes can reveal fundamental centrosome regulation and potential therapies of centrosome-related diseases. We previously found that cells of a resorptive intestinal epithelium, the Drosophila rectal papilla, are extremely tolerant to extra centrosomes and multipolar divisions. As part of normal organ development, papillar cells frequently accumulate extra centrosomes and undergo tripolar division. These effects can be amplified by expression of the centrosome duplication regulator Polo-like kinase 4 (Plk4). Despite this seemingly aberrant centrosome production and subsequent mitosis, no cell death occurs and the tissue develops and functions normally. In order to better understand the mechanism(s) by which these cells tolerate extra centrosomes and the extreme aneuploidy generated by tripolar cell division, we conducted a forward genetic screen for mutants that are lethal in papillar tissue only in the presence of extra centrosomes. To do so, we generated hundreds of recessive mutations on the X chromosome and screened them for papillary tissue phenotypes by generating mosaic homozygous clones specifically in a Plk4-expression background. This screen was rapid, as it took advantage of the ability of wild type rectal papillary tissue to excrete excess salt. Animals with compromised papillae, including large patches of mosaic mutant cells, die rapidly on a high-salt diet. Using this simple feeding assay, we screened 807 EMS-generated lines and identified seven mutants that reproducibly yield phenotypes only when centrosomes are amplified. Results from this screen are beginning to reveal new, physiologically relevant responses to centrosome amplification.

SG273

Understanding the impact of secretory alterations induced by centrosome amplification
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The centrosome is the main microtubule-organising centre in animal cells; important to assemble a bipolar mitotic spindle ensuring proper chromosome segregation and genomic stability. Centrosomal abnormalities, in particular centrosome amplification, are recurrent features of human tumours. Enforced centrosome amplification in vivo plays a role in tumour initiation and progression. However, centrosome amplification occurs only in a subset of cancer cells and thus, partly due to this heterogeneity, the contribution of centrosome amplification to tumours in unknown. We recently demonstrated that supernumerary centrosomes induce a paracrine-signalling axis via the secretion of proteins, which leads to non-cell autonomous invasion. This extra centrosomes-associated secretory phenotype is a consequence of a stress response caused by increased reactive oxygen species (ROS) in these cells. Furthermore, increased ROS not only drives secretion of pro-invasive factors, but also of small extracellular vesicles that change fibroblasts. We are now characterising this diverse secretory phenotype and its impact not only in cancer cells but in the surrounding microenvironment. The discovery that cells with extra centrosomes can manipulate the surrounding cells highlights unforeseen and far-reaching consequences of these abnormalities in cancer.

SG274

Inhibition of centrosome clustering reduces cystogenesis and improves kidney function in ADPKD
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Most cells in the human body, including the kidney, contain a solitary centrosome and cilium. Cells have evolved tight regulatory mechanisms to ensure they contain only one of each organelle. Centrosome amplification (CA), defined as the formation of excess centrosomes per cell, has been noted in renal cells of patients and animal models of Autosomal Dominant Polycystic Kidney Disease (ADPKD). Yet, whether this defect plays a causal role in cystogenesis has remained unclear. Using conditional mouse models with which we could manipulate centrosome biogenesis in the developing kidney, we recently discovered that CA plays a crucial role in cyst development and growth. Increasing centrosome number in vivo perturbed proliferation and differentiation of renal progenitors, resulting in defective branching morphogenesis and renal hypoplasia. CA disrupted mitotic spindle morphology, ciliary assembly, and signaling pathways essential for growth of renal progenitors, highlighting the mechanisms underlying the developmental defects. Importantly, CA alone was sufficient to induce rapid cystogenesis shortly after birth. Based on these findings, we hypothesize that cells with CA behave as “bad actors” that impact not only their progeny during cell proliferation, but also quiescent neighboring tubular epithelia (via paracrine signaling) to drive cyst growth. Thus, eliminating these cells may improve renal morphology and function. CA typically causes abnormal multipolar spindle morphology, leading to mitotic catastrophe and cell death. However, cells with CA achieve a pseudobipolar spindle configuration via centrosome clustering, a key mechanism that cells adapt to circumvent mitotic catastrophe. Here, we show that cystic kidney cells with CA can be selectively eliminated when treated with novel compounds that specifically target centrosome clustering. Inhibition of centrosome clustering in ADPKD cells in vitro promoted the formation of multipolar mitotic spindles, activation of the spindle assembly checkpoint, cell death and elimination of these cells from the population. Inhibition of centrosome clustering in ADPKD mouse models in vivo similarly caused a change from pseudobipolar to multipolar mitotic spindle formation, resulting in mitotic catastrophe and apoptosis. The elimination of these cells reduced levels of genome instability, defective ciliogenesis, and secretion of pro-inflammatory and proliferative cytokines in the subsequent daughter cells. Importantly, this resulted in decreased cyst initiation and growth, improved kidney morphology, and dramatic reduction in the progressive decline of kidney function over time. In sum, our results highlight the potential for targeting centrosome clustering as a new therapeutic strategy in ADPKD.

SG275
orb2-dependent microcephaly reveals a novel role for RNA-binding proteins in centrosome regulation.
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During neurodevelopment, neural stem cells (NSCs) undergo repeated rounds of asymmetric cell division to maintain a population of self-renewing stem cells and generate the differentiating cells required for neurogenesis. In human and Drosophila NSCs, asymmetric centrosome activity ensures error-free mitosis along a defined polarity axis. Accordingly, loss of centrosome asymmetry is associated with errant microtubule organization and defective cell divisions. In humans, aberrant centrosome function is associated with the neurodevelopmental condition microcephaly, characterized by an abnormally small brain and head circumference. Centrosome asymmetries are generated through the asymmetric enrichment of the various proteins that comprise the pericentriolar material (PCM), which dictates the microtubule-organizing activity of the centrosome. The mechanism underlying regulation of centrosome activity is poorly understood. Intriguingly, the enrichment of specific mRNAs to the
centrosome suggests that RNA localization coupled with local translation by RNA-binding proteins (RBPs) may contribute to centrosome regulation. We predicted that an RBP may bind the cognate RNAs of PCM proteins to regulate gene localization and expression. Through 3’ untranslated region (UTR) analysis of RNAs that localize to the centrosome, we identified the cytoplasmic polyadenylation element binding (CPEB) protein, Orb2, as a potential regulator of centrosome activity. CPEB proteins are implicated in mRNA localization and regulation of translation. Consequently, we examined a requirement for Orb2 in regulating NSC centrosome activities and overall neurodevelopment. Our preliminary data reveal that orb2 is a novel microcephaly gene. We found that orb2 mutant brains have a volume of >2 standard deviations below the control mean. Additionally, orb2 mutant NSCs show centrosome-associated phenotypes, including loss of centrosome asymmetry, centrosome amplification, and defective spindle morphology and orientation. Additionally, orb2 mutant brains have a reduced number of NSCs compared to control brains, suggesting the observed microcephaly is a result of failed asymmetric cell division. These data are consistent with a novel role for Orb2, an RBP, in centrosome regulation.

SG276

Coordinated loss of cilia, centrioles and multi-ciliated cells during Xenopus development

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The skin of Xenopus embryos contains numerous multiciliated cells (MCCs) that work to create a directed fluid flow over the epithelial surface. To generate this flow MCCs become extremely specialized, containing approximately 150 evenly-spaced motile cilia each anchored into the cell via a centriole/basal body and an extensive interconnected cytoskeletal network. We have observed that flow begins to decrease at stage 38 (ST38) and ultimately disappears by ST48. It has been proposed that MCCs have the potential to lose their cilia and undergo transdifferentiation into a mucus-secreting cell. By performing detailed lineage tracing experiments we show that these cells instead delaminate from the surface and undergo cell death. Prior to delaminating, these cells eliminate their cilia and extrude their centrioles. Importantly, chemically deciliating embryos earlier in development restarts the ciliogenesis transcriptional program and offers a protection against MCC loss, suggesting that MCCs have a preset life span that is coupled to cilia age. Supporting this, we have performed skin transplants of younger skin onto older embryos and find that MCCs loss cilia/centrioles based on their original age instead of overall embryo age. Collectively, this suggests that cilia loss in MCCs is a cell-autonomous, transcriptionally defined process. This work is funded by the Northwestern University T32 Training Grant in Cutaneous Biology (T32AR060710) and GM089970 from the NIH/NIGMS (BJM).

SG277

SON splicing factor control of centriole assembly through modulation of centrosome trafficking and the cytoskeletal organization

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As the primary microtubule organizing center of dividing cells, control of the number of centrosomes is critical for cell division, intracellular trafficking, and cilium formation. Regulation of centrosome number occurs through the precise assembly of centrioles that reside in the center of centrosomes. There are multiple mechanisms of centriole assembly control including at the transcriptional level. To examine
transcriptional regulation more closely, we utilized a technique that isolates centriole assembly from other cell cycle processes. We tested five splicing factors originally identified as required for centriole assembly (Balestra et al, 2013), and found that amongst these SON is dramatically required for centriole duplication. Although centriole assembly and maturation are abolished when SON is reduced, early centriole assembly events occur. Whole genome mRNA sequencing identified thousands of genes whose expression and splicing is affected by the reduction of SON, with an enrichment of genes involved in the microtubule cytoskeleton. By focusing on proteins associated with centrosomes and centriolar satellites, we investigated the roles of CEP131, PCNT, γ-tubulin and CNTROB (centrobin). SON is required for the proper splicing and expression of these genes and their reduction disrupts centriole assembly to varying degrees. Using fluorescence microscopy and Electron Tomography we observed key differences in the cytoskeletal and satellite trafficking network around the centrosomes that likely contribute to the centriole assembly defects. This establishes that SON is required for centriole assembly, partially through its activity in splicing centrosome components as well as through control of the cytoskeletal network around the centrosome.

Innate Immunity Across Systems

SG278
Signal Transduction Pathways of the Innate Immune System
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The central goal of my research is to understand the earliest events that determine innate immune responses in various multicellular organisms. We aim to create a comprehensive map of the subcellular sites of innate immune signal transduction, and determine how manipulations of early signaling events influence protective immunity. Particular focus is placed on understanding how microbial or self-derived molecules engage pattern recognition receptors, and the functional consequences of this engagement. In this seminar, I will discuss our recent investigations of innate immune signal transduction, with an emphasis on defining how known regulators of signal transduction interact with one another dynamically and functionally to execute effective host defenses.

SG279
Visualisation of IL1 inflammatory signaling reveals the stepwise assembly of MyD88, IRAK4 and IRAK1 into Myddosomes
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The adapter MyD88 connects IL1R/TLR receptors to downstream signalling cascades that activate an innate immune response. Biochemical models of IL1R/TLR signalling have limited consideration for the dynamics of MyD88 and how it associates with IRAK family kinases to signal via an oligomeric complex termed the Myddosome. Here we have developed a live cell approach to visualise IL1R and Myddosome signalling. We find that IL1 engagement with IL1R induces the nucleation of MyD88 oligomers at the plasma membrane. MyD88 oligomerization is initially reversible. However, MyD88 oligomer stability increased with size. The formation of larger MyD88 oligomers consisting of >4 monomers triggered the sequential recruitment of IRAK4 and IRAK1. IRAK4 knock out cells formed larger MyD88 oligomers and revealed that IRAK4 caps the growth and size of MyD88 oligomers. This data reveals Myddosome assembly is inducible, and that a critical size of MyD88 oligomers is required to trigger downstream
signalling. These results show the fundamental role of triggered macromolecular assembly in IL1R signalling. Furthermore, these results reveal how macromolecular assembly and protein oligomerization functions in cellular signaling pathways.

SG280

**Signalosome nucleation enables digital innate immune proinflammatory responses**

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Pathogen sensing by innate immune cells results in binary all-or-none proinflammatory responses. These responses are coupled to the formation of polymeric protein self-assemblies known as signalosomes. However, whether signalosome formation is directly responsible for binary signaling remains unclear. We have previously found that the formation of certain signalosomes involves a rate-limiting nucleation step. Here, we evaluated the existence and functional consequences of a hypothetical nucleation barrier associated with assembly of the CARD9-Bcl10-MALT1 (CBM) signalosome, which activates transcription factor NF-kB upon fungal pathogen sensing. We employed Distributed Amphifluoric FRET (DAmFRET), a recently developed flow cytometry-based method that determines the frequency of protein nucleation in cells using flow cytometry. We determined that CARD9 and Bcl10 form nucleation-limited polymers with saturating concentrations similar to those from in vitro studies. We then dissected the nucleation steps required for signalosome formation. Co-expression of artificially oligomerized CARD9 robustly nucleated Bcl10 to its polymeric form. Remarkably, CARD9 oligomers containing mutations that impair antifungal signaling in humans were unable to nucleate Bcl10, suggesting that the nucleating activity of CARD9 is essential for signaling. To address whether the nucleation-limited polymerization of Bcl10 causes binary activation of NF-kB, we introduced a single cell fluorescent transcriptional reporter of NF-kB activity into the DAmFRET assay. This approach revealed that cells containing Bcl10 assemblies strongly induced NF-kB activation, while cells expressing the same level of Bcl10 in its pre-assembled monomeric state did not. We further showed that rational point mutations that increase or decrease the nucleation barrier for Bcl10 correspondingly impaired or enhanced, respectively, NF-kB activation. Our results indicate a critical role of nucleation barriers in governing the functional outcome of signalosomes. This line of inquiry could uncover a fundamental molecular mechanism of innate immune signaling, which will inform our understanding of the causes of autoinflammatory diseases.

SG281

**DDX3X scaffold function promotes prionoid phase transition and NLRP3 inflammasome activation**

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Defects in stress granule machinery and aberrant NLRP3 inflammasome activation can promote inflammation in the brain. Stress granules can help the cell survive until the stressor is removed. Stress granules are membraneless compartments whose assembly requires liquid-liquid phase separation. NLRP3 inflammasome activation leads to the assembly of another cytoplasmic membraneless compartment called ASC specks and the induction of a form of pro-inflammatory programmed cell death called pyroptosis. ASC speck formation requires prionoid phase transitions. Thus, stress granules and NLRP3 inflamasomes involve radically different phase transitions and provide contrasting cell fate choices to a stressed cell - cell survival or pyroptosis. Cells utilize the limiting number of DDX3X
molecules, the DDX3X cellular valency, to choose between the two cell fate choices. DDX3X is an integral component of stress granules. We have identified DDX3X as a novel regulator of NLRP3 inflammasome activation, making it a common essential factor for both stress granule assembly and NLRP3 inflammasome activation. Our data suggest that induction of stress granules inhibits NLRP3 inflammasome activation by limiting the available pool of DDX3X molecules, sequestering them in stress granules, thereby allowing the cell to make a pro-survival cell fate choice. These findings also suggest that stress granules and the NLRP3 inflammasome compete for DDX3X molecules, which makes DDX3X cellular valency critical for this decision-making process. Our data suggest that the ATPase activity of DDX3X is dispensable while its scaffold function is required for NLRP3 inflammasome activation. DDX3X-NLRP3 interactions are mediated by intrinsically disordered regions in the two proteins. Using an LPS-mediated peritonitis model, we further show that stress granules inhibit NLRP3 inflammasome activation in vivo. Our data point toward a mechanistic paradigm where cells exploit the cellular valency of molecules and competition between biological processes for common essential factors to make cell fate decisions. Funding Source: T.-D.K. is supported by NIH grants AI101935, AI124346, AR056296 and CA163507 and by the American Lebanese Syrian Associated Charities

SG282
The cell biology of inflammasomes: localization, assembly and execution
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Inflammasomes are cytosolic supramolecular complexes that activate caspase-1 and other inflammatory caspases upon stimulation by pathogen- or damage-associated molecular patterns. Activated caspase-1 cleaves IL-1 family cytokines and the pore-forming protein gasdermin D (GSDMD) to induce cytokine maturation, cytokine secretion and pyroptosis. In this presentation, I will elaborate on how inflammasomes are assembled, their cellular localization and how GSDMD forms pores to allow cytokine secretion.

SG283
Regulation of phagosomal size and integrity during infection
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To exert their microbicidal function, phagosomes must maintain their membrane integrity. Some microorganisms, however, survive and can grow within phagosomes. In such instances, phagosomes need to expand to avoid rupture and microbial escape. We used the polymorphic fungus Candida albicans to study whether macrophage phagosomes are able to regulate their size to preserve their integrity during infection. We demonstrate that as C. albicans hyphae elongate within the phagosome, luminal calcium is released, which induces recruitment and insertion of lysosomes -but not of earlier compartments of the endocytic pathway- thereby increasing the phagosomal surface area. As the hyphae grow, the expanding phagosome consumes the majority of free (unfused) lysosomes. Simultaneously, compensatory lysosomal biogenesis is stimulated by activation of the transcription factor TFEB. Preventing lysosomal insertion by chelating cytosolic calcium leads to phagosomal rupture, inflammasome activation, IL-1β secretion and eventual host cell death. Our findings reveal a mechanosensitive process whereby phagosomes maintain their integrity while expanding, which ensures that growing pathogens remain entrapped within this microbicidal compartment.
SG284

**Cellular mechanisms of self versus non-self discrimination by nucleic acid-sensing Toll-like receptors**

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Nucleic acid sensing by the immune system occurs at specific intracellular sites, either inside endosomes or within the cytosol, where the genomic content of pathogens gets first exposed. Members of the Toll-like receptor (TLR) family surveil the endosomal lumen for DNA (TLR9) and RNA (TLR3/7/8) from ingested microbes to initiate protective immune responses. The endosomal localization of TLRs is believed to facilitate the recognition of foreign nucleic acids, while limiting responses to self-ligands present in the extracellular space. Accidental self-recognition by TLRs contributes to a number of autoimmune diseases. Although intracellular localization is critical for self/non-self discrimination, the molecular mechanisms that control TLR trafficking to endosomes and reinforce compartmentalized activation remain poorly understood. Here we report a new function for the TLR trafficking chaperone Unc93b1 that specifically limits signaling of TLR7, but not TLR9, and prevents TLR7-dependent autoimmunity in mice. We find that mutations in Unc93b1 leading to enhanced TLR7 signaling also disrupt binding to Syntenin-1, a protein implicated in MVB/exosome biogenesis. Both Unc93b1 and TLR7 are detectable in exosomes, suggesting that Unc93b1 recruitment of Syntenin-1 facilitates sorting of TLR7 into MVBs to terminate signaling. TLR9 is not subject to this negative regulation as it releases from Unc93b1 in endosomes. Our work establishes that Unc93b1 not only enables proper trafficking of nucleic acid-sensing TLRs but also sets the activation threshold of potentially self-reactive TLR7. Furthermore, we provide the first molecular mechanism by which endosomal TLR activation may be distinctly regulated. Understanding the differential regulation of TLRs will be key to explain the opposing roles that TLR7 and TLR9 play in certain autoimmune diseases.

SG285

**A genome-wide CRISPR/Cas9 screen identifies novel regulators of GSDMD pore formation in engineered macrophages**

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Cleavage of gasdermin D (GSDMD) by inflammatory caspases results in pore formation at the plasma membrane. GSDMD pores are recognized to mediate pyroptotic lysis of the cell or direct translocation of IL-1 family cytokines from the cytosol into the extracellular space depending on the quantity of pores or duration of plasma membrane occupancy. Hyperactive cells display evidence of fewer GSDMD pores compared to their pyroptotic counterparts, and recent work suggests that ESCRT-dependent membrane repair pathways oppose the cell fate towards pyroptosis through removal of GSDMD pores from the plasma membrane. To identify regulators of GSDMD pore formation at the plasma membrane, we utilized a genome-wide CRISPR/Cas9 screening platform in immortalized bone marrow derived macrophages (iBMMDMs). This screen uncovered novel regulators of GSDMD pore formation in the plasma membrane.

SG286

**Nanoscale ligand patterns control macrophage phagocytosis**
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Macrophages protect the body from dangerous and diseased cells through engulfment of antibody-opsonized targets, driven by Fcγ receptors (FcγRs). To prevent phagocytosis of healthy cells, macrophages must robustly ignore sub-threshold antibody stimuli while rapidly triggering engulfment of highly opsonized particles. This all-or-none decision requires the coordination of multiple receptor-ligand interactions. FcγRs on the fluid plasma membrane coalesce into nanoscale clusters in response to antibody binding, yet how this spatial organization affects downstream signaling is unknown. Here, we develop a DNA origami-based engulfment system to directly assess how nanometer-scale ligand patterns affect FcγR-driven engulfment. We find that ligands distributed into nanoscale clusters consisting of at least 8 ligands spaced ≤7 nm apart provide optimal stimulation of phagocytosis in macrophages. This optimal ligand spacing increases the probability of initiating and completing engulfment but does not affect the speed of the engulfment process itself. Macrophages decipher variations in the nanoscale organization of receptor-ligand interactions through changes in PIP₃ concentrations at the phagocytic cup. These results reveal a key decision point early in the engulfment cascade that is modulated by the nanometer-scale spatial organization of receptor-ligand interactions. Moreover, these data suggest that cancer immunotherapies intended to trigger antibody dependent engulfment of cancer cells should be designed to promote clustering of receptor-ligand interactions or be targeted at antigens that exist in pre-clustered states.

SG287
**STING activation and signaling beyond interferon**
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STING-mediated type I interferon (IFN) signaling plays an important role in innate immune response in infection and autoimmune disease. The STING signaling pathway is unique in that it requires STING trafficking from the ER through ERGIC/Golgi to vesicles, during which time STING recruits kinase TBK1 and transcription factor IRF3 which subsequently activates IFN gene expression. STING trafficking also activates other non-IFN pathways under pathophysiological conditions such as cell death and autophagy. We showed that dominant STING gain-of-function mutations constitutively activate STING trafficking in vitro and causes lung disease and T cell cytopenia independently of IFN signaling in vivo. More recently, we showed that mammalian STING possesses widespread IFN-independent activities that are important for restricting HSV-1 infection, tumor immune evasion and likely also adaptive immunity. I will discuss these and other new findings on STING activation and signaling beyond IFN.

SG289
**Transcriptional induction of a broad anti-viral and anti-bacterial innate immune response in the starlet sea anemone Nemastostella vectensis by the STING ligand 2’3’-cGAMP**
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Nucleic acid sensors are critical players in innate immunity, as they allow cells to detect and respond to infection. In vertebrates, cytosolic DNA from pathogens or tissue damage is sensed by cGAS, leading to cGAMP production and STING activation. This leads to the production of type I interferons, the major
cytokines that control antiviral responses. Interestingly, however, core components of the STING pathway predate the emergence of type I interferons in the vertebrate lineage. Our lab has previously demonstrated that the genome of the model cnidarian *Nematostella vectensis*, with which we shared our most recent common ancestor over 600 million years ago, contains functional components of the STING pathway. In this study, we aim to understand the function of this pathway in *Nematostella*. We have found that *Nematostella* express STING throughout life, and treatment of animals with cGAMP leads to the induction of putative immune genes. Some of these genes encode proteins with antibacterial activity whose induction is dependent on the transcription factor NF-κB, while others encode proteins with antiviral activity, a subset of which seem to depend on STAT signaling. We have characterized several of these antiviral and antibacterial effectors, and demonstrated their role during infection. This work indicates that the ancestral functions of STING activation included both bacterial and viral control, even in the absence of interferons.

SG290

**Putting out the fire: an anti-inflammatory path to regeneration**

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How some species can regenerate organs after injuries while others lost this capacity is a question that have been fascinating biologists for more than a century. Yet, we still do not fully comprehend how regenerative species achieve precise tissue replacement. In all cases, tissue injury triggers a sterile inflammation. How this inflammation will be resolved can make the difference between regeneration and fibrosis (scarring). A flurry of studies both in regenerative and non-regenerative species showed that leukocytes (neutrophils and macrophages) are the first responder to tissue injury and the main actors in resolving inflammation. Moreover, macrophages have been shown to provide cues to the damaged organ stem cells population to start proliferation. However, little is known about the dynamics of their activity and the molecular program regulating their function in different injury contexts. To address these questions, we use the neomycin’s induced hair cells death as a paradigm for tissue injury in the regenerating zebrafish. Using high temporal and spatial resolution imaging of macrophages we show that a tissue resident population accumulates very rapidly at the site of tissue injury and clear dead hair cells in a five hours window. Quantification of migration parameters of macrophages in the trunk shows that in addition to the macrophages that are directly involved into hair cells phagocytosis other populations react to hair cell death suggesting a broad activation of macrophages. This is confirmed by high temporal resolution scRNA-seq of macrophages showing several populations transcriptionally reacting to hair cell death. This scRNA-seq dataset also provide us with a sequence of polarization of macrophages that goes beyond the classical M1 and M2 polarization scheme. Overall, this study illuminates, for the first time *in vivo*, a high-resolution sequence of polarization of macrophages during injury of a regenerative organ and will be instrumental in developing strategies to instruct macrophages in non-regenerative species to induce regeneration over scaring.

SG291

**Signaling by cooperative assembly formation (SCAF) by TIR domains in innate immunity and cell death pathways**

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TIR (Toll/interleukin-1 receptor, resistance protein) domains are key components of innate immunity and cell-death signaling pathways in animals and plants (1). Signaling depends on association of TIR domains. We have been able to reconstitute large assemblies of the TLR (Toll-like receptor) adaptor TIR domains and determined the structure of the filamentous assembly of TLR adaptor MAL (2) and the TLR4:MAL complex by cryo-electron microscopy, and of MyD88 by micro-electron diffraction (unpublished). As an unexpected twist, we found that the TIR domains involved in cell-death pathways, including those from the human TLR adaptor SARM1, involved in axon degeneration, and those from plant immune receptors (NLRs), possess self-association-dependent NAD+-cleavage activity (3). Crystal structures of human SARM TIR domain and grapevine NLR Run1 TIR domain in complex with small-molecule ligands shed light on the structural basis of this enzymatic activity. Our studies unify the mechanism of function of TIR domains as "signaling by cooperative assembly formation (SCAF)" with prion-like features that leads to the activation of effector enzymes, and show that some TIR domains can themselves function as effector enzymes (4). The structures will be useful for therapeutic development against neurodegenerative and inflammatory diseases and for development of improved resistance in agricultural crops. 1. Ve et al (2015) Structure and function of Toll/interleukin-1 receptor/resistance protein (TIR) domains, Apoptosis, 20, 250 2. Ve et al (2017) Structural basis of TIR-domain assembly formation in MyD88/MAL-dependent TLR4 signaling, Nat Struct Mol Biol 24, 743 3. Horsefield et al (2019). NAD(+) cleavage activity by animal and plant TIR domains in cell death pathways. Science 365, 793 4. Vajjhala et al (2017). The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways. Mol Immunol 86, 23