An ASCB | EMBO Meeting

CELL BIO 25

December 6-10 | Philadelphia, PA

Abstracts:
Oral Presentations

Table of Contents

Advancing Vision and Change: Examining Student Identity Formation in Science Education

MS1501
Assessing the impact of open science in classrooms on student learning outcomes and attitudes
*Kaitlyn Casimo[1], Madison Meuler[1], Claire Weichselbaum[1], Gideon Dunster[1], Carlos Goller[2]; Allen Institute[1], North Carolina State University[2]

Introduction: The benefits of inquiry-based learning are established but often do not scale well (Govindan et al., 2020). Although quantitative reasoning is one of six core competencies outlined in Vision & Change (2011), previous research suggests that undergraduate biology students display varying degrees of comfortability with quantitative reasoning (Wachsmuth et al., 2017). Growing access to open datasets can provide a no-cost alternative to more resource-intensive inquiry-based activities, but instructors may be hesitant to use these datasets if their students lack substantial prior quantitative reasoning experience. To investigate the use of open data in biology learning, we developed two modules on mitosis and cell structure. Module A engaged students in qualitative data exploration, while Module B guided students to perform a quantitative analysis. We investigated differential impacts of these modules on student performance and affect. Methods: We recruited faculty across the US to test the modules, and randomly assigned them to Module A (15 classes, 120 student respondents) or Module B (17 classes, 287 student respondents). We surveyed students with 1) validated attitudinal surveys (Fraser, 1981; McDonald et al., 2019; Pintrich, 1991), 2) a novel concept inventory (CI) on mitosis, and 3) novel questions on attitudes toward open science. We used a retrospective-pre/post design to address overestimation bias in pre-surveys and attrition (Little et al., 2020). We also surveyed faculty on their experience teaching the module. Results: In both conditions, there was a small but statistically significant gain in self-efficacy, science identity, and attitudes toward science inquiry and open science. However, there were very few differences in student attitudinal gains between modules; differences included self-rated proficiency at carrying out investigations or analyzing data. Students in Module B were more likely to rate it as challenging. Student performance on the CI was comparable between the modules. Conclusions: Because students derived attitudinal gains from both modules, we suggest there is benefit from using authentic datasets with students, and this benefit can arise from either qualitative and quantitative exploration. A deeper understanding of how these experiences impact students can inform uptake of open science resources in education.

MS1502
Examining the influence of Vision & Change and a mentoring network on teaching philosophies and strategies
*Michael Wolyniak[1], Stephanie Mathews[4], Carlos Goller[4], Uma Swamy[3], Anjali Misra[7], Michael Moore[6], Jeremy Hsu[2], Dina Newman[5]; Hampden Sydney College[1], Chapman University[2], Florida International University[3], North Carolina State University[4], Rochester Institute of Technology[5], University of Arkansas at Little Rock[6], University of California at Santa Cruz[7]

The 2011 report Vision and Change: A Call to Action (V&C) resulted from a national effort to rethink biology curriculum. V&C outlines core concepts and core competencies for biology undergraduates and promotes evidence-based pedagogy, undergraduate research, and inclusive practices. However, it is unclear how much

biology educators know about V&C and what motivates educators' development of their teaching philosophy and practices. We leveraged the Promoting Active Learning and Mentoring (PALM) Network, a group that introduced evidence-based instructional practices (EBIPs) to instructors through mentoring, journal clubs, and a community of practice, to investigate how much V&C has influenced educator knowledge and motivation. Through focus groups, 16 mentors and 22 fellows were asked about their motivations to join PALM, familiarity with V&C, how they learned about V&C, and how PALM and/or V&C shaped the development of their teaching philosophies and strategies. We found that the teaching philosophies and practices of these educators align strongly with V&C principles. V&C provided expectancy (established value), while PALM contributed to greater instructor self-efficacy in EBIPs, overall resulting in reformed teaching philosophies and practices. This model highlights the importance of mentorship and community to successfully drive biology education reform.

MS1505

The Council on Undergraduate Research (CUR) Biology Division: A Dynamic Community Promoting Best Practices in Undergraduate Biology Education

*Michael Wolyniak[1], Beth Beason-Abmayr[5], Susan Safford[2], Jinjie Liu[4], Joyce Fernandes[3], Lance Barton[6]; Hampden Sydney College[1], Lincoln University[2], Miami University (OH)[3], Michigan State University[4], Rice University[5], University of North Carolina-Charlotte[6]

The life sciences have long been a fertile testing ground for developing best practices in undergraduate research. The Biology Division of the Council on Undergraduate Research (CUR) has worked to serve as both an agent of change for the development and implementation of such best practices and a clearinghouse for the dissemination of new and exciting trends in undergraduate research to the life sciences community. In this presentation, we describe the current work of the Biology Division to meet these goals. The Division's projects include active award programs to assist students in being able to purchase supplies for research projects and travel to professional meetings to disseminate their research, a professional development program for the integration of course-based undergraduate research experiences (CUREs) into the undergraduate classroom, a recognition program for outstanding research mentors, and an outreach initiative for maximizing the spread of Division work to as wide of an audience as possible. For each initiative, Biology Councilors are tasked with using appropriate assessment tools to maximize the benefits that they bring to the undergraduate life science education community. We encourage our colleagues in the life sciences to consider becoming a Division Representative and joining a dynamic and creative group dedicated to bringing undergraduate research opportunities to all Biology students.

MS1504

Undergraduates' sense of belonging in science is enhanced by a curriculum on preprint peer review

*Rebeccah Lijek[1], Gary McDowell[3], Sarah Fankhauser[4], Meena Balgopal[2]; Mount Holyoke College[1], Colorado State University[2], Lightoller, LLC[3], Oxford College, Emory University[4]

Undergraduates are taught that manuscript peer review is integral to science. Yet, they also learn that undergraduates are not invited to participate in the peer review process, creating an implicit sense of exclusion from the scientific community. Few training opportunities exist in manuscript peer review and none are designed for undergraduates (McDowell et al., eLife, 2019). We envision flipping this paradigm: teaching

students how to peer review manuscripts could be used as a tool to enhance undergraduates' sense of belonging in science (McDowell et al., MBoC, 2021). Intentional engagement in the peer review process would unmask part of STEM's hidden curriculum, the untaught knowledge required for success in STEM which disproportionately hinders minoritized students. With NSF support, we created and evaluated a curriculum in which biology undergraduates write and publish their own peer reviews of preprints. By authentically reviewing works-in-progress created by practicing scientists, undergraduates meaningfully contribute to the scientific literature and community. As a result, we hypothesize that our preprint peer review curriculum enhances students' science identity and sense of belonging (McDowell et al., Learned Publishing, 2022). To test this hypothesis, we used validated pre-post surveys and thematic analysis to measure changes in identity formation as a result of the curriculum. At previous CellBio meetings, we presented results that the curriculum increases students' scientific literacy (now published as Otto et al., JMBE, 2023). Here, we report that students' sense of identity as a scientist also increases significantly as a result of the curriculum ($p < 0.0001$ by RM ANOVA), with a particular impact on enhancing identity prominence and self-efficacy. Gains in science identity formation are most significant in students with minoritized racial and ethnic identities (e.g. BIPOC, Hispanic students). We demonstrate that these gains are transferable to different instructors, persist across diverse educational settings (a liberal arts college, a land grant university), and can be adapted to introductory, intermediate, and advanced courses (100- through 400-level). The curriculum will be freely available through an open educational resource (Fankhauser, in prep). By enhancing students' identity as scientists, this peer review curriculum has the potential to broaden participation in STEM and prepare students for success in the scientific workforce.

MS1503

Using Science Practices to Enact Vision and Change in the Introductory Biology Laboratory

*Lina Dahlberg[1], Katherine Hunter[1], Luka Spring[1], Elayna Worline[1], Jessica Weaver[1], Kaitlyn Bolland[1], Erin Duffy[2], Trà Huynh[1], Norda Stephenson[1]; Western Washington University[1], Solebury School[2]

The three-dimensional (3D) learning model for science teaching and learning, which was introduced by the Framework for Science Education and Next-Generation Science Standards (NGSS), includes guidance towards teaching science practices in biology. The science practices embody both content knowledge and skills, describing activities that scientist participate in their work. As such, science practices are generally aligned with the core competencies described in the Vision and Change and could be used to scaffold learning activities toward those core competencies. We used the 3D-Learning Assessment Protocol (3D-LAP), which defines criteria required for each science practice, to evaluate the extent to which introductory biology laboratory course series have the potential to engage students in science practices. Using the 3D-LAP, we found that 24/25 of the lab sessions we looked at had the potential to engage students in at least one science practice, with a total of 152 instances over the three courses. Across all three courses we found that there was a strong emphasis on Analyzing and Interpreting Data (80% of sessions), Constructing Explanations and Engaging in Argumentation Using Data (64% of sessions), and Engaging in Argument from Evidence (64% of sessions), with others at lower frequencies. However, three practices–Planning Investigations, Communicating Information, and Defining Problems/Designing Solutions–were not identified in any of the biology laboratory sessions. We also found that potential for students to engage in science practices would increase by 89% if activities that failed to meet just one 3D-LAP criterion ('near misses') were modified to meet them all. Moreover, if these 'near misses' were available to students, they would have the opportunity to engage in two otherwise absent science practices. Our analysis provides insight into how instructors can align introductory-level, course-based

laboratory work with the criteria in the 3D-LAP. Through practices-focused curricula, students can gain technical facility and participate in science practices that build towards the course level and curriculum level outcomes described in Vision and Change's core competencies and refined in the BioSkills guide.

Biological Size Control and Scaling

SG605
AI-driven automated microscopy video analysis reveals a major origin of species-level cell cycle diversity
*Kurt Schmoller[1], Benedikt Mairhörmann[1], Pascal Falter-Braun[1]; Helmholtz Zentrum München[1]

High-throughput live-cell imaging with which dynamic cellular processes can be observed for single cells over time is a powerful tool to study cell cycle regulation. However, so far studies based on this technique have been limited in scope due to the lack of fully automated analysis and the need for time-consuming manual annotation work. Here, we lifted this bottleneck by developing a Deep Learning based method for fully automated cell cycle analysis, including gapless detection and tracking of single cells as well as pedigree annotation. Using this end-to-end method, we generated a unique phenotypic dataset consisting of more than 110.000 complete cell cycles of more than 100 different S. cerevisiae isolates. Through integrated analysis with the rich omics data available, we revealed subtelomeric gene expression as a major factor for cell cycle and cell size diversity. Our results demonstrate for the first time how large-scale, automated data analysis can be used to systematically analyse the natural variability of single-cell phenotypes across a large number of strains. Our phenotypic dataset provides a unique resource that together with genome, transcriptome, and proteome data will allow studying complex, cross-generational cell cycle dynamics on an unprecedented scale.

SG601
Cell crowding drives mass density increase through changes in osmotic regulation in epithelial tissue
*Heather Rizzo[1], Andy Zhang[1], Margaret Gardel[1]; University of Chicago[1]

Cell size regulation is crucial for proper tissue development and maintenance in all organisms. We have previously shown that MDCK II cell lines undergo a series of volume-reducing divisions (VRD) as cell crowding increases, followed by G1/0 arrest at a minimal volume of ~1,000 μm3. Increasingly, it has become clear that regulation of cell mass density is an important factor contributing to cell size regulation; however, this is currently underexplored. Changes in cell mass density have been previously associated with changing cell size and cell senescence, yet the mechanism by which cells set their mass density is not understood. Additionally, much of the work concerning cell size regulation has been done in single-cell systems, while regulation of cell size in multicellular, tissue contexts is crucial for understanding tissue-scale size organization. Here we propose that cell crowding induces changes in cellular osmotic regulation affecting both mass density and cell volume. We employed model epithelial cell lines, confocal fluorescence and quantitative phase microscopy (QPM) to measure volume and mass as cell crowding increases leading up to the onset of contact inhibition and cell cycle arrest. Under these conditions, we show that cell density increases by ~1.5-fold while volume decreases by ~2.5-fold. Furthermore, cell crowding induces significant transcriptional changes in osmoregulatory gene transcripts including ATP1A1 (Na+/K+ ATPase), SCNN1A (epithelial sodium channel), and SLC12A2 (Na-K-Cl cotransporter). Based on these results, we propose that cell crowding induces changes in cellular osmotic

regulation, and this mechanism may be crucial to both cell size regulation and cellular mass density control in epithelial tissues.

SG607

CK2 modulation of the Ran pathway drives spindle morphology changes at the meiosis to mitosis transition

*Helena Cantwell[1], Hieu Nguyen[2], Arminja Kettenbach[2], Rebecca Heald[1]; University of California, Berkeley[1], Geisel School of Medicine at Dartmouth College[2]

Negligible changes to overall cell size and shape accompany the transition from meiotic divisions in the oocyte to the first embryonic mitosis in the zygote but the cell division machinery is rapidly, dramatically altered to facilitate the switch in division program. The small, barrel-shaped, peripherally located meiotic spindle is replaced by a large, centrally located zygotic spindle with abundant astral microtubules at spindle poles. We applied a drug treatment approach using Ciona eggs to identify mechanisms regulating spindle morphology at this transition. Inhibition of Casein Kinase 2 (CK2) caused a shift in spindle morphology from meiotic to mitotic-like with nucleation of astral microtubules at spindle poles. CK2 inhibition had a similar effect on spindle morphology in Xenopus egg extracts and CK2 activity decreased at fertilization in both systems. We carried out phosphoproteomics of Xenopus meiotic and mitotic cytoplasmic extracts in the presence and absence of CK2 inhibition which suggested RanGTP-regulated factors were potential targets. Inhibiting the Ran pathway suppressed the effects of CK2 inhibition on spindle morphology in both Ciona and Xenopus. We propose a model in which a change in CK2 activity at fertilization alters Ran pathway activation and thereby induces the switch to mitotic spindle morphology.

SG602

Image-enabled cell sorting identifies molecular determinants of nuclear-to-cell volume scaling

*Evgeny Zatulovskiy[1]; University of Cambridge[1]

The ratio of nuclear volume to cell volume, known as the karyoplasmic ratio, remains remarkably constant for a given cell type, a phenomenon that has fascinated cell biologists for over a century. Despite its fundamental importance, the molecular mechanisms that govern how the nucleus senses and scales its size with the overall cell volume remain poorly understood. Our study sought to comprehensively identify the genes and molecular pathways that regulate this process. We performed a genome-wide CRISPR/Cas9 knockout screen in HeLa cells, leveraging a high-speed image-assisted cell sorter (ICS) to measure nuclear and cellular dimensions simultaneously in hundreds of millions of individual cells. This approach allowed us to identify and isolate cells with aberrant nuclear-to-cell volume ratios, providing a powerful and unbiased method to screen for regulators of this fundamental cellular property. The screen identified about 160 genes whose loss significantly increased the karyoplasmic ratio and another 60 genes whose loss decreased it. The presence of known regulators of nuclear size, such as LMNA, a core component of the nuclear lamina, among our hits validated the sensitivity and reliability of our screening approach. Our analysis revealed that the identified genes often clustered into functional groups, suggesting that maintaining nuclear size is not governed by a single master regulator, but rather by coordinated molecular networks. We found that the hit genes fall into several distinct classes, including those involved in mRNA biosynthesis and metabolism, a finding consistent with previous studies in fission yeast. Among top-scoring hits we also identified regulators of cell growth, including

components of the mTOR pathway, and chromatin modifiers, highlighting the tight link between nuclear architecture and gene expression. Interestingly, our screen uncovered unexpected targets such as F-actin regulators and mitochondrial components, suggesting novel, unappreciated roles for these pathways in nuclear scaling. We are currently performing an in-depth experimental analysis of the identified pathways to dissect the precise mechanisms by which these diverse classes of genes regulate both nuclear and cell size. Our findings provide a new framework for understanding nuclear scaling, with broad relevance to a range of biological processes and diseases where this ratio is often perturbed, including cancer, developmental disorders, and stem cell biology. This work highlights how coordinated molecular networks maintain a fundamental aspect of cell physiology.

SG604

Polyploidization leads to context-dependent effects on cell growth

*Matilde Galli[1], Cindy Geerlings[1], Christa Jordan Ortiz[1], Gabriella Darmasaputra[1]; Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht[1]

Polyploid cells, which contain more than two copies of the genome, are common in many animal tissues, especially tissues that generate nutrients and metabolites for their surroundings, such as the mammalian liver and placenta. It is thought that polyploidization enhances cellular growth and metabolic output, however surprisingly little is known on how having additional copies of the genome influences cellular growth and protein synthesis. Furthermore, although polyploidy is beneficial for the growth of many organs and tissues, forced polyploidization of cells that normally do not become polyploid has detrimental effects on growth. It is unknown why different cell types respond differently to becoming polyploid, and whether cell types that naturally become polyploid have evolved specific characteristics that enable them to better cope with polyploidy. To answer these questions, we study the consequences of polyploidization on cell growth and protein synthesis in four different in vivo and in vitro polyploidy model systems: the C. elegans intestine, human hepatocyte organoids, human RPE1 cells and mouse ovarian carcinoma organoids. By using live imaging and FACS-based assays to measure cell sizes across the cell cycle we find that naturally occurring polyploid cells such as C. elegans intestinal cells and human hepatocytes linearly scale their cell size with increasing ploidy. In contrast, RPE1 cells that are forced to become polyploid by cytokinesis failure show sublinear scaling of cell size with ploidy. Interestingly, forced cytokinesis failure in hepatocytes does not lead to decreased growth rates in the subsequent G1 phase, suggesting that this cell type is inherently better equipped to handle polyploidy. We find that protein synthesis rates scale linearly with ploidy levels in C. elegans intestinal cells and in human hepatocytes, and that reducing the ploidy of C. elegans intestinal cells decreases protein translation efficiency. These results suggest that naturally occurring polyploid cells have evolved mechanisms to scale their protein synthesis rates with increasing ploidies, allowing optimal growth as ploidy levels increase. We are currently analyzing the consequences of induced polyploidization in mouse ovarian organoids with different oncogenic mutations, which will help us understand the different responses to polyploidy in healthy versus cancer cells. Together, this work will help us untangle the complex and context-specific consequences of polyploidy in different cell types.

SG608

RNA Polymerase II-dependent transcriptional activity is a limiting factor for skeletal myofiber growth, size and

myonuclear accretion.
*Fabian Montecino-Morales[1], Cristofer Calvo[1], Casey Swoboda[1], Alena Bruening[1], Vikram Prasad[1], Douglas Millay[1]; Cincinnati Children's Research Foundation[1]

There is a strong correlation between DNA content and cell size, regardless of cell type. In myofibers, growth is correlated with an increase in gene copy numbers through cell fusion (myonuclear accretion). However, the regulation of myofiber size could also involve an increase in the transcriptional output of the resident myonuclei already present in the syncytium. We sought to identify the relationships between DNA copy number and the regulation of biosynthetic capacity that determines myofiber size. We previously demonstrated that reducing myonuclear numbers through a genetic blockade of fusion in postnatal muscle results in increased transcriptional output and larger myonuclear domains, revealing an inverse relationship between myonuclear number and transcription. Through direct measurement of transcription and single-nucleus ATAC-seq analysis, we confirmed that increased transcription is accompanied by more open chromatin in myofibers with fewer nuclei. This elevated transcriptional output is associated with higher expression and activity of RNA Polymerase II (RNAPII). To test whether RNAPII levels can similarly regulate myofiber size in adult mice, we utilized a CRISPR/Cas9-mediated overexpression system. Increased levels of RNAPII resulted in elevated total mRNA levels, no activation of myonuclear accretion, and larger myofiber sizes. Under muscle overload, higher levels of RNAPII reduce the need of newly fused myonuclei but muscle size is larger than the control. Furthermore, the upregulation of RNAPII during postnatal development induces normal muscle growth with reduced myonuclear accretion resulting in larger MND size. Collectively, our data suggest that myofibers establish a setpoint for transcriptional activity based on DNA copy number and that RNAPII levels are a limiting factor for muscle growth. This finding could be exploited to treat muscle pathology where myonuclear accretion is limited as sarcopenia.

SG606
Spatial organization and compartmentalization modulate embryonic cell cycle timing
*Lendert Gelens[1], Liliana Piñeros[3], Daniel Cebrián-Lacasa[1], Daniel Ruiz-Reynés[2], Nikita Frolov[1]; KU Leuven[1], IFISC (CSIC-UIB). Instituto de Física Interdisciplinar y Sistemas Complejos[2], UC Berkeley[3]

Spatial organization is increasingly recognized as a key regulator of dynamic cellular processes. In the early embryo, the pace of the cell cycle is not fixed but tuned by how regulatory networks are arranged in space. Using frog egg extracts, we highlight two complementary examples of self-organization. First, nuclear self-organization slows cycles: the cytoplasm and nucleus are always coupled, but as the nuclear–cytoplasmic ratio increases, nuclear processes take control. This shift in dominance lengthens interphase and slows the overall oscillator (Piñeros et al., Current Biology 35, 1-16, 2025). Second, cytoplasmic self-organization can speed up cell cycles: in very large cells (~ 1 mm), mitosis is usually coordinated by concentric waves that spread outward from nuclei. But spiral waves of Cdk1 activity can also emerge, and these spirals accelerate cycles much more (Cebrián-Lacasa et al., arXiv 2412.16094, 2024). This shows that not only the presence but the pattern of self-organization matters for timing. Together these findings show that both nuclear and cytoplasmic self-organization reshape how regulatory networks operate, tuning the cell-cycle oscillator either slower or faster depending on size and spatial arrangement. This establishes a direct mechanistic link between spatial organization, size, and developmental cell cycle control.

SG603
Yeast growth is controlled by the proportional scaling of mRNA and ribosome concentrations
*Xin Gao[1]; Stanford University - Stanford, CA[1]

Despite growth being fundamental to all aspects of cell biology, we do not yet know its organizing principles in eukaryotic cells. Classic models derived from the bacteria E. coli posit that protein-synthesis rates are set by mass-action collisions between charged tRNAs produced by metabolic enzymes and mRNA-bound ribosomes. These models show that faster growth is achieved by simultaneously raising both ribosome content and peptide elongation speed. Here, we test if these models are valid for eukaryotes by combining single-molecule tracking, spike-in RNA sequencing, and proteomics in 15 carbon- and nitrogen-limited conditions using the budding yeast S. cerevisiae. Ribosome concentration increases linearly with growth rate, as in bacteria, but the peptide elongation speed remains constant (≈9 amino acids s^{-1}) and charged tRNAs are not limiting. Total mRNA concentration rises in direct proportion to ribosomes, driven by enhanced RNA polymerase II occupancy of the genome. We show that a simple kinetic model of mRNA–ribosome binding predicts both the fraction of active ribosomes, the growth rate, and responses to transcriptional perturbations. Yeast accelerate growth by coordinately and proportionally co-up-regulating total mRNA and ribosome concentrations, not by speeding elongation. Taken together, our work establishes a new framework for eukaryotic growth control and resource allocation.

Bridging Structural and Cell Biology with Cryo-EM and Cryo-ET

MS1304
Architecture of HIV-1 capsid bound to the dynein transport machinery
*Michael Cianfrocco[1], Somaye Badieyan[1], J Gentry[1], Martin Fernandez[1], Shyamal Mosalaganti[1]; University of Michigan[1]

HIV-1’s retrograde transport toward the nucleus is mediated by the microtubule motor protein dynein. Recently, we successfully reconstituted dynein-driven HIV-1 trafficking along microtubules in vitro. Our findings revealed that the HIV-1 core directly interacts with dynein and independently recruits an adaptor protein for activation, allowing the virus to engage multiple dynein cargo adaptors for movement. Building on these discoveries, we sought to define the specificity of the interaction between dynein and HIV-1. Using biochemical and single-molecule assays, we demonstrated that in vitro-assembled HIV-1 capsids can be directly recruited to microtubules and transported by dynein. On the dynein side, we identified the tail domain as the primary region engaging with HIV-1, with evidence indicating that the LC8 light chain may play a key role in this interaction. However, mutational and competition experiments ruled out the known canonical binding sites on the HIV-1 capsid as the sole determinants, suggesting that dynein may recognize an alternative site or a distinct structural feature of the capsid lattice. To further pinpoint dynein's binding site on the HIV-1 capsid, we utilized cryo-electron tomography (cryo-ET) on an in vitro complex of dynein, HIV-1 capsids, and microtubules. Unexpectedly, we observed that dynein predominantly interacts with the ends of HIV-1 capsid assemblies, with a strong preference for binding at the open end of HIV-1 tubes. These findings suggest that dynein binding may be mediated through the internal lumen of the assembled HIV-1 capsid rather than its outer surface. To test this, we imaged long HIV-1 capsid tubes to determine whether end-on attachment is utilized during trafficking

in vitro. Interestingly, our results revealed that the majority of motile events involving capsid assemblies are driven by end-on attachment, as observed through fluorescence (TIRF) and label-free (IR) microscopy. Collectively, our data demonstrate that the HIV-1 capsid lattice directly engages the dynein machinery on microtubules. Furthermore, our findings suggest that a specific capsid lumen structure is recognized by dynein for HIV-1 trafficking along microtubules. This supports the notion that partial capsid disassembly within the cytoplasm may be necessary to expose the binding interface required for dynein recruitment.

MS1307

Coordination between mitochondrial fission and mitochondrial DNA replication

*Nitish Dua[1], Boyuan Ma[1], Hamidreza Rahmani[1], Danielle Grotjahn[1]; The Scripps Research Institute[1]

Mitochondria contain their own genomes, which are present in multiple copies and distributed throughout the mitochondrial network. Mitochondrial DNA (mtDNA) encodes for proteins that are involved in oxidative phosphorylation (OXPHOS). Perturbations to mtDNA copy number, distribution, or integrity can lead to OXPHOS dysfunction and are known causes of cardiomyopathies and neurodegenerative disorders. Hence, maintenance of mtDNA is essential for cells and entails proper replication and segregation. Replication of mtDNA has been shown to be linked to mitochondrial division (fission), which ensures segregation of newly replicated mtDNA copies. mtDNA does not encode for fission machineries itself. Fission is carried out via recruitment of cytosolic factors such as DRP1 and endoplasmic reticulum. Although these two events are spatially and temporally coordinated, how mtDNA replication occurring ‘inside’ the mitochondrial matrix is linked to recruitment of mitochondrial fission factors ‘outside’ on the mitochondrial outer membrane is not known. Using correlative light microscopy and cryo-electron tomography, we show that regions of mitochondria containing replicating mtDNA nucleoids have distinct membrane ultrastructure. Our quantitative membrane analysis reveals that these mitochondria display larger membrane patches with a consistent outer-inner mitochondrial membrane distance. These patches of membrane, also contain tether-like densities which appear to “zipper” between the two membranes. We also find, that these tethers are enriched in mitochondria containing replicating mtDNA, as compared to those containing non-replicating mtDNA. We propose that these tether-like densities could represent the ATPase Family AAA Domain Containing 3A protein, ATAD3A. Using Stimulated Emission Depletion (STED) nanoscopy, we show that ATAD3A is enriched around replicating mtDNA nucleoids. Collectively, our study identifies characteristic changes in the local microenvironment of mtDNA that are associated with replication. We speculate, that changes in membrane ultrastructure and sequestration of ATAD3A at replicating mtDNA helps in recruitment of the mitochondrial fission machinery.

MS1302

Cryogenic electron tomography uncovers the native filamentous architecture of inactive lipoprotein lipase

*Kathryn Gunn[1], Anna Wheless[3], Thomas Calcraft[2], Mark Kreutzberger[4], Kareem El-Houshy[3], Edward Egelman[4], Peter Rosenthal[2], Saskia Neher[3]; Stony Brook University[1], The Francis Crick Institute[2], The University of North Carolina at Chapel Hill[3], University of Virginia[4]

Lipoprotein lipase (LPL) is the sole lipase responsible for hydrolyzing triglycerides from triglyceride-rich lipoproteins in the capillaries. LPL is essential for maintaining a healthy triglyceride concentration in the blood

and free fatty acids liberated by LPL go on to provide surrounding tissues with energy for current or future use. LPL is synthesized in an active form in the tissues surrounding the capillaries, including adipose and oxidative tissues. In adipocytes, LPL is stored in specific vesicles for long periods of time prior to secretion in response to specific nutritional signals. LPL must be regulated within these storage vesicles to prevent off-target lipid hydrolysis of phospholipids. However, the precise method of LPL regulation inside storage vesicles has remained elusive. Our previous work suggested that LPL could be forming inactive filaments inside vesicles as a form of self-regulation. To investigate this hypothesis, we used cryogenic electron tomography (cryoET) to image isolated vesicles containing LPL. We discovered that the vesicles contained LPL filaments that are packed in two distinct patterns. With subtomogram averaging we were able to elucidate a low-resolution in situ structure of the LPL filament, showing that the physiological filament is 11 nm in diameter. We then used cryogenic electron microscopy (cryoEM) to capture and solve a high-resolution structure of the 11 nm LPL filament in vitro. This revealed that the helical filaments are composed of repeating LPL dimers with occluded active sites, supporting that the filament is acting as a regulatory structure that prevents LPL activity. Together, cryoET and cryoEM uncovered a previously uncharacterized, filamentous storage form of LPL, offering new insight into its regulation and secretion.

MS1308
Genetically encoded V-tag for molecular-resolution protein localization in 3D cellular imaging
*Qiangjun Zhou[1]; Vanderbilt University[1]

Cryo-electron tomography (cryo-ET) allows imaging of cells and organelles in a near-native, frozen-hydrated state and reconstruction of 3D ultrastructure at nanometer resolution. However, cellular tomograms are dense and noisy, making it difficult to assign specific densities to individual proteins or small complexes: contrast for single proteins is weak at the low electron doses required to preserve native structure, and common molecular markers (e.g., immunogold, fluorescent tags) are poorly suited to vitrified samples or provide only tens-of-nanometers correlation precision. To bridge the gap between structural context and molecular identity, we developed compact, shape-distinctive, EM-visible protein tags that can be genetically fused to targets and detected directly in tomograms. The tags are intentionally small to minimize perturbation and designed to produce an extended, reproducible 3D motif that stands out from background density — effectively "amplifying" the visible signature of a small protein in the same way filamentous actin or tubular microtubules naturally do. One candidate, a V-shaped tag (~60 kDa), was validated by biochemical characterization and single-particle cryo-EM structure determination. We expressed the V-tag as fusions to soluble proteins in E. coli and to a mitochondrial targeting domain in HeLa cells, combining cryo-CLEM for target selection with cryo-FIB milling and cryo-ET of lamellae to acquire high-quality tomograms. The V-tag produces a distinctive, reproducible motif recognizable by visual inspection and template matching, enabling unambiguous identification of labeled proteins and organelles with localization precision approaching ~1 nm in situ. The tag has no detectable effect on trafficking or subcellular localization—for example, fusing the V-tag to the N-terminal targeting domain of TOM70 preserved correct mitochondrial outer-membrane targeting. For cell biologists, this approach provides a practical way to address mechanistic questions that require both molecular identity and native ultrastructure: mapping the precise arrangement of an adaptor protein at a nascent vesicle, distinguishing closely spaced paralogs within a membrane complex, or tracking assembly intermediates inside cells. Because the tags are genetically encoded and biochemically tractable, they integrate readily with standard molecular perturbations (mutants, truncations, rescue constructs), enabling combined functional and structural studies at molecular resolution within the native cellular context.

MS1306

Nanoscale Biopsy of Biomolecular Condensates: SOLIST Unlocks Phase Separation in Cancer and Beyond

*Philipp Erdmann[1]; Human Technopole[1]

Biomolecular condensates (BMCs) formed by liquid–liquid phase separation (LLPS) regulate key cellular processes and are implicated in diseases from cancer to neurodegeneration. Understanding their molecular organization in a native-like state requires high-resolution techniques such as cryo-electron tomography (cryo-ET). However, classical cryo-ET workflows suffer from low throughput, ice contamination, and poor stability, often restricting its use to simple cell culture. Furthermore, classical approaches would destroy the ultrastructure of in vitro reconstituted systems, where all components can be precisely controlled, which would allow us to finally dissect LLPS both biochemically and structurally. To overcome these limitations in cryo-ET sample preparation, we recently developed Serialized On-Grid Lift-In Sectioning for Tomography (SOLIST). SOLIST is a high-efficiency cryo-lift-out method that produces up to tenfold more lamellas per lift-out session, reduces sample contamination, and enables subnanometer resolution in tissues and organoids within a fraction of the time required by older methods. By bridging nanoscale resolution with mesoscale context, SOLIST makes complex, medically relevant systems accessible to cryo-ET on a routine basis. Here, we extend SOLIST with a 3D-correlative targeting pipeline to study BRD4-NUT-induced chromatin condensates, an oncogenic BMC model relevant to NUT carcinoma. Using high-pressure freezing and fiducial-guided fluorescence targeting, we selectively mill and image individual condensates in lamellas – a level of targeting not achievable before SOLIST. Tomograms revealed phase-separated droplets with compact chromatin architecture. Notably, the phase transition is very sharp and different from previous reports with sponge-like structures, which we attribute to artifacts due to sample deformation while plunge freezing in these earlier studies. While our current resolution is still limited, our work is the first demonstration of molecular-resolution LLPS imaging with high-pressure freezing and targeted lift-out. These advances establish SOLIST as a versatile platform for a “biopsy at the nanoscale”. We combine tissue-scale reach with molecular detail and open the door to in situ structural studies of phase separation in both reconstituted systems and native patient-derived samples.

MS1301

Structural Insights into Lysosomal Supercomplexes and Niemann-Pick Type C Pathology

*Daniel Serwas[1], Hannah Siems[1], Norbert Hill[1], Joshua Hutchings[1], Rahel Woldeyes[1], Jonathan Schwartz[1], Elizabeth Montabana[1], Ariana Peck[1], Yue Yu[1], Utz Ermel[1], Nikki Jean[1], Davis Perez[1], Feng Wang[3], Peng Jin[3], Shu-Hsien Sheu[1], Mohammadreza Paraan[1], Garret; Chan Zuckerberg Imaging Institute[1], Chan Zuckerberg Biohub San Francisco[2], UCSF[3]

Lysosomes are central hubs for cellular recycling and metabolic signaling, functions that depend on specialized dynamic protein organization at the lysosomal membrane. A key player in both processes is the V-ATPase, which is proposed to form higher-order supercomplexes with mTORC1, the cholesterol exporter NPC1, and other regulatory proteins. NPC1 is of particular interest, as loss-of-function mutations cause Niemann-Pick Type C, a devastating lysosomal storage disorder. How lysosomal protein complexes are organized at the molecular level, and how this organization underlies lysosome function and disease, remains a major open

question. To probe lysosome function, we used fluorescence lifetime imaging microscopy and found that lysosomal pH dropped in NPC1 knockout cells, an unexpected result given reports of lysosomal leakage. Cryo-blockface imaging revealed large vesicular Lysotracker-positive aggregates in NPC1-deficient cells. Cryo-electron tomography (cryoET) showed that these aggregates consist of individual lysosomes enclosed within a larger membrane compartment, suggesting lysophagy. CryoET further identified mitochondria with altered membrane morphology, likely reflecting cholesterol imbalance and impaired lysosome-dependent mitophagy. To investigate protein organization directly, we developed an affinity-grid system that captures intact lysosomes from human cell lysates, enabling high-throughput cryoET. Combined with an automated pipeline, we now collect >2,000 high-quality tomograms per week, yielding a dataset of ~20,000 tomograms, likely the largest of its kind. To pinpoint protein complexes, we designed probes for cryo-correlative light and electron microscopy (cryoCLEM). This approach revealed V-ATPase and mTORC1 in multiple assembly states, including larger assemblies consistent with supercomplex formation. Dual labeling showed that V-ATPase, mTORC1, and likely additional proteins co-localize within the same supercomplexes, providing the first direct structural evidence for their existence. We are now developing automated computational workflows for subtomogram averaging, aiming to achieve high-resolution maps of lysosomal supercomplexes. Together, these advances establish a framework for analyzing protein organization at the organelle scale and uncovering molecular principles of lysosome function in health and disease.

MS1315

The Eg5 motor, not spindle bipolarity, is responsible for assembling and maintaining interdigitated, anti-parallel microtubule bundles in metaphase spindles

*William Conway[1], Alex de Marco[1], Stefanie Redemann[2]; New York Structural Biology Center[1], University of Virginia[2]

During cell division, the spindle is responsible for segregating a single copy of each chromosome to each daughter cell. The spindle is a football-shaped bipolar structure composed of dynamic, polar microtubules that interact with molecular motors to assemble the spindle. In previous serial-section resin-embedded cellular electron tomography reconstructions, we observed that the spindle is composed of relatively short microtubules (2 µm mean length vs 10 µm pole-pole spindle length) and that the majority of these microtubules do not connect to either spindle pole. It is not well understood how interactions between these short microtubules, which are not directly anchored to the spindle poles, and molecular motors assemble a bipolar spindle and segregate chromosomes. A critical limitation is that resin-embedded electron tomography does not achieve high enough resolution to determine the polarity of individual microtubules or localize motors, which is crucial for understanding how motor-microtubule interactions assemble the spindle. To overcome this, we utilized FIB-milled, cellular cryo-electron tomography to reconstruct thin cross-sections of the spindle at near-atomic resolution and measure the polarity of individual microtubules. We found that the spindle is composed of dense bundles where ~50% of microtubules are anti-parallel with their neighbors. To understand whether these anti-parallel bundles were formed indirectly by branching nucleation from the spindle poles or directly by interactions with molecular motors, we reconstructed microtubule bundles in monopolar spindles formed either by inhibiting centriole duplication or by inhibiting the Eg5 motor. We found that centriole-depleted monopolar spindles, in which the Eg5 motor is still active, maintained anti-parallel, interdigitating microtubules while Eg5-inhibited monopolar spindles did not. This suggests that Eg5, rather than bipolarity, is required for the formation and/or maintenance of anti-parallel bundles. Additionally, we observed in live-cell fluorescence microscopy that the motor-active centriole-depleted monopolar spindles

often self-organized a second spindle pole after initial monopole formation, eventually leading to the formation of a bipolar spindle that entered anaphase while the Eg5-inhibited monopolar spindles remained arrested as monopoles. Taken together, our cryoET reconstructions and light microscopy indicate that the Eg5 motor is responsible for anti-parallel microtubule bundle formation and that these anti-parallel bundles are required to maintain spindle bipolarity. This supports a bottom-up, self-organized picture of spindle assembly, where motor-driven interactions assemble microtubule bundles, which in turn organize the spindle geometry.

MS1303
Tubulin variant-mediated allosteric effects modulate paclitaxel efficacy
*Shih-Chieh Ti[1], Jingyi Luo[1], Chen Jing Khoo[1]; The University of Hong Kong[1]

Therapeutic agents that target tubulin and disrupt microtubule dynamics have shown established clinical efficacy against diverse hematological malignancies and solid tumors for decades. However, the structure-activity relationship between therapeutic drugs and prognosis-associated tubulin variants remains unknown. Here, we employ cryo-electron microscopy (cryo-EM), single-molecule fluorescence microscopy, and genome-edited cancer cells to reveal that the tubulin allostery determines the efficacy of paclitaxel, a WHO-listed essential medicine for cancer treatment. Mutagenesis analyses identify a key residue, distal from the taxane-binding pocket, that plays a critical role in the hypo-sensitivity of human β3-tubulin to paclitaxel. High-resolution cryo-EM microtubule reconstructions (~2.3 Å) uncover that the paclitaxel-sensitizing mutation in β3-tubulin induces conformational changes not only in the paclitaxel-binding site and inter-tubulin interactions but also in the nucleotide-binding pocket. In particular, microtubules with paclitaxel-sensitive mutant β3-tubulin exhibit a reduced catastrophe frequency and increased affinity for end-binding (EB) proteins. The impact on microtubule dynamics is likely due to the reoriented catalytic α-tubulin E254 residue, slowing down the GTP-to-GDP transition upon the lattice incorporation of tubulin subunits. Furthermore, genome-edited cancer cells harboring paclitaxel-sensitive mutant β3-tubulin at the endogenous loci show profound susceptibility to paclitaxel. Together, we dissect how tubulin primary sequences confer allosteric effects that determine the efficacy of paclitaxel, providing a structural and mechanistic understanding for exploiting tubulin allostery to develop novel microtubule targeting agents with improved selectivity and specificity.

Cancer Cell Biology: Cellular Interactions in the Tumor Microenvironment

MS1104
Adipose-Mimetic Granular Hydrogels Reveal Biophysical Cues Driving Breast Cancer Invasion
*Brianna Knode[1], Garrett Beeghly[1], Brittany Schutrum[1], Dong Wang[3], Yitong Zheng[3], Alice Battistella[2], Ruchi Goswami[2], Jochen Guck[2], Salvatore Girardo[2], Corey O'Hern[3], Claudia Fischbach[1]; Cornell University[1], Max Planck Institute for the Science of Light[2], Yale University[3]

Approximately one in eight women will develop breast cancer in their lifetime, and metastatic breast cancer is the leading cause of cancer-related death in women worldwide. In the first steps of metastasis, cancer cells invade out of the glandular structures of breast tissue and into surrounding adipose tissue-rich mammary stroma. While mechanical properties of the tumor microenvironment are known to regulate invasion, the role of adipose tissue mechanics, which are shaped largely by adipocytes and their surrounding extracellular matrix

(ECM), remains poorly understood. This knowledge gap arises, in part, from a lack of appropriate model systems which replicate the mechanical properties of primary adipocytes. Moreover, no methods currently exist to decouple the effects of adipocyte physical and biochemical properties for investigation. To address these shortcomings, we developed an in vitro model system to identify how adipocyte size and stiffness affect ECM microarchitecture and leveraged this system to evaluate breast cancer cell migration through structurally heterogenous engineered tissue conditions. We characterized the stiffness of primary adipocytes and recapitulated these properties using adipocyte-sized polyacrylamide (PAAm) beads with tunable Young's moduli. Embedding these beads in type I collagen, i.e., the principal fibrillar ECM component of breast adipose tissue, yielded a 3D granular hydrogel that mimicked key features of adipose tissue architecture. PAAm bead inclusion facilitated the assembly of collagen networks resembling native ECM structure and enhanced breast cancer cell invasion relative to bead-free hydrogels. This effect was mediated by bead-induced collagen alignment and hierarchical matrix organization. Notably, breast cancer invasion was more pronounced in soft bead hydrogels, whereas stiffer beads physically restricted tumor cell migration. Live cell imaging of cancer cell invasion through granular hydrogels further demonstrated the rate-limiting role of cell nuclei, which deform and generate recoil force as they squeeze between PAAm beads. Lastly, bulk RNA sequencing of breast cancer cells pre-conditioned in mechanically variable granular hydrogels revealed differing transcriptional responses to adipose tissue characteristics. These findings suggest that adipocyte physical properties regulate tumor invasion by coordinating ECM architecture and cellular confinement, and they highlight the utility of tunable PAAm bead-collagen composites as a micromechanical model of adipose tissue.

MS1108

Loss of actin-binding protein Profilin-1 leverages genomic instability to trigger anti-tumor immune response in breast cancer

*Ian Eder[1], Virginia Yu[1], Sudeep Marurya[1], Partha Roy[1]; University of Pittsburgh[1]

The role of actin cytoskeletal remodeling is being increasingly recognized in the context of immune surveillance and modulation of tumor progression. Reduced expression of actin-binding protein Profilin-1 (Pfn1) in tumor cells, a key regulator of actin cytoskeletal dynamics, is a feature of human breast cancer. The goal of this study was to investigate whether loss of Pfn1 in tumor cells has any impact on immune surveillance in breast cancer. We utilized an inducible CRISPR/Cas9 knockout (KO) model to first demonstrate that triggering Pfn1 depletion selectively in tumor cells promotes a highly immunogenic tumor microenvironment marked by a striking increase in tumor infiltration by $CD8^+$ T cells, leading to a robust tumor regression in an immunocompetent mouse model of breast cancer. Notably, tumor regression is not observed in immunodeficient hosts, indicating that it is an immune-dependent effect. Mechanistically, Pfn1-deficient breast cancer cells exhibit features of genomic instability (polyploidy, micronuclei, and DNA damage) and accumulation of cytosolic DNA, leading to the activation of nucleic acid–sensing cGAS-STING pathway and the downstream signaling consequences including type-I interferon response and transcriptional upregulation of major T-cell recruiting chemokines. Based on these findings, we conclude that the loss of Pfn1 in tumor cells leverages genomic instability to foster a T-cell supportive tumor microenvironment and drive an anti-tumor immune response in breast cancer. Therefore, our studies highlight potential opportunities in targeting Pfn1-driven pathways for immunotherapeutic gain for breast cancer in the future.

MS1107
Mechanotransduction Signals from Cancer-Associated Fibroblasts Regulate Nuclear Structure and Invasive Characteristics in Cancer Cells
*Christal Rolling[1], James Holaska[2]; Cooper Medical School at Rowan University[1], Cooper Medical School at Rowan[2]

Emerin is an inner nuclear membrane protein that maintains nuclear structure and rigidity by binding to nucleoskeletal partners at the nuclear envelope. In breast cancer cells, the size and rigidity of the nucleus is inversely correlated with metastasis. Our lab showed triple-negative breast cancer (TNBC) cells had 50% less emerin protein and smaller, dysmorphic nuclei, resulting in increased cell invasion and tumor formation compared to normal breast cells. However, the mechanism of how emerin regulates cancer cell invasiveness and metastasis is unclear. We hypothesize this occurs by mechanotransduction from the tumor microenvironment (TME), as stiffening of the TME decreases nuclear rigidity. We predict emerin detects and/or transduces mechanical signals from the extracellular matrix (ECM) via the linker of nucleoskeleton and cytoskeleton (LINC) complex to alter nuclear structure by reducing emerin levels. Cancer-associated fibroblasts (CAFs) modulate the TME by depositing ECM and secreting soluble factors. To begin testing whether soluble, CAF-secreted factors affect emerin expression and nuclear structure, we incubated multiple cancer cell lines in CAF-conditioned media (CAF-CM) from both wild type (wt CAF-CM) and Yap/Taz KO CAFs (Yap KO CAF-CM). We found that there was a significant decrease in the nuclear size of cells grown in CAF-CM in at least two of these cell lines. Interestingly, we observed differences in impeded cell migration for cells grown in wt CAF-CM compared to Yap KO CAF-CM. Further, emerin expression levels were not significantly different between cells grown in unconditioned media versus CAF-CM (wt or Yap KO), suggesting that an alternate mechanism is driving the changes elicited by the CAFs. To further investigate the mechanism by which nuclear structure is regulated by the CAFs and TME, we are using verteporfin, a Yap/Taz inhibitor, and Y-27632, a ROCK inhibitor, to block biochemical signaling pathways. LINC involvement is also being analyzed using DN-KASH and CRISPR. Collectively, our data show that soluble and/or mechanical signals from CAFs play an important role in regulating nuclear structure to impact cancer cell invasion and metastasis.

MS1105
Mitochondria Anchor Local Translation and Drive Metastatic Migration
*Subhajit Dutta[1]; Universitätsklinikum Hamburg-Eppendorf (UKE)[1]

Background & Aim: Metastasis remains the principal cause of mortality in breast cancer. While cell migration is essential for metastasis, how spatio-temporal mitochondrial dynamics at the leading edge drive this process remains elusive. We hypothesised that spatially polarised mitochondrial potential and VDAC1 activity at the leading edge fuel persistent and high-speed migration (needed for metastasis) through control of local translation. Methods: We combined high-resolution live-cell microscopy, super-resolution immunofluorescence imaging, with optogenetic tools, micropatterning, and various high-dimensional microscopy techniques on breast cancer cell lines with distinct metastatic potentials (MDA-MB-231, MDA-MB-468, MCF7), pharmacological perturbation of mitochondrial potential and VDAC1 oligomerisation, and multi-omics analyses (TCGA, CCLE, proteomics) along with molecular biology to dissect mitochondria–cytoskeleton–

ribosome crosstalk. Results: Highly metastatic MDA-MB-231 cells displayed fragmented mitochondria spatially enriched at protrusions, with elevated mitochondrial membrane potential (ΔΨm) and dynamic ATP flux compared to perinuclear, mostly elongated mitochondria. VDAC1 expression was significantly upregulated in breast cancer patients (TCGA, n>1,000) and >55 BRCA cell lines, correlating with poor survival. VDAC1s also oligomerize and are enriched in protrusions, and conductance via oligomeric VDAC1s is hindered by blocking the pores with microtubule interaction. Fragmented mitochondria transport ribosomes to the leading edge, enabling local translation of focal adhesion and mechanosignaling proteins that sustain high-speed migration. Perturbation of ΔΨm (with FCCP or photoactivable uncoupler) or VDAC1 oligomerisation (with VBIT-12) activity reduces the migration speed and directionality. It closely mimics the photo-activable translational inhibition. These perturbations collapsed mitochondrial polarity, impaired cytoskeletal organisation, and reduced membrane ruffling. Mechanistically, due to impaired ribosome hitchhiking and local translation (reduced expression of EIF1B, EEF1A2), migration speed and persistence were altered. Conclusion: We identify a heretofore unknown mechanism of leading-edge mitochondrial potential dynamics, maintained by VDAC1, as a mechanometabolic driver of metastatic migration. Targeting this axis disrupts local translation and cytoskeletal organisation, offering a new therapeutic avenue to hinder metastasis.

MS1103

Nuclear envelope rupture of breast cancer cells drives epigenetic and inflammatory reprogramming

*Kayla Snare[1], Jeneille Deans[1], Bryce Jurkouich[1], Igor Salerno Filgueiras[2], Diana Cruz[1], Rodrigo Nalio Ramos[2], Guilherme Nader[1]; Children's Hospital Of Philadelphia[1], University of São Paulo[2]

Rapid growth of in situ solid tumors causes the accumulation of compressive stress at the tumor periphery, resulting in cancer cell confinement and nuclear deformation. This can lead to nuclear envelope rupture (NER), which exposes nuclear DNA to the cytosol where nucleases generate extensive DNA damage. These NER events are found at the invasive margins of many solid tumors, including human ductal carcinoma in situ (DCIS), and are thought to promote the progression from pre-invasive to invasive disease. To study these events in vitro, we use 2D microfabricated devices to confine cells to precise heights. Following NER, malignant cells recover and acquire a partial-EMT phenotype characterized by Snail1 upregulation and increased collagenolytic activity. As the nuclear envelope and lamina are known to regulate chromatin accessibility, loss of nuclear envelope integrity due to mechanical stress may trigger extensive chromatin reorganization. Indeed, epigenetic reprogramming has been documented as an adaptive response to mechanical stress in many cell types, as it directly modulates the physical properties of the nuclear envelope and, in addition, supports cell plasticity. However, we do not yet appreciate the extent of the reprogramming, nor its longevity, following NER. Given this, we hypothesized that confinement-induced NER alters chromatin organization, and that this epigenetic reorganization maintains NER-induced behavioral changes. To test this, we performed bulk RNA-sequencing on confined and non-confined DCIS cells, which revealed significant epigenetic remodeling. We observed an increase in histone demethylases and acetyltransferases following confinement-induced NER, confirmed by western blots and immunofluorescence. These results are consistent with an open, active chromatin state. Additionally, we found that DCIS cells maintain this euchromatic, invasive state up to 3 days post-confinement, indicating that NER-induced reprogramming is durable. Interestingly, our RNA-seq also revealed an NER-induced inflammatory response driven by NF-κB. Because myeloid cells represent the largest immune cell population in solid tumors, we focused on DCIS cell regulation of monocyte differentiation and activation. By culturing primary human monocytes in supernatant from DCIS cells previously subjected to confinement-induced NER, we found that NER shapes paracrine signaling of DCIS cells, which directs

monocytes towards a phagocytic and anti-inflammatory macrophage state. This work suggests that NER events drive durable signaling changes that promote both invasiveness and immune evasion.

MS1106
Targeting ACSS2 Induces Immunogenic Cell Death and Enhances Immune Activation in Breast Cancer Brain Metastases
*Riley Young[1]; Drexel University[1]

Brain metastasis in breast cancer represents a devastating, end-stage event, with median survival measured in months. This underscores the urgent need for novel therapeutic strategies. Breast cancers that metastasize to the brain face a nutrient-poor environment and exhibit metabolic reprogramming, relying heavily on acetate as a carbon source. The enzyme acetyl-CoA synthetase 2 (ACSS2) has emerged as a critical regulator of this adaptation. Our studies show that breast cancer brain metastatic (BCBM) cells express significantly higher ACSS2 levels compared to their parental counterparts. Mechanistically, ACSS2 promotes BCBM survival by suppressing ferroptosis, an immunogenic form of cell death, through E2F1-mediated regulation of the cystine-glutamate transporter SLC7A11. Consistent with this role, treatment with the brain-penetrant ACSS2 inhibitor AD-8007 in a syngeneic BCBM model significantly reduced 4T1-BR tumor growth in vivo. Single-cell RNA sequencing (scRNA-seq) revealed increased infiltration of NK and T cells in AD-8007–treated tumors, with elevated expression of CD8a, CD8b1, CD4, and CD28. Furthermore, conditioned media from ACSS2 inhibitor-treated 4T1-BR cells enhanced dendritic cell maturation in vitro, as indicated by upregulation of costimulatory molecules CD86 and CD40. These findings position ACSS2 as a key regulator of immunogenic cell death and immune activation in BCBM. Targeting ACSS2 impairs tumor survival but may also remodel the brain tumor microenvironment to enhance immune responses.

MS1102
ZBTB18 limits tumor cell pliancy and tumor cell-myeloid cell crosstalk to inhibit metastasis
*Myriam Labelle[1], Benjamin Minden-Birkenmaier[1], Ruishan Wang[1]; St. Jude Children's Research Hospital[1]

To successfully metastasize tumor cells must survive the immunological challenges and microenvironmental stresses that occur during transit through the circulation and at the site of distant metastasis. Tumor cells with a high degree of pliancy can readily alter their gene expression profiles to adapt to these various stresses and are more likely to survive and form overt metastases. The objective of this study is to define the mechanisms that confer cancer cells the flexibility needed to appropriately respond to microenvironmental cues. Using novel related murine breast cancer cell lines with different metastatic potentials, we found that highly metastatic cancer cells are characterized by lower activity of the transcriptional repressor ZBTB18 (RP58/ZFN238). Loss of activity of ZBTB18 occurs via its nuclear exclusion, and restoration of its activity via overexpression inhibits metastasis. Furthermore, nuclear exclusion of ZBTB18 is seen in tumors from breast cancer patients and correlates with more aggressive disease and with lymph node metastasis. Investigation of the mechanisms by which ZBTB18 inhibits metastasis revealed that it significantly decreases the ability of disseminated tumor cells to initiate metastatic outgrowth via a myeloid cell-dependent mechanism. At the molecular level, ZBTB18 induces widespread chromatin closing, a global epigenetic adaptation previously linked to reduced phenotypic flexibility. Thus, we hypothesized that ZBTB18 decreases global chromatin

accessibility and thereby prevents tumor cells from establishing pro-metastatic tumor cell-myeloid cell interactions. To test this hypothesis, the gene expression profiles of disseminated tumor cells from mouse lungs and of cells grown in vitro were compared. These analyses revealed that chemokines and response pathways to inflammatory signals are upregulated in tumor cells present in lungs compared to tumor cells grown in vitro. However, consistent with reduced cell pliancy, ZBTB18 overexpression significantly limited the activation of these pathways in vivo and allowed myeloid cells, in particular macrophages, to block rather than promote metastasis. Thus, we propose that ZBTB18 inhibits metastasis by restricting signaling interactions between tumor cells and myeloid cells and thereby prevents the establishment of a pro-metastatic microenvironment.

Cell Adhesion, Migration and the Extracellular Environment

MS2109
Cell confinement aftereffects include heritable loss of chromosomes and a persistently dysregulated division program
*Jeneille Deans[1], Steven Phan[2], Guilherme Nader[1], Dennis Discher[2]; Children's Hospital of Philadelphia and University of Pennsylvania[1], University of Pennsylvania[2]

Cell confinement and mechanical stress are common in solid tumors, but any impact on genetic changes remains unclear. Using chromosome reporters (ChReporters) that reveal heritable losses of chromosomes in live cells, we discover that ChReporter losses are surprisingly equivalent when cells are subjected to mild confinement (6-10μm) or severe confinement (2μm). Severe confinement causes interphase nuclear rupture in most cells, whereas mild confinement suffices to perturb mitotic spindles, prolong pro/metaphase, and eventually induce micronuclei. Indeed, micronuclei that form through chromosome mis-segregation in mitosis do not emerge during mild confinement but do emerge following release – indicating an aftereffect of the confined state. Live cell imaging post-confinement revealed that mitotic cells and their daughters also tend to arrest or die. Delayed induction of micronuclei and ChReporter loss are phenocopied by transient disruption of microtubules which prolongs pro/metaphase. Inhibition of cell cycle entry using a clinically deployed CDK4/6-inhibitor further shows ChReporter loss requires mitosis while inhibitor washout increases micronuclei and ChReporter loss. Consistent with a memory of spindle confinement, single cell and bulk RNA-sequencing demonstrate enrichment of chromosome segregation factors long after confinement.

MS2105
DAAM1 formin and VASP polymerize functionally distinct actin filament populations at focal adhesions
*Xiang Le Chua[1], Parijat Biswas[1], Andrea Vizcaíno-Castillo[1], Sarah Körber[1], Pekka Lappalainen[1]; University of Helsinki[1]

Actin filaments form a large array of biochemically and functionally distinct structures to drive fundamental cellular processes such as endocytosis, migration and morphogenesis in eukaryotic cells. Our recent findings revealed that even specific cellular structures, such as focal adhesions, harbor at least three distinct actin filament layers, which are specified by α-actinin crosslinked filaments, as well as by actin filaments decorated with tropomyosin (Tpm) isoforms Tpm1.6 and Tpm3.2. How such functionally distinct actin filaments are

assembled in cells remains an important outstanding question. Formins and Ena/VASP family proteins are key proteins that promote the elongation of linear actin filaments and have been proposed to contribute to actin filament assembly also in focal adhesions. However, the functional distinction between these two types of proteins in polymerizing linear actin filament arrays have remained elusive. Here, we reveal that depletion of DAAM1/DAAM2 formins from U2-OS cells resulted in focal adhesion defects similar to those reported earlier for Tpm3.1/Tpm3.2 knockout cells, and that Tpm3.2 protein levels were significantly reduced in DAAM1/DAAM2 double-knockout cells. On the other hand, siRNA-mediated silencing of the Ena/VASP family proteins resulted in reduced levels of α-actinin in focal adhesions and in the loss of focal adhesion -associated dorsal stress fibers. Importantly, artificial re-localization of DAAM1 to the outer mitochondrial membrane resulted in the preferential assembly of Tpm3.2-actin filaments on mitochondria, whereas mitochondrial-targeted Ena/VASP proteins (through their focal adhesion resident activator, zyxin) induced the specific assembly of α-actinin cross-linked actin filaments, but not tropomyosin-actin filaments, on mitochondria. Together, these results provide evidence that DAAM1/DAAM2 formins are responsible for the preferential assembly of Tpm3.2-decorated actin filaments, whereas the Ena/VASP-family proteins polymerize α-actinin crosslinked filaments at focal adhesions. More broadly, our study highlights specific roles for formins and Ena/VASP family proteins in assembling biochemically distinct linear actin filament arrays in cells.

MS2104

Dynamics and underlying mechanism of chemotaxis in the green algae model system Chlamydomonas reinhardtii

*Alexis Strain[1]; Louisiana State University[1]

Chemotaxis is a directed migration in response to chemical cues, which allows cells to adapt to changing nutrient conditions as well as more complex processes such as intercellular signaling mediated by pheromones and peptides. The mechanisms of chemotaxis from signaling reception to transduction are well established in most model bacteria and eukaryotic organisms. However, these components are relatively unknown in the green algae, and specifically in the model organism Chlamydomonas reinhardtii. The C. reinhardtii genome is missing several components with consistent homology to known systems such as the bacteria chemotaxis methyl-accepting chemotaxis proteins (MCPs). A previous screen of chemotaxis revealed several strains deficient in chemotaxis towards ammonium, which are further investigated here1. On such strain is a knockout of the membrane-localized protein COP5/HKR1, which does not exhibit chemotaxis toward ammonium, despite maintaining otherwise normal motility and photobehavior. COP5/HKR1 is a membrane-bound protein that encodes an eight transmembrane helix rhodopsin, histidine kinase, response regulator and motifs associated with protein-protein interaction. Microscopic analysis at the single cell level revealed that wild-type cells change the ciliary beat pattern when swimming in an ammonium gradient, while the COP5/HKR1 disrupted strain does not. We also describe a COP5/HKR1-independent negative response that occurs at higher concentration gradients of ammonium (<100 mM of NH4+). The second finding validated here is the role of the calcium voltage-gated channel CAV2 in Chemotaxis. In wild-type strains CAV2 mediates calcium influx into cilia2. The role of calcium and CAV2 was confirmed by showing that exposure to the calcium chelator EGTA reduced chemotaxis. Microscopic analysis further shows the effect of calcium influx on ciliary beat patterns during chemotaxis. These findings reveal two key components which are required for chemotaxis signaling in green algae and provides preliminary insights into similarities and divergences between chemotaxis in green algae and other model systems.

MS2106

Intracellular pH modulates cancer migration phenotypes under compressive environments.

*Carlos Arias-Matus[1], Leah Lund[1], Alice Burchett[1], Meenal Datta[1], Katharine White[1]; University of Notre Dame[1]

Cancer cells exhibit an increased intracellular pH (pHi) compared to normal cells which promotes a variety of cancer cell behaviors. In addition to dysregulated pHi, cancer cells also experience an abnormal mechanical tumor microenvironment, including increased mechanical compression (solid stress). Under compression, Rho/ROCK are part of the central regulators of the actomyosin cytoskeleton, allowing cell movement, invasion and survival to new environments. Both increased pHi and solid stress are associated with aggressive cancer phenotypes, including increased cell migration, however their co-regulation and the molecular mechanisms that link the two are still unknown. Understanding this connection could uncover potential molecular targets that enable cancer cells to adopt aggressive phenotypes. Here, we show that pHi dynamics of cancer cells are sensitive to mechanical compression. Using a 2D in vitro compression model, we show that mechanical compression of 0.14 kPa for 24 hours raises pHi, increases pHi heterogeneity, and causes cells to shift from a mesenchymal to an amoeboid morphological phenotype. We further assessed how compression altered cell behaviors and pHi during cell migration using a wound healing assay. We demonstrated that uncompressed cancer cells exhibited controlled leading lamellipodium protrusive motion and slight retraction on the opposite side, while compressed cells (with high pHi) adopted blebbing, an amoeboid migratory phenotype, with both increased cell speed and migration compared to uncompressed cells. To test whether pHi is required for amoeboid morphology and movement under compression, we used CRISPR knockout cells, lacking the sodium proton exchanger NHE1 (NHE1 K.O.). We found that knocking out the sodium proton exchanger (NHE1) lowers pHi under compression and abrogates amoeboid morphology and migration phenotypes. Importantly, experimentally raising pHi in NHE1 K.O. cells partially restored amoeboid blebbing formation, demonstrating that high pHi is a necessary mediator of the mechanosensitive amoeboid migration under compression. Pharmacological inhibition of Myosin II and ROCK similarly suppressed the amoeboid phenotype, even though pHi was still increased under compression. This implicates RhoA signaling as the downstream regulator of the pH-dependent amoeboid phenotype. Together, these findings suggest high pHi is a necessary regulator of RhoA/ROCK-dependent amoeboid migration phenotypes under compression. By establishing pHi as a regulator of compression-induced amoeboid migration, future work could therapeutically target pHi to limit aggressive and mechanically adaptive cancer behaviors.

MS2102

Long range mutual activation establishes Rho and Rac polarity during cell migration

*Henry De Belly[1], Orion Weiner[1]; University of California, San Francisco[1]

In migrating cells, the GTPase Rac organizes a protrusive front, whereas Rho organizes a contractile back. How these GTPases are appropriately positioned at the opposite poles of migrating cells is unknown. Here we leverage optogenetics, manipulation of cell mechanics, and mathematical modeling to reveal a surprising mechanochemical long-range mutual activation of the front and back polarity programs that complements their well-known local mutual inhibition. Rac-based protrusion stimulates Rho activation at the opposite side of the cell via membrane tension-based activation of mTORC2. Conversely, Rho-based contraction induces

cortical-flow-based regulation of phosphoinositide signaling to trigger Rac activation at the opposite side of the cell. We develop a minimal unifying mechanochemical model of the cell to explain how this long-range facilitation complements local inhibition to enable robust Rho and Rac partitioning. We show that this long-range mutual activation of Rac and Rho is conserved in epithelial cells and is also essential for efficient polarity and migration of primary human T cells, indicating the generality of this circuit. Our findings demonstrate that the actin cortex and plasma membrane function as an integrated mechanochemical system for long-range partitioning of Rac and Rho during cell migration and likely other cellular contexts.

MS2101
Nematic fluctuation of FA elements, energized by active forces, mediates "patrolling" mechanosensing
*Congying Wu[1], Congying Wu[1]; Peking University[1]

Cells not only respond to mechanical perturbation but also actively explore their physical environment, in order to adhere, migrate or just to stay and grow. Focal adhesions (FAs) are membrane-less organelles that connect the cytoskeleton to the extracellular matrix while transmitting force. Although originally described as “plaque-like”, the mechanotransduction within FAs are known to be discontinuous and dynamic. However, knowledge on how discrete FA sub-regions fulfill local mechanosensing remains scarce and can be interpreted from their motions. We utilized super-resolution live-cell imaging to capture the dynamics of FA proteins at the endogenous level and uncovered distinct movement patterns of FA elements from different proteins. Intriguingly, vinculin elements exhibited nematic fluctuations (diffusion-like motion along the long axis of FAs) in low-motility cells, and this behavior was mechanosensitive. Simultaneous super-resolution imaging of vinculin elements and DNA-based force probes revealed that this nematic diffusion correlates with force transmitting integrins. Mathematical simulations suggest that this motion is driven by active fluctuating forces arising from the anti-parallel organization of myosin II within FAs and is independent of actin retrograde flow. Subsequent PREM and STED imaging provided supporting evidence for such structures at FAs. Together, our study identified a patrolling mechanosensing that is propelled by active forces to ensure effective sensing of local forces, especially in low-motility cells.

MS2103
Neutrophil migration patterns change in response to elevated extracellular viscosity
*Daniel Yan[1], Eileen Stiles[2], Jackson Guo[1], Allison Boboltz[3], Gregg Duncan[3], Yun Chen[1]; Johns Hopkins University[1], UC Irvine[2], University of Maryland, College Park - College Park, MD[3]

Highly viscous mucus is associated with diseases such as cystic fibrosis, IPF, and COVID-19. The affected airways are prone to incur epithelial wounds, which expose cells initially protected by the epithelial barriers to pathogens and viscous mucus. Those cells include mesenchymal cells such as fibroblasts and immune cells such as neutrophils. We previously found that mesenchymal cells with active ruffling lamellipodia can sense extracellular viscosity levels. At increased viscosity, cells with ruffles flatten, and focal adhesion turnover increases, counterintuitively resulting in faster migration. In this study, we investigated the effects of elevated mucus viscosity on neutrophils. We used simple healthy and diseased mucus-mimetics made by supplementing long-chain polymer methylcellulose in medium, resulting in a viscosity two orders higher than that of the control. We also used a synthetic mucin hydrogel to physiologically represent mucus in healthy and

diseased airways. We used differentiated HL-60 (dHL-60) to model neutrophil behaviors. In diseased mucus-mimetics, dHL-60 increased their surface area by 2-fold, migrated 4 times faster with 3-fold higher directional persistence compared to healthy mucus-mimetics. Similar trends were observed using mucin hydrogels. We note that dHL-60 and neutrophils only exhibit such persistence in the presence of a chemo-gradient at low viscosity. Strikingly, unlike the case where chemo-gradient is present, each dHL-60 moved persistently in different directions under high viscous environments. We then inspected actin dynamics in dHL-60 expressing Ftractin-mStayGold. When subjected to diseased mucus-mimetic medium, each dHL-60 would not only form one, large, leading pseudopod, but also assemble multiple actin puncta of ~450 nm diameter near the leading edge. We also characterized the changes in the nucleus, and found 17% lower chromatin condensation in cells subjected to diseased mucus-mimetic. These results suggest that mucus viscosity may change actin dynamics and alter epigenetic profiles.

MS2108

Novel spatial arrangement of signaling modules driving mechanics of bleb-based migration.

*Ankita Jha[1], Payam Farahani[4], Jared Toettcher[4], Jason Haugh[3], Clare Waterman[2]; National Institutes of Health[1], National Heart, Lung, and Blood Institute, NIH[2], North Carolina State University[3], Princeton University[4]

Cancer cell migration plays a crucial role in invasion and metastasis. During metastasis, tumor cells migrate through their surrounding microenvironment, navigating various tissue and extracellular matrix (ECM) geometries, including confined spaces, to spread to distant sites. In confined microenvironments with low adhesion, metastatic cells often adopt a bleb-based mode of migration that differs fundamentally from integrin-dependent mesenchymal motility. Using metastatic melanoma cells under non-adhesive confinement, we investigated the mechanisms that govern the formation and stability of the large, pressure-driven leader bleb that defines cell polarity in this context. High-resolution live imaging and molecular perturbations revealed that EGF signaling via PI3K is critical for maintaining a stable, polarized leader bleb. Surprisingly, biosensors showed a rear-to-front gradient of EGFR and PI3K activity, with downstream effectors PIP3 and Rac1-GTP enriched at the rear of the bleb, while PIP2 and RhoA-GTP localized to the front. This spatial organization is inverted compared to classical mesenchymal migration. Disruption of this gradient via optogenetic tools led to bleb collapse, highlighting its functional importance. We further identified that, transmembrane picket protein CD44 and membrane-cortex linker proteins restrict EGFR mobility within a dense actin cortex at the bleb rear, stabilizing the signaling gradient. Combined modeling and experimental data support a feedback mechanism linking cortical architecture to signaling dynamics. Our findings uncover a novel polarity axis in bleb-based migration driven by localized signaling and cytoskeletal interactions. This work underscores how distinct spatial arrangements of conserved signaling modules on the membrane enable plasticity in migration strategies, allowing metastatic cells to adapt to biophysical constraints of the tumor microenvironment.

MS2107

Protein Glycosylation Pathway Mutants of Pseudomonas aeruginosa Show Distinct Phenotypes in Biofilm Formation

*George Trekell[1], Maya Quaranta[1], Elena Fuentes Solano[1], Katherine Palmatier[1], Chenxin Wang[1], Sherry Kuchma[2], George O'Toole[2], Mechthild Pohlschroder[3], Stefan Schulze[1]; Rochester Institute of Technology[1],

Dartmouth College[2], University of Pennsylvania[3]

Pseudomonas aeruginosa is an opportunistic human pathogen commonly acquired in hospitals by vulnerable individuals like those with cystic fibrosis or burn wounds. The increasing prevalence of antibiotic resistance in P. aeruginosa, as well as its ability to form strong biofilms makes it challenging to treat these infections. However, while P. aeruginosa is a model organism for studying biofilm development, structure, and function, the roles of protein glycosylation in this process have not yet been elucidated. Therefore, we have analyzed biofilm formation in knockout mutants of two glycosylation pathway enzymes, FgtA and TfpW, that have previously been described to modify flagella and pili, respectively. Together with their corresponding wild types, PA14 and Pa5196, we performed 96-well plate adhesion assays with crystal violet staining to quantitatively assess their ability to form biofilms. Interestingly, after 1 h, 4 h, and 24 h, decreased biofilm formation in comparison to the wild type was observed for the fgtA deletion strain, but not the tfpW mutant. In addition, Confocal Laser Scanning Microscopy (CLSM) was used to obtain three-dimensional images of the biofilms produced over 24 h. In line with the 96-well plate assay results, CLSM images showed thinner biofilms with fewer characteristic mushroom-shaped cell clusters for the fgtA mutant than for their wild type PA14. Furthermore, even though no quantitative changes were observed for the tfpW mutant, their biofilm structure showed larger clusters of cells than for the wild type PA5196. These findings indicate not only that protein glycosylation plays critical roles in mature biofilm formation of P. aeruginosa, but also that mutants of different glycosylation pathways show distinct biofilm phenotypes. These insights may contribute to potential strategies for combating biofilm-associated P. aeruginosa infections, such as by targeting specific glycosylation pathways.

Cell Behavior and Cognition

SG1003
A morphological circuit optimizes predatory strategy in the unicellular hunter P. collini
*Scott Coyle[1], Zhejing Xu[2]; University of Wisconsin[1], University of Wisconsin, Madison[2]

Cells dynamically manipulate their morphology in response to environmental cues to perform behaviors like motility, feeding, or host invasion. In predatory ciliates, prey capture provides resources required to build cytoskeletal feeding structures, creating feedback between environmental inputs and a morphological output behavior. Here, we describe how such feedback provides the control logic for a cellular morphology circuit that optimizes the predatory strategy of the unicellular suctorian P. collini. P. collini cells trap and feed on large cellular preys using arrays of straw-like microtubule-based tentacle structures that siphon out prey cytoplasm upon contact with their tips. We developed an automated live-cell deep-learning pipeline to digitize the number, length, and three-dimensional arrangement of P. collini tentacles across hundreds of thousands of individual cells grown under diverse environmental conditions and prey densities. This revealed that P. collini cell morphology scales anisotropically with capture history, dramatically increasing or decreasing tentacle number across a narrow range of tentacle lengths. The scaling behavior is implemented at the single-cell level through a simple morphological circuit in which competition between existing tentacle structures for available cellular resources controls the levels of a free resource pool, which in turn regulates tentacle biogenesis and destruction. Using drug perturbation, transcriptomics, and proteomics, we identify distinct molecular processes and pathways responsible for regulating tentacle maintenance and biogenesis. Mathematical modeling shows how the resulting circuit allows P. collini to use its success-rate to estimate predatory density, naturally optimizing its morphology to maximize prey capture efficiency for the local environment. Our work

suggests feedback between metabolic state and subcellular morphology provides a simple control logic for organizing cellular feeding strategies.

SG1007
Decision making without central nervous systems or processors: how cells and robots navigate complex landscapes?"
*Yun Chen[1], Daniel Yan[1], Stefan Hustrulid[1], Kyunmo Choi[1], Nathan Brown[1], Jochen Mueller[1], Chen Li[1]; Johns Hopkins University[1]

Decades of extensive studies combining approaches of genetics, cell biology, biochemistry, imaging, and computational modeling have built up a knowledgebase regarding the biomolecules, signaling pathways, and regulation associated with cell migration. However, important questions remain unanswered, including how cells navigate the extremely heterogenous mechanical environment in vivo, how they change migration modes in response to environmental cues, and how they process conflicting cues. Here we explore whether robophysical modeling can be used to understand how cells traverse the diverse tissue landscapes with such versatility and efficiency. Robophysics is an emerging field, where robots are utilized as active physical models of organisms to provide insights otherwise difficult to obtain in biological systems. This approach has proven useful for macroscopic organismal locomotion, especially in cases where equations of motion are too complex to solve. Because robots enact real physical interactions, they are guaranteed to be governed by physical laws no matter how complex the system and interaction are. We envision the approach of robophysical modeling will complement other methods to accelerate the process of characterization, validation, and discovery. In this work, we evaluated whether it is feasible to construct robots recapitulating migratory behaviors of cells. Our first-of-its-kind, cell-mimic robot consisted of connected segments, with each segment independently adhering to the substrate and creep outward away from the center, emulating cellular locomotion. The robot design allowed us to vary the ligand density to create a gradient across the landscape, mimicking the scenario in which cells undergo haptotaxis. We could also vary the power of the motor to emulate the cellular traction force which increases as actomyosin contractility increases. Moreover, we could vary the stiffness of the inter-segmental connection to evaluate how cell body axial stiffness affects migration speed. Our robot was not equipped with any computing unit to process the mechanical cues and make locomotive decisions. Importantly, we ensured that the robophysical model is dynamically similar to cell migration conditions, traction forces from solid substrates is much larger than fluid drag. When subjecting the robot to conditions equivalent to a substrate stiffness gradient, the robot underwent haptotaxis as cells do. Furthermore, the robot recapitulated the oscillatory patterns of traction forces and stress fiber elongation observed in cells. In summary, we demonstrated the feasibility of studying cell migration by robophysical modeling.

SG1006
Decoding behavioral algorithms in cells
*Ben Larson[1]; Rensselaer Polytechnic Institute[1]

Single cells can display remarkably sophisticated behaviors, managing the flow of information and orchestrating intricate processes to carry out essential functions. Proper execution of cellular behavior is essential not only for survival of microbes in the diverse, fluctuating environments they inhabit, but also for

proper development and function of multicellular organisms. In animals, behavioral control is generally achieved through neural activity. In cells, however, behaviors emerge directly from the joint action of chemical reactions, cellular architecture, and physical mechanisms and constraints within the cell and in its environment. How do cells coordinate the ordered sequences of actions that constitute behaviors in the face of constraints on the precision of sensing and actuation? Despite increasingly sophisticated knowledge of the components of cells, it remains challenging to synthesize this knowledge into understanding of how cells work. To meet this challenge, we are developing tools and approaches for decoding complex cellular behaviors. Applying these methods to diverse eukaryotic cells, we obtain low dimensional phenotypic spaces in which behaviors can be decomposed into stochastic yet stereotyped sequences of sub-behaviors. This perspective on cell behavior allows us to predict and infer functional physiological states of cells and also to interpret effects of perturbative experiments, yielding mechanistic insights into behavioral control. Altogether, our work sheds new light on how geometry and mechanics encodes cell function. In addition to delivering fundamental insights into the physical and molecular mechanisms and principles of behavioral control in eukaryotic cells, our work stands to enable new ways to predict, guide, and engineer cell function and generate new bio-inspired designs for autonomous control systems.

SG1002
Dynamics switches in cilia coordination encode the swimming behaviors of a unicellular predator
*Guillermina Ramirez-SanJuan[1], Aikaterini Kourkoulou[1]; EPFL[1]

Motile cilia are hair-like organelles, conserved across eukaryotes, that beat to generate flows. In most organisms cilia form dense arrays of thousands of filaments, that coordinate their motion into persistent, temporally synchronized patterns known as metachronal waves. Despite their ubiquity, the dynamics of these patterns and their role in tuning propulsion remain poorly understood. Here, we investigate how metachronal coordination shapes the navigation of Didinium nasutum, a highly agile unicellular predator with two circumferential ciliary bands. Using high-speed imaging of freely swimming cells, we capture and quantify the dynamics of metachronal wave coordination and track their evolution across different swimming states and transitions. Combining these measurements with a hydrodynamic model, we uncover how dynamic changes in coordination directly regulate propulsion and maneuverability. We show that stable metachronal waves support persistent directed swimming, local inhomogeneities in coordination give rise to curved trajectories, and global wave reversals accommodate rapid evasion-like reorientations. Our findings reveal that transitions between coordination modes allow Didinium to access a diverse swimming repertoire, highlighting dynamic ciliary patterning as a key mechanism to encode complex microscale navigation strategies.

SG1004
Hierarchical Decision-making in Stentor
*Daniel Cortes[1]; Virginia Tech[1]

Between 1902 and 1906 Herbert Jennings demonstrated that Stentor roeselii, a semi-transparent generally-sessile member of the genus, was capable of enacting different responses to the same noxious stimulus provided in consistent short bursts. Further, Jennings characterized the different response outcomes in a population of stentors and demonstrated that these organisms followed a general hierarchical decision tree;

usually enacting one behavior after another in a very specific pattern. Several decades later, researchers working with S. coeruleus, a much more motile and light-sensitive species, suggested that this stentor defaulted to detachment from substrate whenever presented with the same noxious stimulant rather than exhibiting any other response before detachment, thus throwing Jennings' original work into doubt. Recent work with S. roeselii has validated Jennings' original work and brought these observations into the modern era of cell biology and behavior. We follow up on this recent re-validation of Jennings' work by asking whether other species of Stentor are also capable of demonstrating hierarchical decision patterns in response to noxious stimulants, and if so, whether these always exhibit the same general pattern or whether there is species-specific expression of these behaviors. Using a hydraulic microinjector, we introduced either of two noxious stimulants – 1um magnetic polystyrene beads, or reconstituted powdered milk – in controlled volumes and at controlled intervals of 1 minute to the frontal field of stentor cells anchored to a substrate. In this way, we analyzed the response of four stentor species – light-sensitive S. coeruleus, light-loving S. stipatus, and light-agnostic S. muelleri and S. polymorphus. Interestingly, all species, including S. coeruleus, exhibit all of the primary responses (turning, ciliary reversal, and contraction), but not all exhibit the terminal response (detachment). Furthermore, some species heavily favor certain responses over others, demonstrating that the hierarchical response may be tied to cellular, behavioral, and ecological differences amongst these species.

SG1001

Mechanistic analysis of epithelial barrier dysfunction mitigation by zonulin inhibitor against inflammatory cytokines

*Takumi Konno[1], Maki Miyakawa[2], Hiroki Tanaka[2], Takayuki Kohno[1], Takashi Kojima[1]; Sapporo Medical University - 201387616S[1], Sapporo IBD Clinic[2]

The intestinal epithelial barrier plays a critical role in maintaining gut homeostasis by regulating selective permeability and preventing the intrusion of harmful pathogens, toxins, and antigens. Dysfunction of this barrier, commonly referred to as increased intestinal permeability or "leaky gut", has been strongly linked to the development and exacerbation of inflammatory diseases, including inflammatory bowel disease (IBD), and other systemic inflammatory conditions. In inflammatory conditions, inflammatory cytokines and immune mediators disrupt epithelial tight junction integrity, allowing uncontrolled penetration of antigens and pathogens into subepithelial tissues. Zonulin is a physiological modulator of intestinal barrier function, controlling tight junction permeability between intestinal epithelial cells. Elevated zonulin levels disrupt barrier integrity, increasing intestinal permeability and causing the situation called "leaky gut". Larazotide acetate (AT-1001) is a synthetic peptide that antagonizes zonulin signaling by stabilizing tight junctions and reducing pathological increases in intestinal permeability. Therefore, targeting the zonulin pathway with larazotide acetate is a promising therapeutic approach for impaired intestinal barrier function. However, there are still many unknowns in zonulin signaling. In this study, our group analyzed the effects of larazotide acetate against inflammatory cytokines by using human colorectal adenocarcinoma cell line Caco-2 cells and human colon cancer cell line HT29 cells as human intestinal epithelial cell models. Co-culture model of Caco-2 and HT29 cells was also utilized for epithelial barrier function assay. Treatment with inflammatory cytokines including TNFα and HMGB1 decreased epithelial barrier function of cell culture model, and treatment with AT-1001 mitigated this decrease. Our results indicate that larazotide acetate might be a new candidate for treating inflammatory diseases including IBD in terms of epithelial barrier function.

SG1008
Multicellular collaboration: how do cells work together in the wild?
*Allyson Sgro[1], John Durel[1], Emily Hager[2]; HHMI Janelia Research Campus[1], Boston University[2]

Cells of all kinds work together in multicellular behaviors ranging from collective migration to development. Simple laboratory assays have revealed a number of different behaviors cells engage in and how they're coordinated. However, natural environments are more spatially complex than these assays in ways that change both how cells can coordinate with one another and what behaviors they might need to perform. To understand how the complexity of natural environments shapes multicellular coordination and behaviors, we focus on how a soil-dwelling microbe communicates and behaves in a naturalistic soil model environment during starvation-induced aggregation. We find that to aggregate in synthetic soil, these cells engage in new behaviors such as bridge building and environmental remodeling that resemble how animals behave. These shared strategies for navigating complex spaces suggest there may also be shared coordination strategies that nature implements at both the cellular and organismal scales.

SG1009
What is it like to be a choanoflagellate?
*Thibaut Brunet[1], Núria Ros-Rocher[1], Josean Reyes-Rivera[2], Uzuki Horo[1], Diede De Haan[1], Maite Freire-Delgado[1]; Institut Pasteur[1], UCSF[2]

As the closest living relatives of animals, choanoflagellates hold the promise of revealing crucial information on animal origins. Despite their image as relatively passive filter-feeders, choanoflagellates have been found in the past few years to be capable of a wide range of surprising behaviors in response to their environment - including collective contractility, flagellar gliding, amoeboid motility, as well as fast and reversible disaggregation and reaggregation of cells. I will discuss recent findings on how choanoflagellates perceive and integrate environmental stimuli to implement these surprising phenotypes, as well as implications on the origin of animal sensation, cognition and behavior.

Cell Biology at Scale: New Technologies for Systematic Exploration of Cellular Biology

MS1203
A microscopy-based CRISPR screening platform enables organellar functional genomics and illuminates ciliary biology
*Jingbo Sun[1], Irem Sude Atiş[1], Stéfany L. L. Empke[2], Mustafa K. Khokha[2], David K. Breslow[1]; Yale University[1], Yale University School of Medicine[2]

Microscopy offers an indispensable technique for visualizing diverse biological processes and for defining cytological abnormalities characteristic of disease. However, combining microscopy with the power of pooled

CRISPR screening presents considerable technical challenges, hindering application of systematic genetic analysis to imaging-defined phenotypes. Here, we present a microscopy-based CRISPR screening platform that uniquely combines ease of implementation and compatibility with live-cell or antibody-based molecular markers, including post-translational modifications. Illustrating the value of this methodology, we systematically identify regulators of primary cilium structure and function in human cells through targeted and genome-wide screens. We further show that integration of screens focused on complementary but distinct phenotypes yields multi-dimensional profiles that delineate precise gene functions. Among newly identified hits, we identify a micro-protein at the ciliary transition zone that is required for ciliogenesis in human cells and for ciliary function in Xenopus embryos. Ongoing development of this technology aims to offer increased throughput, more facile application in diverse cellular models, and compatibility with complementary functional genomic assays. More broadly, our approach provides a technological and conceptual strategy for microscopy-based functional genomics with wide applicability.

MS1205

A multiplexed approach for genetic screening of human cells by electron microscopy uncovers an intermembrane space protein impacting mitochondrial cristae-shape

*Sarah Hassdenteufel[1], Yura Bykov[1], Caroline Kolenda[2], Benjamin Dubreuil[1], Nir Cohen[1], Kira Orlovsky[1], Maxim Itkin[1], Ofrah Faust[1], Katja Noll[3], Yoav Peleg[1], Shira Albeck[1], Sergey Malitsky[1], Elena Ainbinder[1], Karina von der Malsburg[3], Michael Meinecke[2], De; Weizmann Institute of Science[1], Heidelberg University[2], Saarland University Medical School[3]

Mitochondria form complex membrane-architectures essential for their diverse functions across physiological contexts. While much is known about the core proteins sculpting mitochondrial membranes, many components remain unidentified, and their orchestration unclear. A major limitation for functional discovery is technological–visualizing ultrastructure (US) diversity relies on low-throughput, high-resolution methods, such as electron-microscopy (EM). We here describe a scalable pipeline for screening human cells by EM. Our method, hMultiCLEM, works by fluorescently barcoding human cells enabling Multiplexing of samples and ultra-structure phenotyping by Correlative Light and EM. This unique approach for systematic US visualization permits routine screening of dozens of samples across a few EM experiments, yielding thousands of images. To showcase the power of hMultiCLEM we performed a genetic screen exploring mitochondrial US – systematically and at scale. hMultiCLEM robustly confirmed proposed cristae modulators and uncovered additional ones. Biochemical and computational analyses for validation of candidates highlighted an uncharacterized intermembrane space (IMS) protein linked to familial Ménière's disease, which we named MISHA (Mitochondrial-IMS membrane-SHApe-impacting protein). hMultiCLEM thus illuminates the protein networks driving cristae organization. More broadly, hMultiCLEM propels the EM field towards an era of genetic/chemical screening in basic research and medical contexts.

MS1204

Ballistic Microscopy (BaM)

*Jijumon A. S.[1], Ray Chang[1], Manu Prakash[1]; Stanford University[1]

Understanding how proteins and their interactomes function in living cells is fundamental to deciphering cellular homeostasis and the origins of disease. Conventional approaches for analysing unknown proteins often require fixed samples or whole-cell lysis, or are restricted to tracking known analytes in living cells. Meanwhile, light or electron microscopy provides structural resolution from cellular to atomic scales but lacks the ability to uncover the de novo molecular composition of unknown structures. Techniques such as mass spectrometry or sequencing excel at molecular identification but sacrifice live-cell and subcellular spatial context. Here, we introduce Ballistic Microscopy (BaM), a new approach that bombards millions of nanoparticles traveling at ultra-high speed (~1000 m/s) into living cells. Each particle penetrates and exits the cell while capturing "attoliters" of cytoplasm onto a capture substrate, preserving the spatial mapping of individual components. Because thousands of particles can traverse a single cell simultaneously, BaM achieves high spatial resolution, enabling comparison of materials from distinct subcellular regions. Extracted materials can be imaged on a gel to preserve spatial distribution or collected for downstream biochemical and structural analysis. We demonstrate the versatility of BaM by integrating it with fluorescence microscopy, TEM, Cryo-ET, mass spectrometry, confocal imaging, and DNA amplification. In live cells, BaM enabled spatially mapped extraction of membrane components, cytoplasm, and phase-separated condensates. Applied to the microtubule plus-end tracking protein CLIP-170, BaM revealed the protein composition of CLIP-170 condensates for the first time, identifying ~641 associated proteins within a strong protein-protein interaction network. This included experimentally validated keratin-18 intermediate filaments. These findings establish BaM as a novel platform for live-cell molecular sampling, offering multiple detection modalities when combined with existing methods. Looking ahead, BaM could be applied to investigate diverse subcellular structures such as cilia, spindles, filopodia, organelles, membranes, and disease-associated protein condensates or aggregates across a broad range of animal, plant, and other cell types.

MS1202

Cell Biology at the Scale of the Organism: Whole Body Cellular Activity Imaging in Vertebrates

*Virginia Ruetten[1], Wei Zheng[7], Igor Siwanowicz[1], Brett Mensh[1], Yunfeng Chi[5], Amy Hu[1], Mark Eddison[1], Yinan Wan[2], Sara Lelek-Greskovic[4], Mark Fishman[4], Florian Engert[4], Mikail Rubinov[6], Philipp Keller[1], Chie Satou[1], Guoqiang Yu[5], Paul Tillberg[1], Maneesh S; Janelia Research Campus, HHMI[1], Biozentrum, University of Basel[2], Gatsby Computational Neuroscience Unit, UCL[3], Harvard University[4], Tsinghua University[5], Vanderbilt University[6], Virginia Polytechnic Institute and State University[7]

Multicellular life depends on the dynamic coordination of cells distributed across the body. Yet current technologies force trade-offs: focusing on small tissue subsets, producing static whole-organism snapshots, or achieving organismal coverage at the cost of cellular resolution. Without whole-organism context, the ultimate goals and consequences of physiological processes are difficult to infer; without temporal dynamics, the algorithm is hidden; without cellular resolution, the implementation is inaccessible. To overcome these barriers, we developed WHOLISTIC: a platform for imaging cellular activity throughout the entire body of live zebrafish at single-cell resolution. WHOLISTIC extends functional imaging beyond the nervous system to all organs and tissues—including gut, skin, liver, pancreas, and more—by combining pan-cellular calcium reporter lines, rapid volumetric fluorescence imaging, new machine-learning pipelines for whole-body registration and activity analysis, and complementary whole-body expansion microscopy. Using this approach, we uncover a host of new cellular properties and inter-organ interactions, including state-dependent oscillatory ependymal-cell dynamics during sleep-like states, meningeal responses to ketamine, and pulsating waves along kidney nephrons. At the whole-organism level, we captured rapid brainstem-controlled blood flow redistribution

during hypoxia, followed by decreased enteric activity, suggesting a novel mechanism of cellular activity regulation via controlled oxygenation. Integration with optogenetics enables all-optical causal mapping of brain–body interactions, revealing a topological correspondence between central and peripheral activity. Together, these advances establish a new paradigm for systems biology, bridging cellular and organismal physiology with implications for fundamental biology, systems neuroscience, and drug discovery.

MS1201
Large-scale functional genetics approaches reveal the mechanistic basis for core cellular processes
*Iain Cheeseman[1]; Whitehead Institute[1]

The ability to conduct functional genetics approaches in human cell is accelerating discovery and transforming the capability to analyze core cell biological questions, from how these questions are conceived to the nature and scale of data that can be generated. For our work, we are combining Cas9-based gene targeting with large-scale approaches to analyze gene requirements using both proliferation-based screens, enrichment strategies, and systematic microscopy-based morphological analyses across thousands of gene targets. I will describe our recent work to identify the requirements for cellular adhesion in cultured human cells using parallel mechanical and optical approaches. Our large-scale analysis has identified multiple factors required for cellular adhesion in interphase cells across diverse biological pathways, including factors that either increase or decrease cellular adhesion. In addition, due to the reduced adhesion of mitotic cells, our strategy provides a robust means to enrich for gene targets that perturb mitotic progression. Unexpectedly, we identified key pathways is potent roles in adhesion that have not been previously implicated in cell-substrate interactions, highlighting the potential for cell biological discovery.

MS1206
Ribosome demographics link molecular aging to chronological aging
*Jordy Botello[1], Lifei Jiang[1], Peter Metzger[1], Aya Abu-Alfa[1], Troy Comi[1], Claire Weaver[1], Ileana Cristea[1], Clifford Brangwynne[1]; Princeton University[1]

Cellular homeostasis depends on a dynamic balance between synthesis and degradation of biomolecules, ensuring renewal of functional components and clearance of damaged ones. While most proteins, RNAs, and metabolites turn over rapidly, certain molecular assemblies persist for weeks to years in post-mitotic cells. Ribosomes exemplify this biomolecular longevity: as the essential nanomachines of protein synthesis, ribosomes are both metabolically expensive to produce and unusually stable. Their stability, however, makes them susceptible to cumulative molecular damage, raising the question of whether ribosome age directly influences translational fidelity and proteostasis. However, methods to track the subcellular localization and purify native cellular complexes like ribosomes over extended timescales have been lacking. Here, we introduce an approach for spatiotemporal mapping of ribosome composition and function, enabling resolution of key stages of the ribosome life cycle, including pre-60S maturation in the nucleolus, nascent 40S maturation in the cytoplasm, and molecular aging of mature cytoplasmic ribosomes. By performing molecular age-selective ribosome profiling, we reveal that intracellular molecular aging of ribosomes modulates the translation efficiency of specific transcripts, limiting the ability of aged ribosomes to synthesize basic proteins. We also identified molecular age-dependent collisions which are driven by specific ribosomal RNA modification

signatures, whose depletion enhanced biogenesis of specific proteins and rescued associated cellular phenotypes. Notably, we found that these deleterious modification profiles accumulate during human chronological aging, and their removal ameliorates aging-related defects. These findings establish molecular age as a key determinant of ribosome function and link ribosomal aging to translational decline with chronological aging. More broadly, our work provides a mechanistic framework for how the persistence of cellular nanomachines shapes proteome integrity in physiology and disease.

MS1207
Toward a software ecosystem for subcellular proteomics
*Duo Peng[1]; Chan Zuckerberg Biohub San Francisco[1]

Proteins serve as fundamental structural and functional units in cells. During cellular perturbations, many proteins undergo physiologically relevant redistributions that remain undetectable by conventional abundance-based assays. Subcellular fractionation and affinity enrichment techniques in combination with mass-spectrometry readouts are commonly employed to discover the organellar placement of proteins. We present grassp (Graph-based Analysis of Spatial/Subcellular Proteomics), a computational framework for extracting insights from raw mass-spectrometry readouts. grassp preserves biological signals through rigorous preprocessing and constructs protein neighborhood graphs to generate subcellular spatial maps, enabling comparisons across cellular states. The framework enforces a new metadata standards and leverages the AnnData format for storage and portability. We reprocess public spatial proteomics datasets with grassp, generating subcellular proteomics maps built on unified ontologies. These resources can be explored through our interactive data portal, with cross-map queries made accessible via natural-language interaction powered by an AI chatbot. By standardizing the processing, analysis, integration, and dissemination of subcellular proteomics data, grassp establishes the computational foundation of an ecosystem for large-scale, comparative, and AI-driven exploration of cell architecture.

MS1208
Toward understanding cellular dynamics at scale: Lessons from the study of actin-based motility
*Julie Theriot[1]; University of Washington[1]

In recent years, a wide range of technological developments in both molecular methods and imaging methods have enabled the collection of very large-scale data sets with high-dimensional quantitative information at the single-cell level. Recognizing that change over time is the only constant feature of living systems, there is substantial interest in developing robust approaches for inferring the dynamic behavior of individual cells from quantitative observations of cell population dynamics. As experimental cell biologists, how can we most effectively use the wealth of emerging large-scale quantitative biological data to drive development of the best theories for explaining the dynamic biology of cells? Research on the biophysical mechanisms of actin-based motility offers a useful test case for exploring the strengths and shortcomings of various approaches to inferring or modeling variations in single-cell dynamic behavior based on high-throughput population data. Although many molecular aspects of actin-based motility are conserved across a wide range of eukaryotic cells, there is substantial variation in emergent motile properties such as cell speed among different cell types, and even among populations of ostensibly identical individual cells. We have used three particularly well-studied

models for actin-based motility (Listeria monocytogenes comet tails, fish basal epidermal keratocytes, and mammalian neutrophils) to compare the predictive value of measurements taken at the population level versus measurements taken for single cells over time. We find dramatic variations in ergodicity for motile behavior over these model systems. Specifically, individual bacterial comet tails and individual fish keratocytes maintain cell-to-cell variations in properties such as speed and directional persistence over very long time spans relative to the intrinsic kinetics of molecular driving processes such as actin filament assembly. In contrast, neutrophils display substantially more behavioral variation over short time spans, such that the distribution of measurements for instantaneous speed and for directional persistence of individual cells over time rapidly approaches the population distribution. In the cases where cell behaviors remain consistent for individuals over time but vary in the population, further exploration of this heterogeneity has yielded important mechanistic insights into the underlying biophysical processes driving motility. However, even in the more ergodic case of neutrophils, population measurements fail to capture important dynamical aspects of motility such as cyclical shape changes.

Cell Biology at the Extremes

SG301
Cell membranes of the extreme: characterizing the biosynthesis and physiological roles of membrane-spanning lipids in archaea
*Andy Garcia[1], Grayson Chadwick[2], Xiao-Lei Liu[3], Dipti Nayak[2], Paula Welander[1]; Stanford University[1], University of California: Berkeley[2], University of Oklahoma[3]

Archaea, the so-called “third” domain of life, are a diverse group of microorganisms that possess many unique features which distinguish them from other lifeforms on Earth. Paramount amongst these defining characteristics are the novel lipids that archaea use to construct their cell membranes. Most notably, archaea commonly fuse the canonical membrane bilayer into a monolayer of membrane-spanning lipids known as glycerol dibiphytanyl glycerol tetraethers (GDGTs) often just referred to as tetraethers. The formation and modification of these lipids is highly dependent upon radical (S)-adenosyl methionine (rSAM) enzymes. GDGTs are formed by the rSAM tetraether synthase and the subsequent cyclization of their lipid tails is performed by the B12-binding rSAM (B12-rSAM) GDGT ring synthase. We sought to identify enzymes responsible for additional GDGT modifications such as crosslinking, methylation, desaturation, and hydroxylation and to determine the physiological roles of such lipids. Utilizing three archaeal model organisms – Sulfolobus acidocaldarius, Thermococcus kodakarensis, and Methanosarcina acetivorans – we identified six enzymes responsible for these modifications, five of which are rSAM enzymes. In particular, we uncovered 1) the rSAM enzyme GMGT synthase (Gms) which covalently crosslinks GDGT tails to form glycerol monoalkyl glycerol tetraethers (GMGTs), 2) three distinct B12-rSAM GDGT methylases which introduce 1-5 additional methyl groups into the tails of specific classes of GDGT lipids (GMGTs, cyclized GDGTs, and acyclic GDGTs), 3) a B12-rSAM tetraether desaturase (Ted) which seemingly reverses previous saturation steps in the archaeal lipid biosynthesis pathway, forming 1-4 double bonds in the saturated hydrocarbon tails of GDGTs without the need for molecular oxygen in contrast to the fatty acid desaturases of bacteria and eukaryotes, and 4) a non-rSAM hydroxy-GDGT synthase (Hgs) which hydrates the double bonds introduced by Ted to form OH-GDGTs. Notably, we show that Gms proteins are widely distributed throughout archaea and unexpectedly in some bacteria, and we demonstrate that the cross-linking of GDGTs by Gms is part of a conserved heat stress response in phylogenetically diverse archaea. Further, we demonstrate that GDGT methylation participates in a novel archaeal stress response triggered by exposure to membrane-destabilizing chemical agents such as hexanoic

acid and penicillin G. These results highlight the value of exploring the rSAM enzyme landscape of archaea – revealing diverse and novel rSAM enzyme activities amongst homologous proteins that invoke new paradigms such as reversibility in the archaeal lipid biosynthesis pathway.

SG304

Cellular Transformers: Surface Glycans Mediate Robust Assembly of Silica Cages in Loricate Choanoflagellates

*Deepak Krishnamurthy[1], Aymeric Chorlay[1], Nicole King[1], Daniel Fletcher[1]; University of California, Berkeley[1]

Controlled, bottom-up assembly of micron-scale 3D structures remains a fundamental challenge in engineering and physics. Loricate choanoflagellates provide a striking counterpoint: within 10 minutes, a single cell constructs an intricate extracellular basket (loricae) from hundreds of biomineralized silica strips through a process that has to balance opposing requirements of free movement and strong adhesion. The building blocks (curved, rod-shaped strips 2–3 µm long and ~100 nm wide) must first remain relatively non-adhesive to move into position yet later adhere strongly to form stable structures. How does a single cell modulate adhesion and microscale manipulation to build robust extracellular architectures in a fluctuating marine environment? Using the loricate D. grandis as a model, we generated high-resolution 3D datasets of intact loricae and quantified key geometric features, confirming the fidelity of assembly. We found that fully assembled loricae are highly resilient to mechanical stress, withstanding significant compressive strain without detectable damage, consistent with strong adhesion between constituent strips. Inspired by other microbial adhesive structures, we hypothesized that glycans (cell-secreted polysaccharides) mediate this adhesion. Using fluorescent lectin labeling and super-resolution microscopy, we identified distinct spatial patterns: O-glycans showed uniform coverage, while N-glycans were enriched at strip junctions, suggesting adhesive roles in guiding and anchoring strips, respectively. In vitro experiments on isolated strips revealed that controlled proteinase treatment sequentially “unmasks” adhesive N-glycan sites at distinct locations, indicating a layered architecture. These surface glycans also impart key biophysical properties, including strong negative surface charges that may enable mobility of the strips during assembly. Interestingly, we found that perturbing strip surface properties during assembly—by altering protein or glycan composition (e.g., charge or sialylation)—produced striking assembly failures, underscoring the importance of surface biophysical properties in lorica assembly. Together, these findings reveal how a single cell encodes information for reliably building complex extracellular 3D structures through glycan-mediated, spatiotemporal control of nanoscale adhesion.

SG303

Cooperative hydrodynamics accompany multicellular-like colonial organization in the unicellular protist Stentor

*Shashank Shekhar[1], Hanliang Guo[2], Sean Colin[4], Wallace Marshall[5], Eva Kanso[6], John Costello[3]; Emory University[1], Ohio Wesleyan University[2], Providence College[3], Roger Williams University[4], University of California, San Francisco[5], University of Southern California[6]

Many single-celled protists exhibit both solitary and colonial existence. An important step towards multicellularity, which is associated with benefits, such as enhanced nutrient uptake, was the formation of colonies of unicellular organisms. However, the initial drivers that favored individual cells aggregating into more complex colonies are less clear. Here, using the ciliate Stentor coeruleus as a model organism, we show that hydrodynamic coupling between proximal neighbors results in faster feeding flows for neighboring ciliates,

such that individuals within a dynamic colony have stronger average feeding flows than solitary individuals. Flows generated by individuals actin together reach higher velocities allowing access to a wider range of prey resources than individuals actin on their own. Moreover, we find that accrued feeding benefits are typically asymmetric: whereas all individuals benefit from acting together, those with slower solitary currents gain more from partnering than those with faster currents. We find that colonial organization in simple unicellular organisms is beneficial for all its members. This provides fundamental insights into the selective forces favoring the early evolution of multicellular organization.

SG306

Extreme membrane invaginations in the largest Campylobacterota reveal novel mechanisms of bacterial cellular organization

*Jean-Marie Volland[1]; University of California Santa Barbara[1]

Cells of most bacteria and archaea are typically regarded as small and uncompartmentalized, typically around 2 µm, lacking the complex endomembrane systems found in eukaryotes. Their DNA, contained within the nucleoid, is not isolated by a surrounding membrane. Yet, with only a small fraction of these organisms cultured, the full scope of their biology remains largely unexplored. Using innovative culture-independent techniques, our lab is contributing to bridging this knowledge gap through the study of uncultivated large sulfur bacteria. Despite prevailing views of bacterial simplicity, our research reveals a remarkable diversity in bacterial cell biology, previously unseen in the microbial world. We have identified and characterized extraordinary species like Thiomargarita magnifica (Pseudomonadota), which spans up to two centimeters in length, and Thiovulum imperiosus, the largest known Campylobacterota species. Both species not only defy conventional size limitations but also constrain their cytoplasm to the cell periphery and organize their multiple genome copies in membrane-bound compartments. Most notably, using three-dimensional electron microscopy, we have meticulously characterized the sophisticated endomembrane system of Thiovulum imperiosus. Our findings reveal that this complexity is facilitated by extreme invagination of the inner membrane, suggesting novel mechanisms of cellular organization and function in bacteria. These insights not only challenge traditional views of bacterial simplicity but also open new avenues for understanding microbial life at the edges of size and complexity.

SG302

Living with a killer: how coevolved Saccharomyces cerevisiae become killer toxin resistant

*Michelle Hays[1], Angelina Chan[2], Gavin Sherlock[3]; University of Michigan[1], Stanford[2], Stanford University[3]

Some yeasts are killers. They secrete toxins that kill neighboring cells but protect themselves with an intracellular antidote. Killers have an advantage in mixed populations, although toxin production comes at a metabolic cost. Many Saccharomyces cerevisiae strains require two viral genomes to be killers. Along with sensitive cells in the environment, this is a complex genetic conflict: yeast and viral genomes alike are capable of adaptation and fitness tradeoffs abound at many levels. Our research dissects the molecular basis of adaptation in the face of these competing selection pressures to understand evolutionary outcomes. We identified beneficial mutations that arose in coevolved killer and sensitive yeast, including mutations responsible for toxin-resistance. Through bulk segregant analysis, we identified a putative gain-of-function

missense mutation in the HOG osmoregulatory pathway component SSK1. Preliminary data suggest this dominant-acting polymorphism is sufficient to protect yeast from the coevolved toxin, and other killer toxins. Ongoing experiments aim to determine the mechanism by which this polymorphism creates toxin-resistance and any host tradeoffs associated with the coevolved allele. Preliminary data suggest this mutation may affect protein-protein interactions, leading to crosstalk with the Cell Wall Integrity (CWI) pathway. We are pursuing the transcriptional and signaling impacts of the resistant allele. SSK1 is required for multiple drug resistance in Candida auris, and our ongoing work will address how this mutant allele might impact phenotypes of fungi associated with human pathogenicity and drug resistance. In future, we aim to understand how killer yeasts counter-adapt in the face of resistant competitors, and how viral genomes evolve as competing host population composition changes. Understanding how fungi adapt, defend themselves, and kill one another has important implications for health during climate change, as fungal pathogens emerge and drug resistance spreads.

SG305

The chytrid fungus Batrachochytrium dendrobatidis differentially regulates actin networks between its animal-like and yeast-like cell types.

*Sarah Prostak[1], Lilian Fritz-Laylin[1]; University of Massachusetts Amherst[1]

Cell morphogenesis is critical for organismal development and physiology. In nearly all eukaryotes, including animal and yeast cells, shape control relies heavily on actin. While animal cells change shape with an actin cortex and protrusions, yeast remodel their walls with directed actin-mediated material delivery and endocytic actin patches. The mechanisms of cell morphogenesis have clearly diversified across animals and fungi, but how this diversification occurred has largely remained unstudied. Chytrids are deep branching fungi that have actin networks resembling those of both animals and yeast, making them great tools for studying morphogenetic diversification. Chytrids develop from motile wall-less “zoospores” into sessile, walled “sporangia” that produce the next zoospore generation. In the “frog-killing” chytrid Batrachochytrium dendrobatidis (Bd), zoospores have animal-like actin structures like a cortex and protrusions, while sporangia have more yeast-typical structures like actin patches. It is not understood if Bd actin structures are homologous to similar structures in animals and yeast, as we would need to know which actin networks incorporate animal- and yeast-like actin regulators, but when and how Bd uses any of its actin network components is unknown. Therefore, to establish when Bd expresses these components, we performed RNA sequencing throughout its lifecycle. By analyzing RNA abundance, we find that actin regulatory and endocytosis-related genes can be divided into two groups: those highly expressed in zoospores and cellularizing stages and those highly expressed in developing sporangia. Several genes in the first category are associated with animal-typical actin structures, including the SCAR/WAVE complex and talin, actin regulators that are important for crawling motility in animal cells. Actin itself also falls into this category, suggesting that Bd may use high monomer levels in zoospores and lower levels in sporangia. In contrast, genes that are highly expressed in sporangia tend to be general actin regulators and endocytosis-associated genes. These data suggest that actin structures in Bd could be homologous to their counterparts in animals and yeast, a finding that we are confirming by determining the localization of SCAR/WAVE, talin, and select endocytosis proteins to the actin structures of interest.

Cell Biology of Functional Condensates: Metabolons and Membrane Interfaces

SG708
Contact sites at the interface of membraneless organelles and membranes
*Dragomir Milovanovic[1]; German Center for Neurodegenerative Diseases (DZNE)[1]

Phase separation is a major mechanism for organizing macromolecules, particularly proteins with intrinsically disordered regions, in compartments not limited by a membrane or a scaffold. The cell can thus be viewed as a dynamic emulsion, composed of numerous biomolecular condensates alongside classical membrane-bound organelles. The condensate-membrane interfaces emerge as chemical microenvironments that couple diffusion and the material properties of condensates to biochemical processes occurring in the membranes. For example, synapsin/synaptic vesicle condensates – a membraneless organelle that is in fact packed with membranes – can harbor ion potential differences at their interfaces and may act as a charge center in synapses. Another prominent example of large membrane areas that are tightly juxtaposed in cells are mitochondria and ER. Our recent data show that PDZD8, the ER-resident protein identified as a tether between the ER and mitochondria, can undergo liquid-liquid phase separation via its intrinsically disordered region. Endogenously-labeled PDZD8 forms condensates on membranes both in vitro and in vivo. These observations highlight how phase separation at membrane interfaces can create adhesion zones that tether vesicle cohorts or stitch neighboring organelles, revealing new principles for mesoscale cellular organization.

SG703
Cytoplasmic Crowding and Diffusion Dynamics Regulate Biomolecular Condensation
*Hui Li[1]; School of Systems Science, Beijing Normal University, China[1]

The physical microenvironment of the cytoplasm—including crowding, fluctuations, and spatial organization—critically influences biomolecular dynamics such as liquid-liquid phase separation (LLPS), yet the underlying mechanisms remain unclear. By combining single-particle tracking with novel approaches to manipulate cytoplasmic properties, we systematically investigate how intracellular dynamics and crowding govern LLPS. We demonstrate that reduced molecular diffusion promotes initial stress granule (SG) nucleation, while microtubule-driven active transport facilitates subsequent droplet coalescence. Intriguingly, the formation of SG droplets contributes to increased spatial heterogeneity within the cell as well. To dissect crowding effects without perturbing cell volume (e.g., via osmotic compression), we develop a method for in situ synthesis of abiotic supramolecular polymers directly in living cells, and reveal that elevated crowding decelerates droplet coarsening kinetics. Our findings highlight how distinct physical parameters of the cytoplasm regulate condensate dynamics, offering insights into the interplay between metabolic activity and membraneless organelle formation.

SG702
Disrupted purinosome assembly in purine de novo synthesis disorders

*Veronika Baresova[1], Vaclava Skopova[1], Olga Souckova[1], Stan Kmoch[1], Marie Zikanova[1]; CHARLES University, Prague[1]

Objectives: The enzymes involved in de novo purine synthesis (DNPS) form the purinosome, a multienzyme complex that assembles in cells under purine-depleted conditions. Understanding how pathogenic variants in DNPS genes affect purinosome formation is essential for explaining how enzyme defects lead to disease. Methods: Using immunofluorescent labeling and colocalization of endogenous DNPS proteins, we detected the purinosome formation in a broad spectrum of cell types, including patient-derived skin fibroblasts, without the need for protein overexpression. To evaluate the functional impact of pathogenic DNPS variants we prepared CRISPR-modified HeLa cells deficient in all enzymatic steps of DNPS and further analyzed the metabolomic profiles of these cells as well as of patients' body fluids. Results: Purinosome assembly was disrupted in fibroblasts and model HeLa cells with pathogenic variants in ATIC (AICARribosuria), ADSL (ADSL deficiency), PAICS (PAICS deficiency), and PFAS (PFAS deficiency). The accumulation of DNPS metabolites (AICAR, SAICAR, SAdo, AIR, and CAIR) in the body fluids of patients correlated with clinical severity, ranging from fatal neonatal encephalopathy to milder intellectual disability. These defects reflect the destabilization of functional enzymes. Transient transfection with the corresponding wild-type proteins normalized both purinosome assembly and metabolite accumulation. Conclusion: Our work identifies the purinosome as a critical, clinically relevant compartment and suggests that modulation of purinosome formation could represent a therapeutic target for inborn errors of purine metabolism. Funding: Grants NU23-01-00500 from the Ministry of Health of Czech Republic, MULTIOMICS_CZ (Programme Johannes Amos Comenius, Ministry of Education, Youth and Sports of the Czech Republic,//ID Project CZ.02.01.01/00/23_020/0008540) – Co-funded by the European Union.

SG705

Jurkat T cell activation requires raft-associated LAT condensates

*Sze Ham Chan[1], Sofia Marigliano[1], Mayukha Nerayanuru[1], Hongyin Wang[1], Jonathon Ditlev[2], Kandice Levental[1], Ilya Levental[1]; University of Virginia[1], University of Toronto[2]

T cells are central effectors of adaptive immunity, mediating critical defenses against infections and cancer. T cell activation must be tightly regulated to elicit proper immune responses while avoiding aberrant activation that could produce autoimmunity. Despite decades of research, the regulatory mechanisms of normal and aberrant T cell activation are not fully understood. Recently, using both in vitro membrane models and Jurkat cells we reported coupling between ordered, cholesterol-rich domains known as membrane rafts and protein condensates driven by multivalent interactions between the transmembrane adaptor Linker of Activation of T-cells (LAT) and its effectors Grb2 and Sos1. Such coupling is mediated via the raft-preferring transmembrane protein LAT and is critical for transduction of T-cell signaling. To investigate how LAT condensates cooperate with membrane rafts to regulate T-cell signaling, we mutated the LAT transmembrane domain (TMD) to target it to nonraft domains and found both LAT phosphorylation and downstream MAPK signaling were completely abrogated. Thus, raft association of LAT and its associated protein condensates is essential for T cell signaling. We hypothesized that membrane rafts facilitate activation by enriching activating signals (e.g. Lck and ZAP70 kinases) and excluding inhibitory proteins (i.e. phosphatases like CD45), thereby promoting robust LAT phosphorylation, its subsequent condensation, and downstream signal propagation. Testing a panel of LAT TMD mutants whose raft affinity was systematically varied, we showed that LAT's raft affinity was tightly and linearly correlated with its phosphorylation. Conversely, targeting the phosphatase CD45 to membrane rafts reduces both LAT condensate density and downstream T cell signaling. Together, these results suggest that

membrane rafts serve as a signaling platform to regulate LAT phosphorylation and condensation, which in turn tunes immune signaling.

SG707
Lipids facilitate protein assembly at membranes through prewetting
*Sarah Veatch[1]; University of Michigan[1]

Proteins that assemble into condensates in solution can also condense into surface phase domains when a subset of condensate components are tethered to or interact with fluid membranes. These domains are dynamic, effectively two-dimensional, and considered “prewet” because they occur well outside of the coexistence region of the condensate system alone. We show that proximity to the membrane phase transition dramatically potentiates biopolymer prewetting through a combination of theory, simulation, and experiments in well-defined reconstituted systems. When taken into cells, we show that protein prewetting at the plasma membrane inner leaflet is sensitive to perturbations of plasma membrane composition. More generally, we speculate that prewetting at membranes represents a general protein assembly mechanism involved in a broad array of cell processes and sensitive to both membrane and protein properties.

SG701
Multienzyme Condensates of Various Sizes Mediate Functional Crosstalk Between Glucose Metabolism and Signaling Pathways in a Size-specific Manner
*Songon An[1]; University of Maryland Baltimore County[1]

A prevailing paradigm of metabolic regulation has held for several decades that metabolic pathways are orchestrated by spatially ‘well-mixed’ enzymes. However, numerous metabolic enzymes have now been characterized to form spatially distinct granules and/or structurally defined filaments, suggesting that metabolism may benefit from spatial organization. In fact, we have demonstrated that rate-determining enzymes in glycolysis and gluconeogenesis are spatially organized into multienzyme condensates of various sizes, termed ‘glucosomes.’ Importantly, these assemblies function to regulate glucose flux between energy metabolism and building block biosynthesis in an assembly size-specific manner. Accordingly, we hypothesize that glucosome assemblies are intricately associated with diverse cellular processes, particularly signaling pathways, in a size-specific manner. Using Luminex multiplexing assays and fluorescence single-cell imaging, we found that epidermal growth factor (EGF), which promotes the formation of large-sized glucosomes, triggers robust temporal activation of extracellular signal-regulated kinases 1/2 (ERK1/2). We then demonstrated that pharmacological inhibition or genetic knockdown of ERK1/2 promote the dissociation of large-sized glucosomes into medium-sized assemblies in HeLa and Hs578T cells. In addition, we developed a quantitative cell-based high-throughput screening (qHTS) assay to assess small-molecule effects on glucosome formation. An initial qHTS with a library of pharmacologically active compounds directed further efforts toward kinase inhibitor-enriched collections. Follow-up knockdown experiments revealed that the cell cycle–associated signaling axis involving cyclin-dependent kinase 2 (CDK2) and Aurora kinase A (AURKA) specifically contributes to the formation of medium-sized glucosomes in HeLa and Hs578T cells. These results strongly suggest that metabolically active glucosomes are regulated by specific signaling cascades in an assembly size-

dependent manner. Therefore, we propose that glucosomes represent novel biological entities that can be targeted to modulate the network of multiple druggable pathways simultaneously for therapeutic intervention.

SG706

Nano-liquid Signal Integration Hubs Promoting Cell Survival and Growth

*Akihiro Kusumi[1]; Okinawa Institute of Science and Technology Graduate University[1]

Super-resolution microscopy has greatly improved spatial resolution, but progress in temporal resolution has been limited, despite its critical importance for the studies of living cells. We recently developed an ultrafast camera system that achieves the highest time resolutions in single fluorescent-molecule imaging to date, reaching the photon-limited regime imposed by fluorophore photophysics. Using Cy3, the optimal fluorophore we identified, the system provided 33- and 100-µs time resolutions with single-molecule localization precisions of 34 and 20 nm, respectively. Furthermore, this camera enables simultaneous data acquisitions for PALM/dSTORM at rates up to 1 kHz, with 29/19 nm localization precisions in the 640 × 640-pixel view-field (Fujiwara et al. 2023a, b JCB; PMID 37278763, 37278764). Using advanced single-molecule imaging, including this system, we identified a nanoscale liquid-like condensate on the plasma membrane (PM) that serves as a platform for integrating signals critical for cell survival and growth in vivo: those downstream from receptor-type tyrosine kinases (RTKs) and CD59, a GPI-anchored receptor involved in immune evasion. This platform is a 34-nm-diameter liquid-like condensate formed by liquid-liquid phase separation driven mainly by zyxin's intrinsically disordered regions. It is mainly composed of integrins, talin, RIAM, VASP, and zyxin, and thus called iTRVZ. These proteins are shared with focal adhesions although iTRVZ is distinct from them. iTRVZ formation is promoted by interactions with PM raft domains and cortical actin (actin-based membrane skeleton that compartmentalizes the PM). iTRVZ recruits both CD59 and engaged RTKs, enhancing RTK signals as well as CD59's dual functions: protection from complement-dependent cytotoxicity and promotion of cell growth. When RTKs and CD59 are co-stimulated, iTRVZ mediates cooperative and non-linear amplification of downstream signals. This effect is likely arises from co-recruitment of Src-family kinases downstream from RTKs and FAK from CD59, enabling mutual chain activation cycles within iTRVZ due to its nano-scale smallness and liquidity. In mice, iTRVZ promotes tumor growth by facilitating both cell proliferation and immune evasion. These findings identify iTRVZ as a nanoscale liquid condensate essential for cell survival and growth signals, with implications for cancer biology.

SG704

Primary partitioning principles of the cell: Cytoophidium assembly, phase separation and membrane enclosure

*Ji-Long Liu[1]; ShanghaiTech University[1]

In the 4.6 billion year history, the Earth we live on has nurtured a diverse range of life forms. The basic unit of life is the cell. Since the discovery of cells 360 years ago, scientists have gained a deep understanding of their structure, function, and heterogeneity. In order to understand the mechanisms and laws of cell structure, here I propose three primary partitioning principles of the cell, namely cytoophidium assembly, phase separation, and membrane enclosure. From the perspective of partitioning principles, I introduce these three principles separately and then compare them. Furthermore, I attempt to elucidate the primary partitioning principles from the perspectives of mathematics, physics, chemistry, evolution, and communications.

Cell Biology of Meiosis

MS305
A Ring-Shaped Chromosome-Associated Protein Complex Promotes Proper Microtubule Organization in C. elegans Oocytes
*Jordy Martinez[1], Sadie Wignall[1]; Northwestern University[1]

Oocytes undergo meiosis, a specialized form of cell division that reduces genetic content by half, mediated by a microtubule-based spindle. Unlike most cells, oocytes assemble their spindles in the absence of microtubule organizers called centrosomes. In C. elegans oocytes, microtubules form lateral bundles alongside homologous chromosome pairs (bivalents) that guide chromosome movements. Moreover, a ring-shaped protein complex (RC) forms at the interface between the two homologs, and this complex is critical for bivalent alignment. We previously found that depletion of the kinase AIR-2Aurora B disrupts RC assembly and causes severe spindle assembly defects, suggesting that the RC may also promote proper microtubule organization. We are therefore investigating the relationship between lateral bundles and the RC to determine if this unique microtubule organization is dependent on the RC. To assess this, we utilized a him-8 mutant, in which the X chromosomes fail to pair, resulting in oocytes containing 5 bivalents with RCs and 2 univalents lacking RCs. We analyzed these mutants on monopolar spindles, to better visualize lateral bundles by eliminating the microtubule overlap zone found in bipolar spindles. This analysis revealed that univalents lacking RCs do not have lateral bundle associations and remain stuck at the monopole. We confirmed this finding using spo-11 mutants, in which double-strand breaks fail to occur, resulting in 12 univalents. Interestingly, only a subset of the univalents recruit RCs in this mutant, and we found that the univalents with RCs had lateral bundles running alongside them, while the univalents without RCs did not. This suggests that RCs promote the formation of lateral microtubule bundles. To further test this hypothesis, we attempted to acutely remove RCs from bivalents and determine if there was an impact on microtubule organization. We therefore acutely depleted AIR-2 using the auxin-inducible degradation system. Upon rapid AIR-2 removal from oocytes, we found the multiple RC components were no longer localized at the midbivalent region, suggesting that the RC was disassembled. Importantly, under these conditions, the spindle began to lose its integrity, suggesting that AIR-2, and potentially other RC components, are required for spindle stability. Altogether, these findings support the view that the RC has a role in generating and maintaining proper spindle architecture in oocytes, revealing important new roles for this protein complex.

MS308
DDX4 and HDLBP Regulate the Progression of Meiosis through Translational Regulation of Emi2 mRNA in Mouse Oocyte.
*Takahiro Sanada[1], Tomoya Kotani[1]; Hokkaido University[1]

In vertebrates, oocytes store more than ten thousand maternal mRNAs in dormant states during oogenesis. Since the transcription becomes quiescent after initiation of oocyte maturation, proteins crucial for the progression of oocyte maturation are synthesized from mRNAs stored in oocytes. EMI2 protein is synthesized from Emi2 mRNA at the end of meiosis I and is essential for the arrest of meiotic cell cycle at the metaphase of meiosis II (Takei et al., 2021). We have identified TDRD3 protein as a regulator of Emi2 mRNA translation. In

TDRD3-deficient oocytes, EMI2 was only slightly synthesized and the meiotic cell cycle was not arrested at metaphase II, and instead the oocytes entered the cleavage stage without fertilization. However, the molecular mechanism by which the translation of Emi2 mRNA is regulated remains unclear. By performing an RNA pull-down assay, we have isolated DDX4 and HDLBP proteins as candidates for Emi2 mRNA-binding proteins. In this study, we aimed to elucidate how these proteins were involved in the translational regulation of Emi2 mRNA. First, we examined the distribution pattern of DDX4, HDLBP, and TDRD3 in mouse oocytes by immunofluorescence. All these proteins formed aggregates in the cytoplasm. We then analyzed the interactions of DDX4 and HDLBP with Emi2 mRNA in vivo by immunoprecipitation followed by RT-PCR. Emi2 mRNA was detected in the precipitations with the anti-DDX4 and anti-HDLBP antibodies. Next, we assessed interaction among these proteins and Emi2 mRNA by in situ hybridization and immunofluorescence. These proteins formed RNA-binding protein complexes (RBP-complexes) including Emi2 mRNA. To assess the functions of DDX4 and HDLBP in the progression of meiosis and Emi2 translation, we knocked down DDX4 and HDLBP in immature oocytes. Both DDX4- and HDLBP-deficient oocytes exhibited abnormal meiosis such as multipolar spindles and parthenogenesis after resumption of meiosis. In addition, the translational activity of Emi2 mRNA decreased in DDX4- and HDLBP-deficient oocytes. Finally, we investigated the structures of the RBP-complexes in DDX4-deficient oocytes. The TDRD3-Emi2 colocalization was disrupted in DDX4-deficient oocytes. These results suggest that DDX4 and HDLBP regulate the progression of meiosis cooperatively with TDRD3 through the translational regulation of Emi2 mRNA.

MS307

Differential contributions of beta-tubulin isotypes to spindle assembly and chromosome separation dynamics during C. elegans acentrosomal oocyte meiosis

*Emmanuel Nsamba[1], Anne Villeneuve[1]; Stanford University[1]

Assembly of bipolar spindles without centrosomes, a universal feature of oocyte meiosis, involves coordination between microtubules (MTs) composed of several different α- and β-tubulin isotypes, MT associated motors and other MT-interacting proteins (MAPs). Here we use high-resolution immunofluorescence microscopy and live imaging of Caenorhabditis elegans strains with altered β-tubulin isotype composition to investigate the contributions of and interplay between MT composition, motors, and MAPs during oocyte meiotic spindle assembly and chromosome segregation. We found that “TBB-2 only” oocytes (containing only one of two partially-redundant germline-expressed β-tubulin isotypes) exhibit a synthetic genetic interaction with klp-18(ts), a missense mutation causing partial loss-of-function of the plus-end directed kinesin-12 MT cross-linking motor KLP-18, resulting in a high incidence of embryo lethality, X-chromosome missegregation, and monopolar oocyte spindles. Moreover, these synthetic spindle assembly and chromosome segregation defects can be suppressed either by a tbb-2(E439K) mutation that confers partial resistance of MTs to the MT-severing enzyme katanin or by a mei-2(A235T) mutation that decreases katanin activity. We further found that both spindle length and the spatial distribution of katanin along spindles are affected by β-tubulin isotype composition, with “TBB-1 only” oocytes exhibiting longer meiotic spindles and a dearth of katanin at spindle centers relative to poles. Through live imaging, we uncovered hidden contributions of β-tubulin isotypes to spindle morphology and chromosome separation dynamics. We found that TBB-2-only oocyte spindles achieve bipolar metaphase with normal timing despite assembling shorter spindle structures, and they exhibit accelerated spindle elongation and 67% faster chromosome separation velocities during anaphase B. Together, our data support a model in which β-tubulin isotype composition helps to maintain a balance between microtubule crosslinking and severing activities during oocyte meiosis. We further propose that this balance is

crucial for establishing spindle bipolarity, maintaining spindle structures, and modulating the dynamics of chromosome separation.

MS304
ESCRT recruitment to meiotic nuclear envelope hole
*Arnav Pandey[1], Charlotte Shore[1], Kianna Satterwhite[1], Nicholas Ader[1]; University of North Carolina, Greensboro[1]

Meiotic nuclear division necessitates an underappreciated challenge for cells, where the homologous recombination of meiotic chromosomes under intense mechanical stress must be coordinated with rapid nuclear envelope (NE) remodeling. While work in the last decade has provided insight into the mechanism of mitotic NE remodeling, including the contribution of the endosomal sorting complexes required for transport (ESCRT) to membrane restriction and sealing, a mechanism of meiotic NE remodeling has been comparatively lacking. Though superficially similar, meiosis presents unique challenges to the cell. Notably, in the model yeast Schizosaccharomyces pombe, there is a transient loss the RanGTP gradient, without a full disassembly of the NE, in what has been termed a “virtual” NE breakdown. However, the full extent of nuclear compartmentalization loss remains unknown. Moreover, it is not clear how the current model of ESCRT recruitment to a NE hole, which relies on an intact NE permeability barrier, can be reconciled with the meiotic landscape. We have used live-cell fluorescence microscopy of GFP-ESCRT proteins to determine their role in NE remodeling during meiosis. We determine that GFP-ESCRTs are recruited to a meiotic NE following extrusion of the yeast microtubule organizing center, the spindle pole body (SPB), in both meiosis I and meiosis II. We find that Cmp7-GFP is recruited 78 seconds following SPB extrusion from a NE hole in meiosis I and 108 seconds following SPB extrusion in meiosis II. Unexpectedly, we find that ESCRT proteins persist asymmetrically at a single site of a meiotic NE hole from meiosis I and into meiosis II. Further, using a fluorescent reporter of passive diffusion in the nucleoplasm, MGM4, we determine the extent to passive diffusion is disrupted during “virtual” NE breakdown. Our results demonstrate the adaptability of the ESCRT machinery to respond to a NE hole in different cellular contexts and further elucidate the molecular mechanism of this protein complex.

MS302
Essential roles of BRCA2 in spermatogenesis and oogenesis
*Neil Hunter[1], Sushil Khanal[1], Sumit Sandhu[1]; University of California Davis[1]

BRCA2 (FANCD1) is essential for gamete formation and human alleles have been linked to early menopause. However, its functions in fertility remain poorly defined relative to those in somatic cells. BRCA2 plays a central role in homologous recombination, a chromosome-repair process that is defective in many cancers and is essential for meiosis, the specialized cell division that produces eggs and sperm. The major function of BRCA2 is to load the homologous pairing and DNA strand-exchange protein RAD51 onto the ends of broken DNA molecules. To understand BRCA2 functions in fertility, we have exploited a conditional allele of the Brca2 gene in mouse to inactivate its mediator function at different stages of gametogenesis in spermatogonia (stem cells), spermatocytes, and oocytes. Inactivation in spermatogonia reveals an essential function for BRCA2 in the proliferation and differentiation of germline stem cells. When Brca2 is inactivated early in meiosis, assembly of strand-exchange complexes is defective and the early events of meiotic prophase-I fail, including chromosome

pairing and synapsis. These observations are consistent with an essential role for BRCA2 in loading RAD51 and DMC1 (the meiosis-specific RAD51 ortholog) onto DNA ends. Later inactivation of BRCA2 mediator function reveals an unanticipated function after initial strand exchange and chromosome synapsis have been achieved. At this stage, BRCA2 mediates the de novo loading of RAD51, but not DMC1, to complete the repair of a subset of ~40 residual DNA breaks. Without BRCA2 mediator function, these breaks persist and become excessively resected. Surprisingly, in spermatocytes, these persistent DNA lesions do not impede the progression of meiosis, and repair is eventually completed during diakinesis/metaphase I. Sperm are produced efficiently but fertility is low suggesting that aberrant DNA repair may cause lethal mutations. Consistently, genome sequencing reveals the formation of de novo mutations, including large deletions, in the progeny of Brca2 mutant fathers. By contrast, in females, Brca2 mutant oocytes undergo apoptosis revealing a sexually dimorphic response to BRCA2 dysfunction and DNA damage arising during meiotic prophase I. Unexpectedly, BRCA2 dysfunction also disturbs the patterning of crossovers – the exchanges between homologous chromosomes that are essential for their accurate segregation. We conclude that BRCA2 functions throughout gametogenesis and mediates distinct modes of DNA repair during the early and late stages of meiosis. Our analysis also reveals sexually dimorphic responses to BRCA2 dysfunction with implications for gametogenesis and germline mutation.

MS306

Illuminating actin-driven mechanisms of chromosome segregation in mouse oocyte meiosis

*Andrea Rix[1], Federica Giannini[1], Binyam Mogessie[1]; Yale University[1]

Accurate chromosome segregation in oocytes is fundamental for the formation and development of healthy embryos. Chromosomes are segregated using a microtubule-based spindle apparatus. We previously discovered that a unique actin-based structure (spindle F-actin) underpins chromosome segregation in oocytes by mediating robust chromosome-microtubule connections. More recently, we revealed that spindle F-actin also prevents premature separation of sister chromatids in eggs of reproductively older females, providing a new explanation for the molecular origins of aneuploidies associated with human infertility and genetic conditions. While these findings have advanced our understanding of chromosome segregation mechanisms in female meiosis, how spindle actin is assembled and exerts its function at the chromosome-microtubule interface remains a mystery. To address this, we are combining advanced microscopy of female meiosis with targeted protein degradation strategies to test candidate spindle actin assembly factors in mouse oocytes. Our emerging studies highlight a potential role for Septins, a conserved family of GTP-binding proteins, in regulating actin organization during oocyte meiosis. We are now building on these data to uncover how spindle actin assembly is controlled by Septins and how this regulation safeguards chromosome segregation in female meiosis.

MS303

Real-time visualization of crossover designation in meiosis

*Simone Koehler[1], Ivana Čavka[1]; European Molecular Biology Laboratory[1]

Crossover formation is essential for meiosis to ensure the correct pairing and segregation of homologous chromosomes and to increase the diversity of the progeny. To produce crossovers, an excess of double-strand

breaks is introduced and processed into recombination intermediates. However, only a limited subset of these recombination intermediates is selected to become crossovers. Any imbalance in the distribution of crossovers may lead to genomic instability and errors in chromosome segregation with devastating consequences for the progeny. However, the main regulatory mechanisms and when exactly crossovers are designated remain highly disputed questions. We developed an in vivo imaging and automated image analysis pipeline that allows us to directly visualize crossover designation in C. elegans meiosis. Our analysis uncovers surprisingly fast dynamics of crossover factors at recombination intermediates and directly shows a multi-layered regulation of crossover events: A subset of recombination intermediates is selected very early for a crossover fate while other intermediates must be repaired by non-crossover pathways. However, this early selection cannot guarantee the extremely robust selection of just one crossover per pair of homologous chromosomes that is typically observed in C. elegans. Therefore, an additional regulatory layer removes supernumerary precursors a few hours later. Our results show how meiotic cells can precisely regulate how crossovers are distributed between homologous chromosomes, which is required to guarantee genome stability for future generations.

MS301

Transcription factor Ovo orchestrates an inducible response to episodes of transposon threat in the male germline

*Amelie Raz[1], Andrew Van Dusen[2], Yukiko Yamashita[1]; Whitehead Institute for Biomedical Research[1], Massachusetts Institute of Technology[2]

Germ cells are uniquely immortal and require specialized mechanisms to safeguard the heritable genome. Transposable elements (TEs) can threaten genomic integrity by excising and/or inserting themselves throughout the genome; germ cells throughout the Metazoa counter this threat through deployment of the piRNA pathway. This pathway serves as an “immune system” for the genome, preventing further replication and genomic infection by TEs through production of complementary interfering RNAs. Notably, the risk posed by TEs is not constant over developmental or generational time. High-threat scenarios include conditions in which some TE repression is reduced to allow for a so-called "domesticated" TE to be functionally expressed, as well as cases in which cells lack maternally deposited piRNAs against a genomically-encoded TE. Inducible responses to heightened TE risk remain poorly understood. Here, we identify a dynamic, essential role for the transcription factor Ovo in the Drosophila male germline during episodes of TE threat. While ovo mutant testes appear morphologically normal, they lack germline expression of the piRNA factor Piwi, leading to mild global TE derepression under basal low-threat conditions. Under high-threat conditions, however, loss of Ovo results in severe global TE upregulation, extensive double-strand breaks, and largely agemetic and sterile testes. Furthermore, we find Ovo is required for production of de novo piRNAs against genomically-encoded invading elements. Notably, Ovo expression itself is elevated in these high-threat conditions, suggesting its transcriptional autoregulation is responsive to TE challenge. We propose that Ovo functions as an inducible regulator of the male germline “immune system”: dispensable when increased TE infection risk is low, but rapidly upregulated and essential when TE activity threatens genomic integrity. This work uncovers the first known role for Ovo in the male germline, and reveals how the male germline can harness domesticated TEs while simultaneously preventing invasion by non-domesticated elements.

Cell Biology of Organelles

MS503
A molecular switch for coordinating kinesin and dynein transport of mitochondrial cargo
*Christina Gladkova[1], Maria Paez-Segala[2], William Grant[5], Samuel Myers[3], Mark Kittisopikul[2], Yuxiao Wang[4], Ronald Vale[2]; Sloan Kettering Institute, MSKCC[1], HHMI Janelia Research Campus[2], La Jolla Institute for Immunology[3], Myeloid Therapeutics[4], Shrike Research[5]

Kinesin and dynein, motor proteins that move in opposite directions along microtubules, govern the spatial distribution of mitochondria in response to cellular stress and local energy needs. However, the mechanism for switching between kinesin- and dynein-driven transport in response to these cues remains unknown. We develop a cellular synthetic-cargo transport assay to understand directional switching by the adaptor protein TRAK, which links both motors to mitochondria. Using AlphaFold2-guided mutagenesis, we identify a regulatory helix in TRAK that determines transport direction. In its “closed” conformation, the helix blocks a binding site critical for dynein activation, and the synthetic mitochondrial cargo is transported by kinesin with inactive dynein bound. When the helix is “opened”, dynein transport is activated and kinesin is excluded from the complex. We show that the distinct regulatory helix sequences of TRAK1 and TRAK2, two closely related isoforms, govern their distinct transport properties. In response to cellular stress, dynein transport to the perinuclear region is activated by phosphorylation of a unique site adjacent to the TRAK2 regulatory helix by JNK family MAP kinases. These results reveal a molecular mechanism for regulating and coordinating the switching between kinesin- and dynein-driven transport of mitochondria and establish the utility of the cellular synthetic-cargo transport assay to study physiological regulation of cargo transport by molecular motors.

MS505
A Sir2-dependent pathway regulates nuclear lipid droplet biogenesis
*Lauren Carson[1], Hong-Guo Yu[1]; Florida State University[1]

The nuclear envelope (NE) is composed of two phospholipid bilayers termed the inner (INM) and outer (ONM) nuclear membrane. While the ONM is continuous with the endoplasmic reticulum, the INM maintains a distinct proteome and lipid composition. Recent studies in budding yeast have shown that the INM is enriched in diacylgycerol (DAG) and serves as a crucial site for nuclear lipid droplet synthesis. However, the mechanism regulating nuclear lipid droplet biogenesis remains to be further elucidated. Here we show that in S. cerevisiae the INM-localized protein Csa1 regulates the nuclear localization of the acyltransferase Lro1, which converts DAG to triacylglycerol to produce nuclear lipid droplets. We show that Csa1 contains an amphipathic helix which mediates its interaction with the INM. In response to nutrient depletion and at the onset of meiosis, both Csa1 and Lro1 concentrate to a subdomain of the INM adjacent to the nucleolus which we term the n-INM. In the absence of Csa1, Lro1 forms foci along the nuclear periphery. We identify the NAD+-dependent deacetylase Sir2 as a regulator of Csa1 and Lro1 at the n-INM. Disrupting the enzymatic activity of Sir2 causes both Csa1 and Lro1 to localize evenly along the nuclear periphery. Loss of Csa1 or Sir2 results in a decrease in lipid droplet formation. Our findings therefore reveal a novel Sir2-Csa1-Lro1 signaling pathway at the INM that regulates nuclear lipid droplet biogenesis and nuclear envelope remodeling.

MS506
Lipid Droplet-Associated Opa1 Regulates Fatty Acid Release
*Xiao Li[1], Jayce Weesner[1], Rikshita Bhattacharyya[1], Chi-Lun Chang[1]; St. Jude Children's Research Hospital[1]

Mitochondria and lipid droplets (LD) are organelle alliances that dynamically coordinate fatty acid consumption and storage, respectively. Opa1, a mitochondrial protein that promotes inner membrane fusion, plays a key role in maintaining mitochondrial morphology, and thereby regulating multiple aspects of mitochondrial function. Opa1 has also been reported to regulate LD turnover, possibly via modulating mitochondrial fatty acid oxidation. Interestingly, Opa1 has been detected on LD in cultured adipocytes and adrenal carcinoma cells, as well as in LD proteomes, suggesting a potential direct role of Opa1 in regulating LD turnover. However, how Opa1 targets to and Opa1's functional impact on LD remains elusive. Here we identified a role of Opa1 in regulating fatty acids release from LD by directly localizing to the surface of them, and loss of Opa1 slowed down the breakdown of LD in U2OS cells. By a battery of imaging techniques and biochemical assays, we demonstrated a subpopulation of Opa1 resides on LD in multiple cell lines. Through domain mapping and AlphaFold prediction, we further identified that the exon4 coding sequence adapts an amphipathic helix and the hydrophobic grove within it is required for LD targeting. Intriguingly, a naturally occurring splicing variant lacking exon4 does not localize to LD, suggesting an intrinsic cellular mechanism regulating Opa1 targeting to LD. The localization of Opa1 to LD can also be regulated by the concentration of free fatty acids and pyruvate. Furthermore, we found Opa1 on LD can reduce the lipid peroxidation of LD depending on it GTPase activity. Collectively, these data reveal that Opa1 directly localizes to LD through exon4-dependent mechanisms, where it safeguards LD lipids from peroxidation and regulates fatty acid release.

MS502
Mitochondrial presequences harbor variable strengths to maintain organellar function
*Natalie Niemi[1], Youmian Yan[1], Baigalmaa Erdenepurev[1], Ian Collinson[2]; Washington University in St. Louis[1], University of Bristol[2]

Hundreds of mitochondrial-destined proteins rely on N-terminal presequences for organellar targeting and import. While generally described as positively charged amphipathic helices, presequences lack a consensus motif and thus likely promote the import of proteins into mitochondria with variable efficiencies. Indeed, the concept of presequence "strength" critically underlies biological models such as stress sensing, yet a quantitative analysis of what dictates "strong" versus "weak" presequences is lacking. Furthermore, the extent to which presequence strength affects mitochondrial function and cellular fitness remains unclear. Here, we capitalize on the high-throughput and kinetic nature of the MitoLuc mitochondrial protein import assay to quantify multiple aspects of presequence strength. We find that select presequences, including those that regulate the mitochondrial unfolded protein response (UPRmt), impart differential import efficiencies during mitochondrial uncoupling. Surprisingly, we find that presequences beyond those classically associated with stress signaling promote highly variable import efficiency in stressed and basal (i.e., non-stressed) conditions in vitro, suggesting that presequence strength may influence a broader array of processes than currently appreciated. We exploit this variability to demonstrate that only presequences that promote robust import in vitro can fully rescue defects in respiratory growth in Complex IV-deficient yeast, suggesting that presequence

strength dictates metabolic potential. Collectively, our findings demonstrate that presequence strength can describe numerous metrics, such as total imported protein, maximal import velocity, or sensitivity to uncoupling, suggesting that the annotation of presequences as “weak” versus “strong” requires more nuanced characterization than is typically performed. Importantly, we find that such variability in presequence strength meaningfully affects cellular fitness in processes beyond stress signaling, suggesting that organisms may broadly exploit presequence strength to fine-tune mitochondrial import and thus organellar homeostasis.

MS507

Mitophagy amplitude is regulated by an interplay between receptor protection and turnover

*Abhishek Kumar[1], Jonathan Friedman[2]; University of Texas, Southwestern Medical Center[1], UT Southwestern Medical Center[2]

Mitochondria are multifunctional organelles required for several critical cellular processes, including energy generation. Cells need to maintain mitochondrial health as accumulation of damaged mitochondria lead to energy failure, oxidative stress, and pathological diseases. Mitochondrial homeostasis is maintained by multiple quality control pathways, including mitophagy, which removes defective mitochondria from the mitochondrial network. However, a major outstanding question is how cells precisely control the timing, position, and duration of mitophagy episodes. During receptor-mediated mitophagy, the mitochondrial proteins BNIP3 and NIX directly recruit autophagy machinery to the mitochondrial surface, though their regulatory mechanism is still unclear. In recent years, two new BNIP3- and NIX-interacting proteins have been identified. PPTC7 targets BNIP3 and NIX for turnover by the proteasome to keep levels of the receptors low, whereas TMEM11 is proposed to spatially control mitophagy by forming a complex with BNIP3 and NIX at active mitophagy sites. However, it is unclear how each of these interactions are controlled and how they interplay with each other. Here, we identify a new negative regulator of mitophagy, ARMC1, which forms a complex with TMEM11 and modulates its association with BNIP3 and NIX. During mitophagy activation, TMEM11 dissociates from ARMC1, forming a complex with active BNIP3 and NIX. Importantly, in gain-of-function studies, we find that the presence of TMEM11 in a BNIP3/NIX complex impedes the ability of PPTC7 to target the receptors to the proteasome, causing BNIP3/NIX stabilization and prolonged mitophagy activation. Together, these findings support a two-stage mitophagy model: a pre-initiation step where mitophagy is inhibited by constitutive receptor turnover and an activation step where receptors are temporarily protected from degradation, allowing mitophagy to proceed freely.

MS504

Structural insights into GTP-coupled conformational changes in Mfn1 revealed by time-resolved transition metal ion FRET

*Sophie Hurwitz[1], William Zagotta[1], Sharona Gordon[1], Suzanne Hoppins[1]; University of Washington[1]

Regulation of mitochondrial shape, size, and cellular distribution is achieved through a balance of fusion, division, and microtubule-based transport. Outer mitochondrial membrane fusion is mediated by the mitofusin paralogs: Mfn1 and Mfn2. Dysregulation of mitochondrial fusion has been implicated in several neurological diseases including Charcot Marie Tooth Type 2A (CMT2A). CMT2A is caused by mutations in MFN2, which alter mitochondrial fusion and transport. The mitofusins are members of the dynamin

superfamily, large GTPases that couple conformational changes with the catalytic cycle to remodel cellular membranes. Molecular characterization of CMT2A variants indicate that the disease variants are impeded at different steps in the fusion mechanism. We predict that aberrant allostery is a major component of the diverse dysfunction in the CMT2A variants. Universally in the dynamin superfamily, allosteric regulation is critical to advance the process of membrane remodeling. To elucidate the conformational dynamics of Mfn1, we applied a novel transition metal fluorescence energy transfer system to a minimal construct called mini-Mfn1. We used time correlated single photon counting (TCSPC) to measure time-resolved FRET. This has the advantage of recording the distribution of FRET efficiencies of the donor-acceptor pair from an entire population of protein in solution. This allows calculation of the distance distributions of the protein sample and reveals structural heterogeneity. We performed our analysis after incubating the mini-Mfn1 with different nucleotides to represent the progressive steps in GTP hydrolysis. Past work has identified the structure of the same mini-Mfn1 when incubated with GDP and GDP•BeF3. This revealed a large conformational change when the free phosphate is released from the binding pocket. However, the conformation of mini-Mfn1 with GTP bound and nucleotide free Mfn1 remain unknown. Our data agrees with the known crystal structures when Mfn1 is bound to GDP and GDP•BeF3, demonstrating we can recapitulate structural data in solution. We identified the conformation of GTP-bound Mfn1, which is comparable to the GDP conformation. This indicates that Mfn1 undergoes two large conformational changes: first when hydrolysis occurs and then again when the free phosphate is released. Our data show that when GDP is released Mfn1 is in a novel conformation, which would be linked to the disassembly of the fusion complex. Our data reveal novel insights into the structure and energetics of GTP-driven conformational changes of Mfn1 and created a powerful system for future studies.

MS501

The ER crossing guard: Djp1 facilitates peroxisomal membrane protein trafficking

*Megan Balzarini[1], Zixuan Yuan[1], Hilla Weidberg[1]; University of British Columbia[1]

Peroxisomes can be formed de novo from the endoplasmic reticulum (ER). In this process, peroxisomal membrane proteins are inserted into the ER, sorted to subdomains and bud off into vesicles that mature into functional peroxisomes. While passing through the ER, peroxisomal proteins must escape being recognized as “foreign” and eliminated by ER quality control processes. However, the mechanisms that protect and sort peroxisomal membrane proteins in the ER prior to their exit remain unclear. We found that an ER-localized cochaperone, Djp1, facilitates these early steps of peroxisomal biogenesis in yeast. Deletion of DJP1 destabilized certain peroxisomal membrane proteins, including Pex13 and Pex15. Additional deletion of the ER quality control factor SPF1 rescued the observed dysregulation of Pex13 and Pex15, suggesting that Djp1 protects these proteins from ER quality control processes. The exposure to quality control systems could be due to their presence in the ER outside of specific subdomains. Using a split-GFP system, we visualized the transient ER localization of peroxisomal proteins. Pex13 and Pex15 showed a broad ER signal only in absence of Djp1. This supports a role for Djp1 in the sorting of these proteins to the correct ER subdomain by preventing their unspecific insertion into the ER. Induction of peroxisomal biogenesis, via growth in oleic acid-containing medium, revealed broad defects in both peroxisomal formation and function without Djp1. Altogether, our results suggest Djp1 functions to sort and protect peroxisomal proteins in the ER, thereby promoting efficient peroxisomal biogenesis.

MS508
VPS13C interaction with GATOR2 is required for mTORC1 activation in plasma membrane-endoplasmic reticulum contact sites
*Wim Annaert[1], Thomas Leroy[1], Roberto Dias[4], Gen Li[1], Xiaojuan Yang[1], Pakita Van Puyenbroeck[1], Christine Van Broeckhoven[5], Francis Impens[6], Judith Klumperman[3], Peter Vangheluwe[2]; KULeuven&VIB[1], KULeuven[2], UMC[3], University of Coimbra[4], VIB&UAntwerp[5], VIB&UGent[6]

Mutations in VPS13C have been linked to Lewy Body diseases, including early-onset Parkinson's disease and Dementia with Lewy Bodies. VPS13C functions as a lipid transfer protein in contact sites between late endosomes/lysosomes and the endoplasmic reticulum (ER) as well as lipid droplets. In this study, we used co-immunoprecipitation followed by mass spectrometry to identify the endogenous VPS13C interactome. We reveal an association with GATOR2, a key complex regulating mTORC1 activation. This interaction appears not to occur on lysosomes but at the cell surface, where VPS13C is recruited to de novo formed plasma membrane-ER contact sites, particularly in response to nutrient availability. In the absence of VPS13C, mTORC1 is still recruited to membrane ruffles but fails to become activated due to an impaired GATOR2 complex recruitment. Further, VPS13C functionally cross-talks with the small GTPase, ARF5, required for the upstream recruitment of mTORC1 to the plasma membrane. Mutations in VPS13C recapitulate these defects underscoring a loss-of-function in Dementia with Lewy Bodies. Pharmacological activation of mTORC1 signaling rescued mitochondrial dysfunction in VPS13C deficient and mutant cells, highlighting a role for non-lysosomal mTORC1 in downstream mitochondrial fitness. Defects in lysosome positioning, mTORC1 activation and mitochondrial function could be recapitulated in VPS13C deficient iPSC-derived human induced dopaminergic neurons, placing VPS13C central in pathways shown to be vulnerable in Lewy Body diseases.

Cell Biology of Organelles and the Cytoskeleton

SY301
A Tour of the Pore: Structural and Functional Mapping of the Nuclear Pore Complex
*Michael Rout[1]; Rockefeller University[1]

Nuclear Pore Complexes (NPCs) mediate the specific and active exchange of transport factors that mediate transport of cognate RNAs and proteins between the cytoplasm and nucleoplasm. We have used an integrative approach to determine structures for the entire 52 MDa yeast NPC, revealing the NPC's functional elements in unprecedented detail. The NPC is surprisingly modular, consisting of only 30 proteins of the nucleoporin family (Nups), which assemble into relatively rigid higher-order scaffold whose modules are held together by flexible connectors, imbuing the NPC with both strength and flexibility. The scaffold surrounds a central channel from which multiple intrinsically disordered Phe-Gly (FG) repeat motifs project which mediate selective nucleocytoplasmic transport through specific interactions with nuclear transport factors. I will describe our latest findings, including the architectures of the membrane-anchoring domains in the inner ring of the NPC and of the nuclear basket, involved in chromatin organization and processing and transport of mRNPs. I will also describe how the organization of the FG repeats mediates rapid and specific macromolecular transport.

SY302
How RNA Can Encode Physical Information for Biomolecular Condensates
*Amy Gladfelter[1]; Duke University School of Medicine[1]

Biomolecular condensates are vessels to spatially and temporally control biochemistry in cells for a variety of processes. Despite their ubiquitous roles in the entire lifecycle of RNA, how RNA impacts the composition, properties and functions of condensates remains poorly understood. The chemical properties and sequence of RNAs are generally considered in light of housing or regulating the genetic code. However, most amino acids are encoded by multiple codons, making the genetic code degenerate. Synonymous mutations in RNA sequences affect protein translation and folding, but their impact on RNA itself is often neglected. We developed a genetic algorithm that introduces synonymous mutations to control the conformational heterogeneity of structures sampled by an mRNA. The behavior of the designed mRNAs reveals physical information layered in the genetic code. We find that mRNA conformational heterogeneity impacts the physical properties and functional outputs of RNA-protein complexes and biomolecular condensates. The role of structure and disorder of proteins in biomolecular condensates is well appreciated, but we find that RNA conformational heterogeneity is a major contributor to condensate properties. This feature of RNA enables both evolution and engineers to build cellular structures with specific material and responsive properties.

Cell Biology of the Nucleus - Form and Function

MS1905
Architectural proteins organize replication into multiphasic condensates
*Yuan Yao[1], Ashish Tangade[3], Quentin Livingston[2], Surya Penna[1], Nidhi Parikh[1], Anupam Mondal[3], Jeetain Mittal[3], Marina Feric[1]; Centre of Eukaryotic Gene Regulation, Department of Biochemistry and Molecular Biology, Pennsylvania State University[1], Pennsylvania State University[2], Texas A&M University[3]

Genomes are organized across several hierarchical levels. Recently, phase transitions have been shown to play a role in condensing genomes, localizing fundamental genomic processes, including transcription and DNA repair. However, less is known regarding how phase transitions influence replication, in which long strands of both double- and single-stranded DNA (dsDNA, ssDNA, respectively) need to be coordinated. Here, we investigated the molecular interactions driving the phase behavior of the core biopolymers associated with mitochondrial replication. We designed several DNA templates varying in sequence features, length, and strandedness, and combined them with the dsDNA-binding protein TFAM and the ssDNA-binding protein mtSSB to induce in vitro phase separation. With super-resolution imaging, we observed multiphasic droplets, in which each architectural protein co-localized preferentially with its cognate nucleic acid. Indeed, we observed similar degrees of miscibility within mt-nucleoids as a function of their replication activity. To better understand the molecular driving forces, we performed single-molecule experiments using dual optical traps and generated force-extension curves, showing how the two architectural proteins cooperate to package tethered DNA into a condensate. Coarse-grained molecular dynamics simulations captured the formation of multiphasic condensates in protein-nucleic acid mixtures, providing molecular-level insight into our experimental findings. Together, these studies shed light on how phase separation of the architectural proteins

facilitates segregation of the exposed single-stranded DNA from double-stranded DNA. Overall, the multiphasic organization of mt-nucleoids has implications for how replication can be spontaneously organized in cells.

MS1906

Decoding Enhancer-Mediated Control of Transcription Dynamics through Live-Cell Super-Resolution Imaging

*Aniket Jana[1], Le Xiong[1], Liangqi Xie[1]; Cleveland Clinic[1]

Eukaryotic transcription is a highly stochastic, discontinuous process occurring in intermittent 'bursts'. Conventional RNA sequencing provides only static snapshots of gene expression, missing this dynamic behavior. Here, we used the MS2-MCP system in live mouse embryonic stem cells to visualize nascent transcription of the Klf4 pluripotency gene. A distal super-enhancer ~70 kb downstream of the Klf4 promoter is essential for its transcription, as we observed that deletion of a Sox2 binding site within this enhancer region reduces gene expression by ~85%. To investigate such long-range enhancer-mediated transcription dynamics, we combined the photostable mStaygold protein with gentle super-resolution structured illumination microscopy (SR-SIM) to track Klf4 transcriptional bursting for over 12 hours. Hidden Markov modeling of transcription-site intensity trajectories revealed two bursting states, active ("On") and inactive ("Off"), and enabled quantification of burst duration, frequency, and amplitude. To probe bursting regulators, we used CRISPR genome editing to introduce a dTAG degron and acutely (<2 h) deplete four classes of factors: Brd4 (co-activator), cohesin (structural), Med14 (Mediator), and Sox2 (transcription factor). Burst frequency was significantly reduced by depletion of all regulators (Brd4: 63%, Med14: 67%, cohesin: 56% and Sox2: 47%) while the impact on burst duration was relatively mild with only Brd4 and Sox2 depletion resulting in a 30-35% reduction. Except cohesin (6%), depletion of other regulators led to a significant (~35-60%) reduction in burst amplitude. The reduction in bursting parameters was in good agreement with our TT-seq (Transient Transcriptome Sequencing) results, which showed strong reduction (>50%) in nascent transcription for Brd4, Med14, and Sox2, but only a modest effect for cohesin depletion (~20%). To probe enhancer regulation, we fluorescently labeled the Klf4 enhancer (E) and promoter (P) using the tetO/tetR system and performed fast dual-color SR imaging, revealing transient (~5–30 s) E–P contacts with long periods of separation. Cohesin depletion increased average E–P separation by ~40%, while other factors had only minor effects (<15%). Using three color volumetric SR imaging, we observe that during gene bursting, the enhancer is stably associated (>10 min) with a well-defined proximal Brd4 condensate (~270 nm away, ~400 nm in size), which gradually dissociates after the end of the burst. Taken altogether, our multi-color labeling and SR imaging of regulatory DNA elements, proteins, and nascent RNA reveal dynamic mechanisms involving long range enhancer-promoter communication and condensate association toward regulation of transcription bursting.

MS1903

Measuring Force Propagation and Mechanical Properties of the Chromatin in Live Cells

*Ivan Surovtsev[1], Emily Clarke[1], Misheal Saah[1], Ryan Ogasawara[1], Thomas Moss[1], Nikaila Campbell[1], Akua Agyemang[1], Teniola Obayomi[1], Contessa Contessa[1], Simon Mochrie[2], Megan King[1]; Yale School of Medicine[1], Yale University[2]

In cells, long DNA polymers are highly compacted into a spatially organized, yet dynamic, three-dimensional chromatin structure. Chromatin dynamics underlies core genomic processes such as transcription, replication

and DNA repair. The dynamics of chromatin is determined by the response of the DNA polymers and associated factors to a combination of thermal fluctuations and active processes. Chromatin also plays a mechanical role by contributing mechanical stiffness against nucleus deformations from the outside forces. Here, we are seeking to infer mechanical properties of the chromatin in its natural context by measuring how mechanical agitation applied outside of the nucleus propagates through chromatin fibers. To this end, we have developed a live cell assay that utilizes natural agitation of the centromeres by cyclic, microtubule-dependent motion of the spindle pole body (SPB) in fission yeast S. pombe. Using fluorescent microscopy and high-precision single particle tracking of individual chromosomal loci, we measure the correlation in motion between the agitation point (centromere) and a distant 'probe' locus in a library of strains with varied genomic distances between the centromere and the 'probe' locus. Unexpectedly, we observe correlation in SPB and chromatin locus motion far from the source - at least several hundred kilobases away - much farther than one can expect from the current estimates of chromatin persistence length (250-6,000 bp). Moreover, some correlation is observed even for telomeric regions which are more than 1 Mb away. Our results also suggest that at the shorter scale (~ few hundreds kilobases), the agitation propagates mostly through individual chromatin fiber, while at the longer scale (~1 Mb) the agitation propagates through the chromatin meshwork. We use theory of semi-flexible polymers to estimate persistence length (rigidity) of the chromatin in the nucleoplasmic environment. We also investigate how perturbations of heterochromatin which are known to mechanically soften or stiffen nuclei impact chromatin rigidity and the scale of the force propagation. Our results show that mechanical stress can be directly transmitted to the genome, describing both physical and genomic length scales over which the chromatin is capable of directly responding to the mechanical stress exerted on the nuclear envelope.

MS1901
Quantitative analysis of the impact of local chromatin organization on transcriptional dynamics
*Noel Buitrago[1], Bomyi Lim[1]; University of Pennsylvania[1]

Transcription, the conversion of DNA to RNA, is a precisely-controlled process that is essential for proper development and maintenance of an organism. Gene promoters and their regulatory enhancers must interact in spatial proximity to initiate transcription. However, in the eukaryotic genome, enhancers and their target promoters are often separated by large linear genomic distances containing numerous non-target genes, calling into question how these elements reliably find each other in the crowded nuclear environment. Insulator elements serve a prominent role in this long-range enhancer-promoter communication by organizing local chromatin into loop-like domains, facilitating interaction within the loop and silencing interaction with elements outside of it. Despite their importance, systematic analysis on the properties of insulators and their effect on transcription is lacking. This study employs single-cell live imaging techniques to elucidate the dynamics of insulator-mediated chromatin looping and its role in transcriptional regulation. Our system consists of a single enhancer that regulates two equidistant promoters in cis. We show that a single enhancer can co-activate two target promoters in cis, such that transcriptional kinetics from the two reporter genes are coordinated. Insulators are then introduced in various positions and orientations to analyze their effect on transcription. Insulator-mediated looping of the system further synchronizes gene activity and increases transcriptional output. Interestingly, bidirectional insulator conformations produce a more pronounced effect than unidirectional ones, indicating an orientation-dependent stability in insulator pairing. Insertion of an insulator between enhancer and promoter results in a sharp decrease in transcriptional output of the blocked reporter gene. Surprisingly, the blocked promoter is able to escape silencing and often exhibits coordinated

transcriptional activity with the unblocked promoter. These results challenge the traditional view that insulator-mediated chromatin loops are largely static and suggest that the dynamic nature of insulator pairing contributes to transcriptional regulation to varying degrees, based upon their relative positions and orientations. Taken together, this work enhances our understanding of the relationship between local 3D chromatin architecture and transcription, providing new insights into the complex mechanisms governing gene expression.

MS1904

The material properties and organization of the synaptonemal complex regulate protein diffusion along meiotic chromosomes

*Lexy von Diezmann[1], Aspen Hughes[1], Victoria Kurdyumov[1]; University of Minnesota[1]

Crossover recombination between homologous chromosomes is a defining event of meiosis. The physical linkages formed by crossovers are necessary for chromosome segregation into haploid gametes, and meiotic DNA repair is tightly regulated to ensure a small number of well-spaced DNA breaks mature into crossovers. To choreograph this process, DNA repair sites must coordinate their choice of repair pathway while separated by millions of base pairs, yet the mechanism enabling this feat remains unclear. To directly observe how the signals that control crossover fate are transduced along meiotic chromosomes, we recently introduced correlative, live-cell single-molecule tracking to the germline of the nematode worm C. elegans. We discovered that the synaptonemal complex, a liquid-like assembly that forms a ~100-nm thick interface between homologous chromosomes, facilitates the rapid diffusion of pro-crossover signaling molecules along the linear chromosome axis. This allows the synaptonemal complex to act as a conduit, or “molecular antenna,” for signaling proteins to move along chromosomes and communicate between distant breaks to ensure crossovers are well-separated. We hypothesized that genetic and environmental perturbations to the synaptonemal complex known to disrupt crossover regulation do so by altering its material properties and its behavior as an “antenna.” To investigate this possibility, we tracked single molecules of the synaptonemal complex central region protein SYP-3 in the context of mutations in the synaptonemal complex scaffold and at extreme temperatures. By classifying and class averaging hundreds of individual molecular trajectories, we quantitatively described the impact of these perturbations on diffusion and (un)binding kinetics. Remarkably, we observed that disruption of the synaptonemal complex increased the proportion of rapidly moving SYP-3 molecules and their overall rate of relocalization throughout the nucleus. However, we also found that SYP-3's linear excursions along the chromosomes were much shorter in disrupted conditions, and while proteins moved more rapidly overall, the proportion of time spent exploring the chromosomes was reduced. Overall, these results indicate that the organization of the synaptonemal complex directly impacts the ability of proteins to diffuse and transmit information along the length of chromosomes, providing a biophysical mechanism to explain how mutations in meiotic proteins result in errors in crossover formation and thus infertility.

MS1908

Topbp1 mediates the response to DNA damage and acentric chromosomes in inviable Xenopus hybrids

*Cordell Clark[1], Arielle McMullan[1], Rebecca Heald[1]; University of California, Berkeley[1]

Inviable hybrid embryos generated by fertilizing Xenopus tropicalis eggs with Xenopus laevis sperm lose the long arms of chromosomes 3L and 4L and die at gastrulation, but the molecular mechanisms driving the hybrid-specific genomic instability are poorly understood. Combining nuclei isolated from X. laevis embryos with metaphase-arrested X. tropicalis egg extract revealed that a subset of X. laevis chromosomes lose centromere foci marked by the H3 centromeric histone variant CENP-A. Rather than a failure of tropicalis centromere assembly factors to properly load new CENP-A onto laevis chromosomes, we have found that CENP-A is actively removed from this subset of laevis chromosomes cycled in tropicalis cytoplasmic extracts. This defect could be rescued by adding the RNA polymerase II inhibitor triptolide to hybrid reactions, suggesting that replication-transcription conflicts at metaphase leads to CENP-A loss from laevis chromosomes 3L and 4L. Despite the consistent loss of CENP-A on a subset of chromosomes in vitro, a low percentage of hybrid embryo cells display micronuclei in vivo. As has been observed in human cells, topoisomerase II binding protein 1 (TOPBP1) localized to the acentric chromosomes in hybrid extract reactions, suggesting a role for TOPBP1 in responding to acentric chromosomes during metaphase. Immunodepletion of TOPBP1 from hybrid extract reactions prevented CENP-A loss, but led to increased DNA damage marked by gamma H2AX, as well as chromosome alignment defects in hybrid reactions. Addition of recombinant TOPBP1 rescued chromosome alignment, yet CENP-A remained localized to chromosomes and elevated levels of gamma H2AX persisted, indicating DNA damage repair factors co-deplete with TOPBP1 and are required for centromere loss. Inhibition of MMEJ-mediated repair enzyme Pol theta also rescued CENP-A loss in hybrid reactions. Altogether, these results are consistent with DNA repair by TOPBP1 and MMEJ leading to gross chromosomal rearrangements at the centromeres of 3L and 4L, and we are currently pursuing a dCas9-based FISH approach to better understand the nature of these rearrangements and determine whether long arm loss is accompanied by isochromosome formation in inviable Xenopus hybrids. Such a phenomenon would likely reconcile the observation that a low percentage of cells form micronuclei in vivo while a subset of chromosomes consistently lack CENP-A in vitro. Ongoing work is further investigating whether this mechanism is hybrid specific, or if non-hybrid embryos utilize similar pathways to maintain proper chromosome alignment and genome integrity when challenged with DNA damage or acentric chromosomes prior to gastrulation, when canonical cell cycle checkpoints are largely inactive.

MS1902

Transcriptional activity generates chromatin motion that drives nuclear blebbing

*Andrew Stephens[1], Kelsey Prince[1], Nick Borowski[1], Katie Lin[1], Andy Li[1]; University of Massachusetts Amherst[1]

Abnormal nuclear blebbing is a hallmark of human diseases, including cancers and age-related disorders. Previous work has outlined that nuclear blebbing is due to an imbalance of nuclear strength resisting actin confinement and contraction. However, our recent work revealed that inhibiting RNA polymerase II suppresses nuclear blebbing independent of altering nuclear spring constant and actin confinement and contraction, but the mechanism remains unknown. Through removing cell culture media serum and then adding it back, we can decrease and then restore transcriptional activity. Decreasing transcriptional activity decreases nuclear bleb formation, stability, and rupture while returning transcriptional activity increases nuclear blebbing. These modulations of transcriptional activity did not alter nuclear or actin mechanics. Instead, we find that transcription activity regulates chromatin motion measured by tracking the mean square displacement (MSD) of chromatin domains labeled via transfected Cy3-dNTPs. To determine if increasing chromatin motion is a mechanism to increase nuclear blebbing, we used an established RAD51 inhibitor BO2 to increase chromatin motion. We verified BO2 increases chromatin domain motion which resulted in increased nuclear blebbing. We

reveal the mechanism by which transcriptional activity drives nuclear blebbing is through chromatin motion. Thus, two hallmarks of human disease are directly linked via transcriptional activity and abnormal nuclear shape.

MS1907
Understanding the role of CENP-A in modulating responses to DNA damage and R-loops formation in human cancers
*Elena Di Tommaso[1], Simona Giunta[1]; University of Studi La Sapienza[1]

The human centromere is the primary constriction within mitotic chromosomes made up of repetitive alpha-satellite DNA hierarchically organized in megabase-long arrays of near-identical higher order repeats (HORs). Centromeres are epigenetically specified by the presence of the centromere-specific histone H3 variant, CENP-A, which enables the assembly of the kinetochore for microtubule attachment. Centromere integrity is crucial to ensure the correct chromosome segregation and preserve the stability of intra and inter-generational inheritance of the genetic information. When errors occur at centromere level, the cells undergo aneuploidy, a major contributor to cancer development. Despite being recognized as a hallmark of human cancer, the exact role of aneuploidy as a “driver” is still largely unknown. Here, we describe how replication stress triggers the non-random segregation (NDS) of DNA damage (γH2AX) in hTERT-RPE-1 cells as a mechanism to preserve the genomic information in one of the two daughter cells. Using a Doxycycline inducible RNaseH1 and an Auxin inducible CENP-A degron, we observed an increase in NDS after CENP-A depletion and a reduction after R-loops removal. We also observed how the homologous recombination protein Rad51 manifests the non-random segregation. Using a chemical inhibitor of Rad51 we observed a strong reduction of cells manifesting the NDS of both γH2AX and Rad51, suggesting an active role of the homologous recombination pathway. Here, we propose that the replication stress triggers the homologous recombination pathway to accumulate the damaged newly synthetized strands in only one of the two sister chromatids. We also hypothesize that the accumulation of R-loops affect how the kinetochore proteins bind to the centromere inducing mechanical and/or epigenetic signals that influence the orientation of the sister chromatids on the metaphase plate. This mechanism can be fundamental to maintain the genetic information; however, the impact of the accumulation of DNA damage on the cell fate remains elusive.

Cell-Cycle Mechanisms and Cancer Vulnerabilities

MS601
CDC7 and APC/CCdh1 Gate Distinct Routes to Initiate DNA Replication
*Samsara Upadhya[1], Nalin Ratnayeke[2], Katherine Ferrick[1], David Rosenthal[1], Tobias Meyer[1]; Weill Medical College/Cornell University[1], Memorial Sloan Kettering Cancer Center[2]

Precise temporal control of DNA replication initiation is essential for faithful cell division, yet there are conflicting prevailing models for how cells trigger origin firing. Here, we demonstrate that human origins are not fired at fixed thresholds of cell cycle regulators E2F, APC/CCdh1, CDK2/1, and CDC7. Instead, origin firing is triggered at a tunable CDK2/1 threshold that is gated by either CDC7 or APC/CCdh1 activity. CDC7 phosphorylates MCM helicase, enabling Cyclin E-CDK2 to trigger origin firing at low CDK2/1 activity,

independent of APC/CCdh1 inactivation. In contrast, APC/CCdh1 inactivation enables Cyclin A-CDK1 to phosphorylate MCM at distinct CDK2/1-specific sites and trigger origin firing, independent of CDC7 activity. Strikingly, this requires much higher CDK2/1 activity, which CDK2 cannot normally reach alone. Thus, having two distinct routes necessitates blocking both routes, through inhibition of CDC7 or CDK2 together with reinforcing APC/CCdh1 activity or inhibiting CDK1, to prevent S phase.

MS606

Characterizing a Novel Class of Antimitotic Compounds

*Keming Li[1], Albert Liu[3], Jin Zhu[1], Bin Sheng Wong[1], Hui Ting ONG[1], Brian Burke[2], Rong LI[1]; The Mechanobiology Institute, National University of Singapore[1], Agency for Science, Technology and Research[2], Johns Hopkins University School of Medicine; The Mechanobiology Institute, National University of Singapore[3]

Microtubule-stabilizing or -destabilizing agents (e.g., paclitaxel) are effective against rapidly dividing cells, such as cancer cells but often cause dose-limiting neurotoxicity because they directly target tubulin, which is intrinsically linked to neurological side effects. This has prompted interest in developing drugs that disrupt microtubule dynamics through alternative targets that are more specific to mitotic cells. In an unrelated screen, we identified four structurally related small molecules (WUF1–WUF4) that impair the microtubule-organizing center (MTOC) and spindle morphology during prometaphase. Treatment leads to loss of astral microtubules, altered spindle length, chromosome misalignment, and subsequent multinucleation, consistent with disruption of a regulator of spindle assembly. These findings suggest that the compounds target a regulator of the mitotic spindle during mitosis. Using synchronized prometaphase cells and the peptide-centric local stability assay (PELSA)—which detects ligand-induced protection of protein regions from proteolysis—we identified potential MTOC-associated mitotic regulator(s) as candidate targets of WUF compounds. Ongoing studies aim to validate the putative drug target and to uncover the mechanism of action. We will then evaluate the antitumor potential of these compounds as mitosis-selective chemotherapeutic agent with a reduced risk of neurological liabilities.

MS605

Cytokinesis failure can be predicted before mitosis in mammary epithelial cells

*Maryam Kohram[1], Phoebe Marshall[1], David Frankel[1], Celeste Nelson[1]; Princeton University[1]

The stability of the cell division cycle is essential for maintaining genomic integrity. Perturbations in the mechanisms that regulate the cell cycle can result in abnormal cellular phenotypes, such as multinucleated cells, which cause genomic instability, cancer progression, and metastasis. We hypothesized that cytokinesis failure and multinucleation are preceded by measurable changes in cellular morphology and behavior. To test this hypothesis, we took advantage of MCF10A human mammary epithelial cells, which undergo epithelial–mesenchymal transition (EMT) and cytokinesis failure when treated with transforming growth factor-beta (TGFβ). We performed long-term time-lapse imaging with fluorescent reporters to track mitochondrial activity, cell-cycle progression, nuclear morphology, and motility at single-cell resolution. This analysis revealed distinct phenotypic alterations in mother cells that undergo cytokinesis failure and produce multinucleated progeny as compared to those that divided normally and produce mononucleated daughters. While mitochondrial membrane potential remained preserved, mothers of multinucleated cells displayed irregular cell-cycle

durations, namely prolonged G2 and M phases, alongside nuclear pleomorphism and aberrant size regulation. Motility analysis revealed a higher frequency of persistent migration and altered polarization among mothers of multinucleated progeny. Our findings reveal that multinucleation is preceded by disruptions in cell motility, nuclear morphology, and cell-cycle duration, providing a functional signature that predicts cytokinesis failure. These insights suggest that the observed variations could serve as predictive markers for aneuploidy, offering insights into the cellular instabilities that initiate cancer progression.

MS602

Fluorescent cross-correlation spectroscopy reveals the affinity of cyclin-CDK complexes using green and near-infrared fluorescent proteins

*Aika Toyama[1], Yuhei Goto[3], Yuhei Yamauchi[3], Hironori Sugiyama[2], Yohei Kondo[4], Atsushi Mochizuki[3], Kazuhiro Aoki[3]; The Graduate University for Advanced Studies[1], Institute of Science Tokyo[2], Kyoto University[3], Nagoya University[4]

Protein–protein interactions are central to diverse biological processes, including cell cycle progression, differentiation, and signal transduction. However, quantifying the strength of specific interactions in living cells remains technically challenging. Fluorescence cross-correlation spectroscopy (FCCS) enables direct measurement of molecular interactions in living cells by tagging two proteins with spectrally distinct fluorescent proteins and simultaneously monitoring their fluorescence fluctuations. Because photobleaching reduces measurement accuracy, photostability of fluorescent proteins is critical for FCCS. While the green fluorescent protein mNeonGreen (mNG) is sufficiently photostable, a comparably stable red fluorescent protein has been lacking. Here, we demonstrate the advantage of the near-infrared fluorescent protein miRFP670 for FCCS. Compared with red fluorescent proteins such as mCherry2 and mScarlet-I, miRFP670 exhibited markedly higher photostability, enabling more reliable FCCS measurements in living cells. Using the mNG and miRFP670 pair, we quantified binding affinities between cyclins and cyclin-dependent kinases (CDKs) in living HeLa cells. CDK is a master regulator of the cell cycle, which is activated through cyclin binding. In mammals, multiple cyclins and CDKs form specific combination of complexes during each cell cycle phase, which is an essential mechanism ensuring cell cycle phase–specific regulation. However, the extent of these interactions in living cells has remained elusive. We measured the dissociation constants of 36 cyclin–CDK complexes. As expected, conventional complexes such as cyclin D–CDK4/6, cyclin E–CDK2, cyclin A–CDK1/2, and cyclin B–CDK1 showed high affinities. Intriguingly, we also detected unconventional interactions, including cyclin D1/D3–CDK2 and cyclin B1–CDK2, suggesting that the cyclin–CDK interaction network is broader than previously appreciated.

MS603

Phosphoproteomics identifies a new role for Cdr2 kinase in cell division

*Veronica Garrido[1], Hannah Oplako[1], Elora Greiner[1], Arminja Kettenbach[1], Jamie Moseley[1]; Geisel School of Medicine at Dartmouth College[1]

Signaling pathways coordinate the events of cell division in time and space. Proteins that act in multiple steps of cell division act as master regulators of this process. In the fission yeast Schizosaccharomyces pombe, the protein kinase Cdr2 is required for timely entry into mitosis and proper formation of the division septum. Cdr2

regulates mitotic entry through Wee1, but its targets in other processes including septation are unknown. We performed a large-scale phosphoproteomic screen to identify new targets of Cdr2 signaling. From multiple candidates, we focused on Cnt6 as a potential substrate of Cdr2. Cnt6 belongs to the centaurin family of ADP-ribosylation factor GTPase-activating proteins (Arf-GAP). Cnt6 phosphorylation was reduced in cdr2Δ but increased by Cdr2 overexpression. Mutation of phosphorylation sites identified in our phosphoproteomic screens prevented this regulation. cnt6Δ mutants exhibited septation defects similar to cdr2Δ, suggesting that Cdr2-Cnt6 signaling regulates septation. By live-cell microscopy, we found that Cnt6 localized to the cell periphery and the spindle pole body (SPB). During mitosis, Cnt6 localized asymmetrically to old SPB but not the new SPB. This asymmetry is similar to components of the Septation Initiation Network (SIN), a signaling pathway that regulates the timing and position of cytokinesis. Consistent with this possibility, a series of genetic and live-cell imaging experiments indicated that Cnt6 functions as a negative regulator of SIN signaling. Our data identify Cnt6 as a novel target of Cdr2 signaling with functions in the SIN pathway and septation. These findings suggest that Cdr2 acts as a master regulator of cell division through distinct targets for mitotic entry and septation.

MS607

Targeting cyclin-dependent kinases to overcome therapy resistance in breast cancer

*Sungsoo Kim[1], Hee Won Yang[1]; Columbia University[1]

Cyclin-dependent kinases 4 and 6 (CDK4/6) are frequently dysregulated in cancer, and CDK4/6 inhibitors have transformed treatment for breast cancer. Nonetheless, clinical benefit is often curtailed by acquired resistance. CDK4/6 normally phosphorylate the retinoblastoma protein (Rb), releasing E2F transcription factors to drive cell-cycle gene expression. We recently found that, under CDK4/6 inhibition, cells can bypass this blockade by promoting proteolytic degradation of Rb. This alternative, incomplete Rb inactivation yields subthreshold E2F activity that requires additional transcriptional amplification. We identify c-Myc as the principal amplifier of this E2F program and a candidate predictive biomarker of CDK4/6 inhibitor response. Because direct c-Myc targeting remains challenging, we curtailed c-Myc activity by inhibiting the downstream RNA polymerase II transcriptional machinery. As CDK7 is a core activator of RNA polymerase II–dependent transcription, dual inhibition of CDK4/6 and CDK7 in breast cancer models with an intact Rb/E2F axis suppressed c-Myc–driven amplification, restored durable cell-cycle arrest, and reduced resistance. Moreover, co-targeting CDK4/6 and CDK7 enhanced antitumor immunity. Together, these findings define an adaptive resistance route to CDK4/6 inhibition and support a rational combination strategy that disables compensatory proliferative circuits while concurrently engaging the immune system.

MS604

Untimely Fibrous Corona Expansion Driven by MPS1 Overexpression Underlies Chromosomal Instability in Colorectal Cancer

*Ana Pinto-Teixeira[1], André Oliveira[4], Ana Sousa[4], Gonçalo Gouveia[4], Marco Grillo[5], Sergi Rodriguez-Calado[2], Marin Barisic[2], Claudio Sunkel[4], Daniele Fachinetti[5], Karoly Szuhai[3], Noel De Miranda[3], Carlos Conde[4]; i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal and Programa Doutoral em Biologia Molecular e Celular, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto,

Porto, Portugal[1], Cell Division

Chromosomal Instability (CIN) is a prevalent feature of human cancers involved in tumour progression and intimately associated with poor patient prognosis. Yet, the mechanistic cause of CIN remains poorly understood. Here, we narrow this knowledge gap by establishing MPS1 overexpression as a driver of CIN and by uncovering the underlying molecular details. RNA-sequencing of colorectal tumours disclosed a correlation between increased aneuploidy and overexpression of the mitotic checkpoint kinase MPS1. Functional studies in patient-derived colon cancer cells (PCCC) revealed an elevated frequency of merotelic kinetochore-microtubule attachments and chromosome segregation errors (lagging chromosomes) in CIN+ cells expressing higher levels of MPS1. Experimentally inducing MPS1 overexpression in RPE-1 and low-passage MSI cells (CIN-) phenocopied these defects and promoted continuous karyotypic heterogeneity and micronuclei formation in previous near-diploid stable cultures. To determine the mechanism by which MPS1 drives CIN, we screened for phospho-epitopes responsive to MPS1 overexpression. The phosphorylation of ROD Thr13/Ser15, required for fibrous corona assembly, stood out as being uncommonly elevated at metaphase kinetochores of MPS1-overexpressing cells. Accordingly, we observed an abnormal accumulation of SPINDLY, CENP-F and ZW10, suggesting that the fibrous corona was atypically expanded. Strikingly, preventing fibrous corona assembly through inhibition of Spindly farnesylation with FTI-277, or partial inhibition of MPS1 activity with suboptimal concentrations of Cpd-5, efficiently decreased the frequency of merotelic attachments and restored the fidelity of chromosome segregation in both RPE-1 cells overexpressing MPS1 and PCCC CIN+ lines. Our results demonstrate that an untimely expanded fibrous corona, driven by excessive MPS1 activity, provides a structural basis for merotely, chromosome missegregation and CIN. These findings not only advance our understanding of the mechanisms driving CIN but also underscores the potential significance of targeting MPS1 and the fibrous corona as therapeutic strategies in colorectal cancer.

Cellular Quality Control and Homeostasis

SY501
Shedding light" on Autophagy-mediated Quality Control: Developing Optogenetic Tools and Genome Wide Screens to Uncover Novel Mechanisms
*Christina Towers[1]; The Salk Institute for Biological Studies[1]

Autophagy is a highly regulated and conserved cellular recycling process that mediates protein and organelle quality control. It involves targeted degradation of cytoplasmic material to autophagosomes which is then trafficked to lysosomes to maintain cellular homeostasis under both basal conditions and during cells stress. The full extent of autophagy substrates has yet to be elucidated. In one project, we seek to uncover the most tightly regulated autophagy substrates that are constitutively turned over and the mechanisms behind these homeostatic pathways. To this end, we developed an optogenetic tool to inhibit autophagy rapidly and selectively with light, coined Autophagic Snare for Abrupt Photomanipulation (ASAP). We confirm that light inhibits autophagy within minutes in ASAP expressing cells and works faster than pharmacological agents that target autophagy. We combined ASAP with TMT proteomics to identify proteins that immediately accumulate after rapid autophagy inhibition. We found that selective mitochondrial proteins and nuclear pore complex (NPC) proteins are constitutively degraded by autophagy. We are characterizing the exact mechanisms and consequences of these autophagy targets. In a second project, we seek to understand new mechanisms of mitochondrial quality control that compensate for the loss of canonical mitophagy pathways. We performed a genome-wide screen coupled with flow cytometry as a read out of mitochondrial delivery to lysosomes in cells

that lack the classic autophagy machinery. We found a new link between epigenetic regulation and non-canonical LC3- independent mitophagy. Together these studies uncover new insights into selective autophagic degradation and maintenance of organelle quality control that could be relevant across diverse pathologies including cancer, neurodegeneration, and aging.

SY502
Stress, Rupture and Repair: Cellular Responses to Lysosomal Damage
*Phyllis Hanson[1]; University of Michigan[1]

Lysosomes function as the terminal degradative compartment of the endomembrane system and are continually challenged by environmental fluctuations and the accumulation of potentially damaging material. Membrane permeabilization disrupts lysosomal integrity and cellular homeostasis and has emerged as a common event in disorders ranging from neurodegeneration to immune dysfunction and cancer. Cells respond to such stress through coordinated pathways that reinforce, repair, or remove damaged membranes. This talk will highlight recent insights into the molecular mechanisms underlying these responses – including how ESCRT machinery, lipid signaling, and related processes cooperate to restore lysosomal integrity and maintain homeostasis.

Closing keynote: Visualizing Form and Function in the Cell - Eva Nogales, Jennifer Lippincott-Schwartz

K201
Insights into microtubule dynamics regulation by glutamylation from mechanistic studies of tubulin code erasers
*Jiayi Chen[1], Elena Zehr[1], James Gruschus[2], Agnieszka Szyk[1], Yanjie Liu[3], Martin Tanner[3], Nico Tjandra[2], Antonina Roll-Mecak[1]; National Institute of Neurological Disorders and Stroke[1], National Heart, Lung and Blood Institute[2], University of British Columbia[3]

Microtubule function is modulated by the tubulin code, diverse posttranslational modifications that are altered dynamically by writer and eraser enzymes. Glutamylation, the addition of branched glutamate chains, is the most evolutionarily widespread tubulin modification. It is introduced by the tubulin tyrosine ligase-like enzymes (TTLLs) and erased by the cytosolic carboxypeptidases (CCPs). Glutamylation marks stable microtubules, such as those in axons and axonemes, and its homeostasis, achieved through the balance of writers and erasers, is critical for normal physiology. Disruption of this balance by mutations in CCP1 and CCP5 leads to human pathologies. Despite its importance, how glutamylation affects intrinsic microtubule dynamics remained unclear. By generating tubulin with short or long glutamate chains, we showed that glutamylation is a negative regulator of microtubule growth. Thus, the stabilizing effect observed in cells should come from effectors. We found that CCP1 preferentially deglutamylates soluble tubulin, in contrast to the TTLLs that prefer modifying microtubules. CCP5, by contrast, showed similar activity on soluble tubulin versus microtubules, while it is inactive on α tubulin and a major β tubulin isotype in the brain. Cryo-EM structures of CCP5 in complex with the microtubule, and X-ray structures in complex with transition-state substrate analogues, explain tubulin/microtubule recognition and reveal that CCP5 deforms the tubulin main chain into a

unique turn that enables branch glutamate recognition. CCP5 binding of the sequences flanking the branch point primarily through peptide backbone atoms enables processing of diverse tubulin isotypes with glutamate branches at different positions, as well as non-tubulin substrates. Our work provides the first atomistic view into glutamate branch recognition and resolution, and sheds light on homeostasis of the tubulin glutamylation syntax and its effects on microtubule dynamics.

Compartmentalized Signaling in Neurons

MS803
A Potential Role for Par3 in Organizing the Postsynaptic Scaffold
*Hanna Caiola[1], Maya Salameh[1], Mengying Deng[2], Paola Torres[1], Zheng Shi[2], Huaye Zhang[1]; Rutgers Robert Wood Johnson Medical School[1], Rutgers University[2]

Synaptic dysfunction and dendritic spine abnormalities often underlie the cognitive symptoms of neurodevelopmental disorders. Within the dendritic spine, synaptic proteins such as receptors, signaling molecules, and scaffolding proteins are organized into sub-compartments called nanomodules. Previous studies have found that the number of nanomodules increases in proportion to synaptic strength and spine size, with immature spines having fewer nanomodules compared to mature spines. Nevertheless, the molecular players driving the formation of nanomodules in the synapse are not fully characterized. The scaffolding protein Partitioning Defective 3 (Par3) is known to promote subcellular compartmentalization. Furthermore, recent work in our lab has found that loss of Par3 in mice results in an increased pool of immature dendritic spines. Therefore, we hypothesize that Par3 may promote dendritic spine maturation and stability by driving the formation of nanomodules within the postsynaptic density. Unexpectedly, we discovered that Par3 interacts with PSD95, a key postsynaptic scaffolding protein. We have narrowed this interaction down to be between the C-terminal 4N2 domain of Par3 and the N-terminus of PSD95. Moreover, Par3 colocalizes with and promotes the clustering of PSD95 in heterologous cells. Within these clusters, Par3 exhibits higher mobility than PSD95, and co-expression of Par3 and PSD95 results in reduced Par3 mobility. Currently, we are working to further characterize the Par3-PSD95 interaction by exploring whether this interaction relies on phase separation, a common mechanism for subcellular compartmentalization of proteins. Furthermore, we are examining the biophysical properties of Par3 and PSD95 condensates using micropipette aspiration and whole-cell patch clamp (MAPAC). Lastly, we have found that Par3 and PSD95 colocalize within the dendritic spine using protein-retention expansion microscopy (ProExM) to visualize the nanostructure within the synapse and are further investigating this interaction under plasticity-inducing conditions.

MS806
BAI1/ADGRB1-mediated Regulation of Mitochondria Morphology in Axons
*Kimberley Tolias[1], Joseph Duman[1]; Baylor College of Medicine[1]

Neurons rely on precise structural and functional polarization, and mitochondrial specialization is central to this. Axonal mitochondria are uniquely small, motile, and tuned to deliver localized metabolic support, Ca^{2+} buffering, and redox signaling that sustain presynaptic activity and guide axonal branching. Yet, the signaling

mechanisms that dictate axonal mitochondrial morphology remain poorly understood. Our work has previously revealed that the adhesion-GPCR BAI1/ADGRB1 is essential for excitatory synapse and dendrite development. BAI1, a member of the brain-specific angiogenesis inhibitor family implicated in glioblastoma, schizophrenia, bipolar disorder, and autism, promotes excitatory synaptogenesis by recruiting the Par3/Tiam1 complex to dendritic spines, activating Rac1, and driving actin remodeling. BAI1 also promotes presynaptic differentiation via trans-synaptic signaling and restricts dendritic arbor growth late in development by engaging Bcr's cryptic RhoA-GEF and Rac1-GAP activities to terminate arborization. Here, we identify a new role for BAI1 in regulating axonal mitochondrial morphology. Loss of BAI1 in cultured hippocampal neurons and in vivo mouse models leads to abnormally elongated axonal mitochondria, accompanied by altered ATP production without changes in baseline motility. Rescue experiments show that BAI1's PDZ-binding motif, canonical GPCR domains, and its cryptic Stachel autoagonist sequence are all required for this regulation. Pharmacological data further implicate RhoA and Gα12/13 signaling downstream of BAI1, pointing to a previously unrecognized Stachel–GPCR signaling axis that controls axonal mitochondria. Together, these findings expand BAI1's repertoire beyond its established roles in promoting synapse formation and restricting dendritic/axonal growth. We propose that BAI1 integrates extracellular and trans-synaptic cues with mitochondrial plasticity to coordinate axonal structure and presynaptic metabolic support. This represents the first demonstration of BAI1 acting through its Stachel–GPCR axis in neurons and reveals a new signaling mechanism linking adhesion-GPCR pathways to axonal specialization and brain function.

MS801
Decoding Synaptic Signaling Dynamics Underlying Plasticity
*Ryohei Yasuda[1]; Max Planck Florida Institute for Neuroscience[1]

Neuronal computation depends on the ability of dendritic spines to compartmentalize biochemical signals, allowing synapses to change independently while maintaining circuit stability. However, the rules by which signaling pathways are confined or integrated across space and time remain poorly defined. We have developed and applied two-photon fluorescence lifetime imaging microscopy (2pFLIM) to measure signaling activity in living brain tissue with molecular specificity and sub-micron resolution. Using FLIM-based biosensors, we monitor diverse signaling pathways—including CaMKII, Ras family small GTPases, and ERK— in single spines during plasticity. These studies reveal that many signaling events are restricted to individual spines, yet others can spread into dendrites or neighboring synapses, providing insights into how neurons balance input specificity with signal integration. To interrogate the causal role of signaling dynamics, we use a photoactivatable CaMKII inhibitor, paAIP2, which allows second-scale temporal control of kinase activity in vivo. This approach has enabled us to dissect when CaMKII activity is required during plasticity induction and consolidation, highlighting distinct temporal windows of kinase function. In parallel, we are developing a miniaturized two-photon FLIM microscope (mini-2pFLIM) that enables imaging of signaling activity in freely moving animals. Early applications suggest that this technology will allow us to extend our studies from isolated synapses to naturalistic contexts, providing a new window into how biochemical signaling contributes to learning-related processes. Together, these efforts begin to establish a framework for understanding compartmentalized signaling in neurons—from molecular dynamics confined within individual spines, to their regulation across dendritic networks, and ultimately to their potential contribution to behavior.

MS807
Endocytic machinery maintains active zone assembly at presynaptic nerve terminals
*JAVIER EMPERADOR MELERO[1]; New York University Grossman School of Medicine[1]

Neurotransmitter release relies on the side-by-side assembly of two machineries: the active zone, which generates release sites, and the endocytic apparatus, which restores neurotransmitter-filled synaptic vesicles at the adjacent periactive zone. Our goal is to understand the mechanisms that position these two machineries next to one another at presynaptic terminals. We previously found that endocytic proteins remain deployed at the periactive zone when the active zone is disassembled or when evoked neurotransmission is blocked, arguing against a model in which endocytic machinery is mainly recruited in a release-dependent manner. We have begun testing the complementary hypothesis that endocytic machinery positions the active zone. Simultaneous ablation of the endocytic proteins Dynamin-1, -2, and -3 in hippocampal neurons resulted in a 50% reduction in the synaptic levels of two key active zone proteins, RIM and Munc13-1, while the levels of other synaptic proteins were not diminished. At the nanoscale level, a preliminary assessment showed that the number of Munc13-1 nanoclusters per synapse was similarly reduced, and that these nanoclusters were positioned further apart from voltage-gated CaV2 channels. Matching these architectural defects, the releasable pool of synaptic vesicles and their probability of being released by an action potential were substantially diminished. We conclude that Dynamin maintains active zone assembly, indicating that endocytic machinery organizes the active zone at presynaptic terminals.

MS802
Live Super-Resolution Imaging Reveals Regulated Phase Separation of Postsynaptic Proteins
*Matthew Dalva[1], Rachel Cain[2]; Tulane University[1], Thomas Jefferson[2]

Neuronal synapses are ordered on the nanoscale, and this order is essential for their function. Here, we demonstrate that nanoclusters of proteins critical for synaptic development and plasticity undergo dynamic association and segregation by liquid-liquid phase separation (LLPS) in living neurons. Live-cell super resolution STED imaging, FRAP, and chemical disruption of LLPS of endogenous post-synaptic proteins tagged with EGFP by CRISPR-Cas9 knock-in, demonstrate that PSD-95 and AMPARs, but not NMDARs, form dynamic LLPS conjugates in living neurons. The phase states of PSD-95 and AMPARs are dynamically regulated by neuronal development, extracellular calcium, and structural plasticity, linking phase separation to synaptic remodeling. These live-cell data of endogenous proteins suggest that LLPS forms distinct synaptic microenvironments that concentrate proteins critical for synaptic plasticity and development.

MS805
Synapses drive local mitochondrial ATP synthesis to fuel plasticity
*ILIKA GHOSH[1], RUOLIN FAN[1], MONIL SHAH[1], OJASEE BAPAT[1], VIDHYA RANGARAJU[1]; Max Planck Florida Institute for Neuroscience[1]

Our brain constantly forms new memories and stabilizes existing memories. To achieve such cognitive flexibility, the brain is wired with plastic synapses that are hotspots of energy consumption. Supplying energy to distant synapses is challenging as they are distributed throughout dendrites and axons, hundreds of microns from their cell body. Synapses, therefore, require an instant and local energy supply provided by locally stabilized mitochondria. However, the mechanisms by which synapses communicate their energy demands to mitochondria, drive local energy production, and sustain synaptic plasticity is unknown. Using highly sensitive spine- and mitochondrial ATP reporters and two-photon glutamate uncaging to stimulate individual spines, we find that synaptic plasticity drives instant and sustained increase in spine ATP levels, provided by local ATP synthesis in ~10-20 µm spatially confined mitochondrial compartments. This ATP generation is driven by a spatially localized mitochondrial calcium influx independent of the endoplasmic reticulum. Notably, the initial spine ATP increase is instantaneous and is independent of CaMKII activation. Without local calcium signaling and mitochondrial stabilization, spines fail to meet their instant and sustained energy needs, resulting in synaptic plasticity defects in neurological disorders.

MS808

Synaptojanin1 supports VPS35-dependent trafficking of dopamine D2 autoreceptors at presynaptic terminals

*Pingyue Pan[1], Nirmal Kumar[1], Elnaz Khezerlou[1], Justin Cai[1], Hanna Caiola[1], Huaye Zhang[1]; Rutgers University[1]

Dopamine release is regulated by synaptic vesicle recycling and gated by dopamine D2 short autoreceptor (D2S). Whether and how presynaptic membrane trafficking regulates dopamine release remains poorly understood. Here, we identify a functional cooperation between two Parkinson's disease genes, Synaptojanin1 and VPS35, in regulating endosomal sorting of presynaptic D2S thereby influencing dopamine signaling. We show that Synaptojanin1 deficiency results in intracellular retention of D2S, impaired gating of dopamine release and reduced behavioral responsiveness to a D2-like agonist. VPS35 is recruited to D2S-containing and Rab7a-positive endosomes in a Synj1-dependent manner, and VPS35 overexpression overcomes Synj1 deficiency-associated impairment in D2S surface delivery in axons. Moreover, Synj1 regulates VPS35 expression and localization in dopaminergic axons, indicating their broader roles in presynaptic cargo sorting. These findings reveal a previously unrecognized cooperation between a synaptic vesicle endocytic regulator and a retromer component, providing new mechanistic insights into presynaptic trafficking and its disruption in Parkinson's disease.

MS804

Temporal-specific degradation of activity-induced nascent proteins by membrane-associated proteasomes in the developing visual circuit

*Haiyan He[1]; Georgetown University[1]

Proteostasis plays critical roles in cellular functions. In neurons, both protein synthesis and degradation have been shown to be required for synaptic plasticity, the core mechanism underlying important cognitive functions such as learning and memory. However, our understanding of the cellular machinery and mechanisms underlying activity-dependent proteostasis remains incomplete, as highlighted by recent discovery of the neuronal membrane proteasome (NMP). Unlike the well-known intracellular 26S proteasome which targets misfolded or other unwanted proteins through the ubiquitin-dependent proteasome pathway,

NMPs were found to specifically degrade nascent proteins in response to pharmacologically elevated neuronal activity, and may in turn be involved in regulating activity and experience-dependent plasticity in neurons. To further elucidate how NMP activity is recruited by plasticity-inducing experience in vivo, we examined the temporal dynamics of NMP-mediated degradation of nascent proteins and their proteasome activity in the midbrain of Xenopus laevis tadpoles in response to a brief period of enhanced visual experience (VE). As predicted by prior studies, 30 min of VE induced multiple waves of acute protein synthesis in the midbrain, which is the visual processing center in tadpole brain. Intriguingly, inhibition of NMP activity at different time points following VE revealed temporal-specific degradation of VE-induced nascent proteins mediated by NMPs, which was accompanied by concomitant increase in NMP-specific proteasome activity. Functional analysis by time-lapse Ca imaging showed that blocking NMP activity during the NMP-active periods following VE resulted in significant disruption of visually-evoked Ca2+ responses in tectal neurons, suggesting NMP function play a critical role in maintaining normal neuronal function following plasticity-inducing experience. These results provide a fine-scale delineation of the dynamics of activity-induced synthesis and degradation of nascent proteins in response to physiologically relevant sensory experience in the brain. It also shed light on cellular mechanisms underlying the temporal-specific proteostatic regulation of the nascent proteome that may be critical for maintaining circuit stability in experience-dependent plasticity.

Dynamics of the Actin Cytoskeleton Across Physiological States

MS102

A new family of pointed end actin regulators (Aip5/SH3BGRL) conserved between animals and fungi

*Joseph Magliozzi[1], Lucas Runyan[1], Adah Welsh[1], Shae Padrick[1], Bruce Goode[1]; Brandeis University[1]

The two ends of the actin filament (“barbed end” and “pointed end”) have distinct properties and are differentially regulated in cells. While our mechanistic understanding of actin regulation at barbed ends is reaching maturity, our understanding of pointed end regulation in cells is comparatively limited. For instance, pointed end cappers have been found in the animal kingdom but not the fungal kingdom, calling into question how universal a role pointed end capping plays in controlling cellular actin dynamics. Here, we identify yeast Aip5 as a member of a new family of thioredoxin-related domain pointed end-associated proteins (Aip5/SH3BGRL), conserved between the animal and fungal kingdoms. This discovery was made by studying actin nucleation mechanisms in yeast. We found that two fungal proteins, Aip5 and Bud6, directly interact to form a novel 'composite nucleator', in which the pointed end of the nascent actin seed is bound by the thioredoxin-related domain of Aip5, and the sides by two WH2-related domains in Bud6. The Aip5-Bud6 complex assembles actin seeds with free barbed ends and recruits formins to processively elongate and protect the barbed ends from capping protein. Live imaging reveals that the actin interactions of Aip5 and Bud6 are critical for maintaining proper thickness of actin cable bundles in vivo, which in turn prevents premature cable detachment from the bud neck and defects in secretory traffic. Single molecule imaging shows that after actin nucleation Aip5 remains associated with pointed end of the filament, stabilizing it against depolymerization. Further, live imaging reveals Aip5 puncta streaming inward from cortical polarity sites toward the back of the mother cell via actin cable retrograde flow, which depends on Aip5-actin interactions. From a structural homology search, we found that the thioredoxin-related domain of Aip5 is very similar to the widely-expressed vertebrate protein SH3BGRL. Further, other groups have recently shown that SH3BGRL interacts with the pointed ends of actin filaments. Together, these findings expand the known diversity of actin nucleation mechanisms and reveal a new family of pointed end-associated proteins (Aip5/SH3BGRL) conserved between yeast and metazoans.

MS108

Arp2/3-mediated bidirectional actin assembly by SPIN90 dimers

*Tianyang Liu[1], LuYan Cao[3], Miroslav Mladenov[3], Guillaume Romet-Lemonne[2], Michael Way[3], Carolyn Moores[1]; Institute of Structural and Molecular Biology, Birkbeck College, London, UK[1], Institut Jacques Monod, Université Paris Cité, CNRS, Paris, France[2], The Francis Crick Institute, London, UK[3]

Branched actin networks play critical roles in various cellular processes, from cell migration to intracellular transport. At the core of these networks is the Arp2/3 complex. When activated by VCA-containing nucleation-promoting factors, the complex nucleates the formation of a daughter filament from the side of a pre-existing mother filament. In contrast, when activated by WHSH/DIP/SPIN90 family proteins, Arp2/3 nucleates the formation of an unbranched actin filament. However, it remains unclear how the relatively small interface between WHSH/DIP/SPIN90 family proteins and ArpC4 is sufficient for full activation, especially given that branch formation requires extensive contacts with the mother filament. To address this question, we examined the interaction of human SPIN90 with human Arp2/3 complex using biophysical and structural approaches. We found that human SPIN90 is a dimer that can nucleate bidirectional actin filaments. To understand the basis for this, we determined a 3 Å resolution structure of human SPIN90–Arp2/3 complex nucleating actin filaments. Our structure shows that SPIN90 dimerizes through a three-helix bundle and interacts with two Arp2/3 complexes. Each SPIN90 molecule binds both Arp2/3 complexes to promote their activation. Our analysis demonstrates that single-filament nucleation by Arp2/3 is mechanistically more like branch formation than previously appreciated. The dimerization domain in SPIN90 orthologs is conserved in metazoans, suggesting that this mode of bidirectional nucleation is a common strategy to generate antiparallel actin filaments. Our work uncovers a new paradigm for how Arp2/3 is activated to shape actin cytoskeleton architecture.

MS109

Beyond Branching: Structural Basis of Arp2/3 Complex Activation for Linear Actin Filament Nucleation by a SPIN90 Dimer

*Justus Francis[1], Kankipati Shyam[1], Saikat Chowdhury[1]; CSIR-Centre for Cellular & Molecular Biology[1]

Arp2/3 complex is a central actin nucleator whose activity is tuned by diverse nucleation-promoting factors (NPFs) to generate specialized actin architectures. WASP-family NPFs require pre-existing filaments to seed branches, but the WISH/DIP1/SPIN90 (WDS) family of proteins uniquely activates Arp2/3 complex to nucleate linear filaments de novo. The molecular basis for this unconventional mode of activation has remained elusive. Using single-particle cryo-electron microscopy, we determined near-atomic resolution structures of human SPIN90 bound to activated Arp2/3 complex at filament pointed ends. Our findings reveal that SPIN90 functions as a dimer, mediated by a metazoan-specific Middle Segment Dimerization Region (MSDR). This dimerization is indispensable, as mutants defective in dimerization fail to activate Arp2/3 complex. Strikingly, the two SPIN90 protomers engage Arp2/3 complex through distinct, non-redundant mechanisms. One protomer engages with the ARPC2–ARPC4 clamp, inducing helix bending and clamp twisting that repositions Arp2 closer to Arp3, into a nucleation-competent short-pitch conformation. The other SPIN90 molecule inserts a conserved loop into Arp3's pointed-end cleft and contacts ARPC3, promoting Arp3 flattening and stabilizing the active state.

Disruption of either interface abolishes nucleation, underscoring their functional necessity. Unexpectedly, we also captured a higher order “doublet” architecture, in which a single SPIN90 dimer bridges two Arp2/3 complexes arranged head-to-head. This geometry nucleates filaments of opposite polarity with ~154° angular separation, providing a direct structural mechanism for bidirectional nucleation. Such an activity is unprecedented among known NPFs and represents a potential metazoan innovation for rapidly generating symmetric actin networks. Together, our structural and biochemical analyses redefine the paradigm of Arp2/3 complex activation. SPIN90 employs a cooperative, dimer-dependent mechanism that expands the architectural repertoire of actin beyond branched junctions, enabling de novo filament seeding and bidirectional network formation. These insights position SPIN90 as a unique metazoan-specific regulator of actin dynamics with implications for lamellipodia extension, endocytosis, and morphogenetic processes.

MS107

CAP1-driven actin filament disassembly organizes epidermal morphogenesis in vivo

*Chen Luxenburg[1], Joby Issac[1], Krishnanand Padmanabhan[1], Eidan Loushi[1], Arad Soffer[1], Sharbel Eid[1], Tal Laviv[1], Marco Rust[2]; Tel Aviv University[1], University of Marburg[2]

Actin filament turnover is central to cell shape remodeling and adhesion, yet how F-actin disassembly contributes to epithelial tissue development in vivo is poorly understood. Cyclase-associated protein 1 (CAP1) has long been recognized as a multifaceted regulator of actin dynamics and signaling in cell-free and cultured systems. However, its function in mammalian tissues has remained poorly defined. Here, we bring CAP1 biology into the context of in vivo epidermal development, providing the first demonstration that its actin-disassembly activity is indispensable for skin morphogenesis. Using in utero gene manipulation in mouse embryos, combined with live imaging and mutational dissection, we show that CAP1 is essential for maintaining adherens junction (AJ) integrity, apicobasal polarity, and proper cell shape. CAP1 loss did not alter cAMP levels but led to elevated F-actin content, reduced actin turnover, and severe disorganization of junctional actin networks. CAP1 was broadly cytoplasmic, consistent with a role in global actin dynamics. Notably, pharmacological stabilization of F-actin phenocopied CAP1 depletion, highlighting that dynamic actin disassembly, not polymerization, is the critical driver of morphogenesis in this system. Structure–function rescue experiments in vitro and in vivo revealed that the helical folded domain (HFD), which enables CAP1 to cooperate with proteins such as cofilin and ADF (actin depolymerization factor/destrin) to sever and depolymerize filaments, is required for AJ organization and morphogenesis. In contrast, a domain supporting G-actin nucleotide exchange was dispensable. Thus, CAP1 promotes epidermal tissue architecture primarily through its F-actin disassembly activity in vivo. Our findings position CAP1 as a central actin regulator in a developmental setting and provide direct evidence that regulated filament disassembly is a key organizing principle for epithelial tissues. By establishing CAP1 function in a mammalian morphogenetic context, this work bridges actin biochemistry with tissue-scale biology, underscoring the importance of actin turnover for development, homeostasis, and disease.

MS106

Cofilin controls the sorting of tropomyosin to distinct actin networks

*Shane McInally[1], Dominic Radcliffe-Hines[1], Ruben Bonilla-Santiago[1], Brian Hercyk[1], Alex Reading[2]; Worcester

Polytechnic Institute[1], Massachusetts Institute of Technology[2]

Proper cell physiology requires the co-assembly of multiple actin cytoskeletal networks that are tailored for specific functions. To distinguish between these networks, cells decorate them with distinct sets of actin-binding proteins (ABPs). This selective decoration is thought to arise from competitive and cooperative binding of ABPs to filament sides or growing barbed ends, but the role of actin disassembly factors is less well understood. Here, we used inducible CRISPR interference and quantitative live-cell imaging to test how disassembly factors control the size and ABP composition of different networks. We show that knockdown of cofilin (Cof1), a potent and highly conserved disassembly factor, leads to the formation of large, disordered actin networks decorated by inappropriate ABPs. Specifically, tropomyosin (Tpm1) is inappropriately recruited to branched networks due to defects in Cof1-mediated disassembly. Contrary to prevailing models of ABP sorting, these networks are co-decorated by Tpm1 and fimbrin (Sac6), and their assembly is independent of formin activity. Moreover, the formation of these disorganized, improperly decorated networks severely disrupts myosin-based intracellular transport. Finally, although dual knockdown of Tpm1 and Cof1 partially rescues polarized cable assembly, these cables fail to support transport. Together, our findings demonstrate a critical role for actin disassembly factors in maintaining cytoskeletal structure and function by regulating ABP sorting across distinct networks.

MS105

Cyclase Associated Actin Cytoskeleton Regulatory Protein 2 (CAP2) Organizes the Actin Cytoskeleton to Influence the Biomechanical Properties of the Ocular Lens

*Sepideh Cheheltani[1], Velia Fowler[1]; University of Delaware, Newark, DE[1]

Cyclase Associated Actin Regulatory Protein 2 (CAP2) is a conserved actin-binding protein that promotes filament turnover by facilitating monomer dissociation from ADF/cofilin-decorated pointed ends. This activity enables actin remodeling and the formation of diverse filamentous actin (F-actin) networks, which are critical for cellular architecture and mechanics. In the ocular lens, fiber cells continuously differentiate throughout life and develop F-actin-rich membrane protrusions that are essential for maintaining lens transparency and biomechanical properties. While actin-binding proteins such as Tropomodulin and Tropomyosin are known to influence lens fiber cell F-actin networks and whole lens biomechanics, the role of CAP2 remains unexplored. To assess CAP2's function in the lens, we generated a lens-specific CAP2 knockout (CAP2cKO) mouse model using Tg (Cryaa-Cre)10Mlr mice. We examined lens morphology, cytoskeletal organization, and mechanical properties using western blotting, immunostaining, confocal microscopy, and mechanical compression testing. Our results showed that CAP2cKO lenses were similar in size, shape, and transparency to control lenses. However, they exhibited significantly increased stiffness under compression and enhanced recovery after load removal. Interestingly, total actin levels and the F-to-G-actin ratio remain unchanged. In contrast, immunofluorescence of individual fiber cells revealed higher levels of Tropomodulin 1 (F-actin pointed-end capping), Tropomyosin3.5 (F-actin stabilizing), and T-Plastin (F-actin-bundling) in the F-actin-rich membrane protrusions of mature fibers, while levels of α-actinin-1, a more flexible actin crosslinker, were reduced. These findings suggest that CAP2 loss alters the balance of F-actin-associated proteins, favoring proteins associated with the assembly of stabilized filaments that form rigid bundles. This reorganization likely underlies the increased lens stiffness observed in CAP2cKO mice. This study provides the first evidence that CAP2 regulates cellular and tissue biomechanics in a non-muscle tissue through modulation of F-actin-associated protein levels and localizations.

MS103
EPS8 dampens the growth dynamics and prolongs the lifetime of actin-based protrusions
*Alexandra Mulligan[1], Matthew Tyska[1]; Vanderbilt University[1]

Actin-based protrusions such as filopodia, microvilli, and stereocilia support a variety of cellular functions, ranging from nutrient absorption to mechanosensation. Although their structure and composition are well characterized, mechanisms that promote their growth and stability remain unclear. The actin filaments that support these structures are bundled in a parallel and unipolar 'barbed-end out' manner, creating a core that is mechanically rigid enough to support membrane protrusion. At their distal ends, individual filopodia, microvilli, and stereocilia exhibit an electron dense tip complex, which could contain factors that gate actin assembly at filament barbed ends. One such factor is Epidermal Growth Factor Receptor Kinase Substrate 8 (EPS8), which localizes robustly to the distal tips of all three protrusions. Loss of EPS8 in cell culture and mouse models results in shorter microvilli and stereocilia, suggesting roles in elongation. Previous work from our group also showed that in epithelial cells, EPS8 puncta appear at the apical membrane prior to the growth of a new microvillus, remain at the distal tip for the lifetime of the structure, and then disappear right before the microvillus collapses, suggesting a role in protrusion survival on the cell surface. To more directly examine the role of EPS8 in protrusion elongation and survival, we used CRISPR/CAS9 to tag the endogenous copy of EPS8 in HeLa cells with mStayGold. As expected, endogenous EPS8 puncta are found at the distal tips of growing filopodia, which were highly dynamic. Using a transfection approach, we increased levels of mStayGold-EPS8 and examined the corresponding impact on filopodial growth and dynamics. Increasing the EPS8 concentration enhanced the amount of EPS8 per tip punctum, significantly reduced rates of filopodia elongation, and increased the lifetime of individual protrusions. Cytochalasin D (CytoD), which caps barbed ends, displaced endogenous EPS8 from filopodial tips, and cells expressing higher levels of EPS8 were protected from this effect. Together, these data suggest that EPS8 binds directly to filament barbed ends where it likely plays a role as a 'leaky capper', allowing slow incorporation of new monomers, and offering long-term protection against actin bundle disassembly. In epithelial systems, such activity could promote the accumulation of large numbers of protrusions during differentiation.

MS101
Mechanism of processive pointed-end elongation of actin filaments by Leiomodin2
*Shashank Shekhar[1], Sudipta Biswas[1], Tania Larrinaga[3], Carol Gregorio[2]; Emory University[1], Icahn School of Medicine at Mt. Sinai[2], University of Arizona[3]

The actin cytoskeleton drives essential processes like cell migration and muscle contraction. While barbed-end polymerization is well-established, pointed-end elongation was long considered impossible in vivo. We demonstrate that Leiomodin 2 (Lmod2), localized to thin-filament pointed ends in striated muscle, functions as a processive actin polymerase. Single-molecule and single-filament imaging reveal that Lmod2 stably associates with pointed ends in vitro, enabling elongation even under profilin-rich, cytosol-like conditions that otherwise promote depolymerization of free pointed ends. Strikingly, Lmod2's activity persists in the presence of tropomyosin, confirming its physiological role. In mouse hearts, Lmod2 governs thin-filament length regulation, dependent on its WH2 domain for processivity and polymerization regulation. A human dilated

cardiomyopathy-associated mutation ablates Lmod2's polymerase activity, directly linking this mechanism to cardiac dysfunction. Our work establishes pointed-end polymerization as a novel cellular mechanism of actin assembly in eukaryotes and provides a mechanistic basis for Lmod2-related muscle diseases.

MS104
Role of Heterogeneous Nuclear Ribonucleoprotein HNRNPU in Maintenance and Differentiation of Pluripotent Stem Cells
*AMITABH DEBNATH[1], INDRANI TALUKDAR[2], RV Shaji[3]; Birla Institute of Technology, KK Birla Goa Campus[1], BITS PILANI KK BIRLA GOA CAMPUS[2], Centre for Stem Cell Research,Vellore, unit of inStem[3]

Embryonic Stem cells are distinguished by high pluripotent plasticity, continuous self-renewal, and the ability to generate multilineage cell populations. The perpetuation of pluripotency is intricately orchestration intrinsic gene regulatory networks, metabolic pathways, epigenetic control mechanisms, alternative splicing (AS) machinery. Reports from previous studies suggest that AS is intricately involved in cell fate decisions and embryonic development. In this study, we unveil that some heterogeneous nuclear ribonucleoproteins (hnRNPs) show high expression in pluripotent mouse embryonic stem cells (mESCs) compared to mouse embryonic fibroblasts (MEFs), as evidenced by RT-PCR analysis. We extended our investigation of hnRNP expression in human pluripotent stem cells (TJC8 hiPSC (indiced Pluripotent Stem cells) by qRT-PCR analysis which validated the elevated hnRNP expression in hiPSC cells. To find the inter-association of these hnRNPs and how they are regulated, promoter regions of these hnRNPs were analyzed by in silico approach. We performed PCR validation which revealed their elevated indegenous expression in mESCs as well as hiPSCs . Literature reviews illuminated among the hnRNPs, hnRNPU's pivotal role in important biological process including cranial and cardiac development. To decipher its regulatory significance in stem cell biology, we explored lentiviral-mediated dual-guide RNA CRISPR-Cas9 technology to generate hnRNPU- knockout human iPSC (icas9) lines. Subsequent molecular analyses demonstrated alterations in pluripotency markers (Oct4, Sox2, C-Myc), muscle progenitor markers (Myogenin, Myf5), and apoptotic gene expression. Future directions aim to unravel the mechanistic intricacies of hnRNPU's influence on pluripotency, lineage commitment, and apoptotic pathways. Through RNA sequencing, organoid model generation, apoptosis assays, splice junction arrays, and overexpression studies, we are moving forward to delineate the molecular underpinnings governing hnRNPU's regulatory landscape in stem cell fate decisions

Education Awards Session: Bruce Alberts Award and Innovation in Education Award

AW601
Molecular Animation: Past, Present, and Future
*Janet Iwasa[1], Dillon Lee[1], Hui Liu[1], Rachel Torrez[1]; University of Utah[1]

In recent years, there has been a rapid growth in the use of animation as a means to communicate complex biological processes to a wide range of audiences. My group and others have seen that 3D animation software can be used to synthesize spatial and dynamic information from a variety of experimental modalities, and that this process can provide important scientific insights and provide a contextualized view of how molecular and

cellular systems operate. These visualizations have served not only to make molecular concepts more accessible to students and the public, but have also proven to be extremely useful for researchers seeking to build and refine their hypotheses. In this talk, I will describe projects that have used animation to communicate and explore different topics in cell biology and discuss new tools my group is developing to enable molecular biologists to create, share, and annotate animated three-dimensional models of molecules.

Emerging Leaders Awards Session

AW502
crafting neural messages: the symphony of form, force, and function
*Shigeki Watanabe[1]; Johns Hopkins University[1]

Neurons are known for their intricate cellular morphology. Axons in particular are exceptionally long (100-1000 mm) and ultrathin (100 nm). Their cable-like morphology is essential for conduction of electrical signals, or action potentials, throughout the brain and body. Thus, it has been long assumed that axons are tubular structures with occasional synaptic varicosities. However, our work has challenged this assumption. Using high-pressure freezing to preserve membrane morphology for electron microscopy or super-resolution imaging of live neurons, we performed ultrastructural analysis of axons in Caenorhabditis elegans motor neurons, mouse hippocampal neurons, and human cortical neurons. We discovered that axons are not simple tubes but rather exhibit a pearls-on-a-string morphology through their entire length, with the pearls being ~250 nm and the strings ~70 nm in diameter. This morphology is reminiscent of membrane tubes undergoing tension-driven instability. Consistent with this notion, the pearled area becomes smaller when hyperosmotic solution is applied and larger when hypoosmotic solution is applied. Interestingly, pharmacological perturbation of the cytoskeleton did not greatly alter axon morphology, suggesting that membrane mechanics drives axon morphology. In further support of this, increasing the membrane fluidity by cholesterol depletion from the plasma membrane led to a shrinking of the pearled membrane regions. Functionally, when axon morphology is altered with pharmacological cholesterol depletion, action potential velocity decreases. Similarly, neuronal stimulation that induces plasticity alters the pearled axon morphology. Our in silico modeling further supports our experimental data that membrane mechanics can cause pearled axon morphology and that pearled morphology greatly impacts action potential conductance. These data have revealed for the first time that axons are pearled not tubular, and that pearled axon morphology has an important functional role in neuronal activity and plasticity. I will discuss our initial discovery and current work.

AW503
Illuminating organelle interaction networks in differentiation and disease
*Sarah Cohen[1]; UNC-Chapel Hill[1]

A key feature of eukaryotic cells is their compartmentalization into membrane-bound organelles. This compartmentalization separates incompatible biochemical processes, but nevertheless organelles must communicate for the cell to function as a unit. Much of this inter-organelle communication happens at membrane contact sites (MCSs): sites of close apposition between organelles that facilitate the exchange of ions, lipids, and information. I will describe my lab’s work using novel biosensors, multispectral imaging, and

computational approaches to elucidate the dynamics of organelle interaction networks. We developed Contact-FP, a suite of dimerization-dependent fluorescent proteins, to visualize the dynamics of inter-organelle interactions. Contact-FP probes can be titrated to visualize and minimally perturb the system or overexpressed as a tool to induce MCSs. We further developed a multispectral imaging and analysis approach to analyze "organelle signatures" comprised of >1000 features of organelle morphology, distribution, and interactions. Multispectral imaging of primary neurons and astrocytes revealed that each cell type has a distinct organelle signature and response to neurodegenerative disease-associated stress (e.g., oxidative stress or ER stress). We reasoned that cell-type specific organelle signatures must be set up during differentiation. Indeed, we discovered that organelle morphology, dynamics, and contacts all dramatically rewire throughout differentiation of stem cells to neurons. Specifically, mitochondria-organelle contacts remodeled early during neuronal differentiation, while peroxisome-organelle contacts remodeled during synaptogenesis. These changes correlated with the changing metabolic needs of the cells. For example, ER-peroxisome contacts increased to facilitate synthesis of lipids such as plasmalogens during synaptogenesis. Strikingly, organelle interactions were more predictive of differentiation stage than any other organelle feature. Together, our results indicate that organelle communication networks are a key feature of cell identity that remodel to support metabolic needs during development and in response to stress or disease.

AW501
The private life of lipids: small chemical changes that shape cell membranes
*Itay Budin[1]; University of California San Diego[1]

Our lab investigates how – and why – cells control the composition of their lipid membranes. In this talk, I will focus on our efforts to uncover how lipid composition is controlled in intracellular membranes and describe emerging biophysical models to explain its chemical diversity in cells. First, I will present new chemical biology approaches we have developed to map phospholipids in cell membranes and determine factors that control their trafficking. A key advance has been the use of fluorogenic dyes for biorthogonal labeling reactions that allows compartment and leaflet-specific detection of phospholipid pools. I will then highlight recent studies that demonstrate show surprisingly small chemical changes to lipid chemical structures drive membrane function. In one, we found that chemical remodeling of sterols during their biosynthesis shapes these molecules' capacity to organize membranes. In the other, we uncovered how the shape of phospholipids – described by their spontaneous curvature – is a biophysical parameter that has driven adaptation across organisms and is actively maintained by cellular metabolism.

Epithelial Cell Polarity and Polarized Trafficking in Organ Health and Disease

MS1703
Actomyosin cortex integration with plasma membrane topography over the one-cell Drosophila embryo
*Rowan Naidoo[1], Rebecca Tam[2], Tony Harris[1]; University of Toronto[1], The Rockefeller University[2]

The actomyosin cortex governs shapes and interactions of cells and is regulated by associated Arp2/3 actin networks. Schematic models typically depict a thin actomyosin cortex below a flat plasma membrane (PM), but most animal cells have complex PM topographies. How actomyosin networks generate cortical tension in

association with highly folded PM, and how they are regulated in such contexts, remains unclear. To study these questions, we examined the one-cell Drosophila embryo with quantitative live-imaging, genetic perturbations, inhibitor injections, and laser cutting experiments. We found actomyosin networks intertwined across PM folds of the one-cell embryo surface. Additional evidence indicated that a composite material of actomyosin networks and PM folds conveys cortical tension: (i) the PM topography oscillated between expanded and condensed states in concert with known assembly-disassembly cycles of cortical myosin; (ii) the folded PM gained myosin-dependent tension with each cycle; (iii) with perturbations of myosin activity, the PM folds expanded with excessive heights and widths, and gained compressive stress; and (iv) with partial F-actin inhibition, actomyosin networks and PM folds co-aggregated into fragmented patches with myosin dependence. Additional experiments showed that regulation of the actomyosin cortex by Arp2/3 involved changes to the associated PM topography. Injection of fluorescent dextran into the extracellular space showed that the cyclic expansions between PM folds occurred primarily on the cytoplasmic side. These expansions coincided with initial cortical assembly of myosin, but also with cyclic accumulations of the Arp2/3 activator SCAR, and F-actin. Perturbation of Arp2/3 hindered the expansions between PM folds and altered the associated myosin localization. Instead of a relatively thick distribution of myosin over broader depths of normal PM folds, myosin became focussed at an abnormally thin plane with dense PM, from which misshaped PM folds emanated above and below. This abnormal plane displayed excessive tension, and the surfaces of Arp2/3-depleted embryos exhibited unusual inward contractions. Overall, our study characterizes a composite of actomyosin networks and PM folds that conveys cortical tension, and that regulation of cortical actomyosin activity by Arp2/3 involves modulation of the associated PM topography.

MS1705

Adapter sequestration and EGFR suppression are widespread features among oncogenic RTK fusions

*Carol Gao[1], David Gonzalez-Martinez[1], Sofia Wissert[1], Hana Bader[1], Nidhi Sahni[2], Anh Le[3], Robert Doebele[3], Lukasz Bugaj[1]; University of Pennsylvania[1], The University of Texas MD Anderson Cancer Center[2], University of Colorado School of Medicine[3]

Regulation of cancer cells by their environment contributes to tumorigenesis and drug response, though the extent to which the oncogenic state can alter a cell's perception of its environment is not clear. EML4-ALK is a receptor tyrosine kinase (RTK) fusion oncoprotein that forms condensates and suppresses transmembrane EGFR signaling in cancer cells. ALK inhibition restores EGFR signaling, thereby promoting survival and drug tolerance. Here we tested whether such modulation of EGFR activity was common among other RTK fusions, which collectively are found in ~5% of all cancers, and whether modulation is linked to oncoprotein condensation. Using live- and fixed-cell microscopy in isogenic and patient-derived cell lines, we indeed found widespread suppression of EGFR by active RTK fusions. However, suppression did not correlate with the formation of condensates but instead corresponded to the formation of low-order assemblies and oncogenic kinase activation. These assemblies triggered fusion activity and binding to Grb2, competitively inhibiting Grb2-mediated EGFR signaling from the membrane. Optogenetic probes confirmed that cytoplasmic sequestration of Grb2 was sufficient to suppress perception of EGFR stimuli, even in the absence of condensation or signaling downstream of the fusion. Our study uncovers shared functional consequences among RTK fusion oncoproteins that could be exploited for novel biomimetic therapies that suppress transmembrane RTK signals.

MS1708

EGFR S442 Mutation Drives Cetuximab Resistance via Impaired Antibody Binding and Sustained Signaling, Reversed by ERBB2 Co-Targeting

*Bhuminder Singh[1], Neeraj Joshi[3], Sarah Harmych[2], Hidenori Tanaka[3], Galina Bogatcheva[3], Marisol Ramirez-Solano[3], Ramona Graves-Deal[3], Claiborne Tydings[2], Claudia Wahoski[2], Chelsie Sievers[3], James Higginbotham[3], Zhiguo Zhao[3], Jeffrey Franklin[3], Xingyi Guo[3]; Vanderbilt University Medical Center Medical Center[1], Vanderbilt University[2], Vanderbilt University Medical Center[3]

Resistance to EGFR-targeted therapies remains a major challenge in colorectal cancer (CRC) and head and neck squamous cell carcinoma (HNSCC). While EGFR ectodomain (ECD) mutations have been implicated in acquired resistance, their functional impact and therapeutic implications remain poorly defined. Here, we identify EGFR S442 mutations – particularly S442I – as recurrent alterations in cetuximab-treated CRC patients and demonstrate their mechanistic role in resistance. Using 3D collagen cultures, we generated a cetuximab-resistant CRC line (DiFi-CR) and identified an S442I mutation in EGFR ECD domain III. Inducible expression of S442I in CRC and HNSCC models conferred resistance to cetuximab and panitumumab in vitro and in vivo. FACS-based cell surface binding assays revealed reduced cetuximab binding in S442I-expressing cells, confirming impaired antibody engagement despite EGFR expression. These findings were supported by structural modeling and thermodynamic integration. Importantly, S442I also diminished EGF-induced EGFR activation, suggesting a neomorphic role in receptor activation and trafficking. In vivo, S442I tumors retained pERK activity and showed reduced intratumoral antibody enrichment, while WT EGFR-expressing tumors exhibited central necrosis and high cetuximab enrichment. To overcome S442I-mediated resistance, we co-targeted ERBB2 using trastuzumab deruxtecan (T-DXd). In cancer lines with EGFR–ERBB2 co-expression and physical interaction (co-immunoprecipitation), T-DXd restored cetuximab sensitivity. This synergy was absent in ERBB2-low HNSCC models, underscoring the importance of receptor context. Our findings thus establish EGFR S442 mutations as resistance drivers that can be specifically targeted. Co-targeting EGFR and ERBB2 offers a promising strategy to restore anti-EGFR efficacy in resistant tumors, while 3D culture systems provide a more physiologically relevant platform for modeling drug response.

MS1702

ESCRT-0 Condensation and Smoothened Clustering drive Extracellular Vesicle Budding from Cilia

*Mingli Zhu[1], Yash Raj[1], Gary A. Bradshaw[3], David U. Mick[2], Jaclyn Goldstein[1], Marian Kalocsay[3], Maxence V. Nachury[1]; Department of Ophthalmology, University of California San Francisco; CA 94143, USA[1], Center of Human and Molecular Biology and Center for Molecular Signaling, Department of Medical Biochemistry and Molecular Biology, Saarland University School of Medicine

The central function of primary cilia is to transduce signals by dynamically concentrating signaling receptors and regulators. Proteins exit cilia by retrieval and ectocytosis. Ciliary ectocytosis has been implicated in both the disposal of excess ciliary material and cell-cell communication. However, how ciliary proteins are selected for inclusion into budding ciliary EVs remains an open question. Mechanistic understanding of cargo packaging will provide critical insights into the physiological and pathological roles of ciliary EVs. Here, we leverage quantitative mass spectrometry to identify the proteome of cilia-derived extracellular vesicles and uncover the

ciliary GPCR Smoothened (SMO) as the major cargo and the ubiquitin reader ESCRT-0 (HRS/STAM) as a candidate sorting machinery. Notably, 2% of the total cellular HRS/STAM and 5% of SMO are packaged into EVs via cilia. We show that K63-linked ubiquitin chains serve as the sorting signal for SMO, as SMO is highly K63-ubiquitinated in EVs, and targeting a K63-specific deubiquitinase to cilia abolishes SMO secretion. While ESCRT-III is known to mediate almost all instances of reverse-topology budding events in cells, functional analysis of the full ESCRT machinery shows that the ESCRT-0 complex—rather than other ESCRT components, including the ESCRT-III remodeling enzyme VPS4—is specifically required for packaging SMO into ciliary EV. Instead, ESCRT-0 undergoes condensation within cilia and is co-packaged with SMO into EVs. Importantly, condensation of ESCRT-0 is essential for SMO secretion into EVs. Furthermore, FRAP analyses show that SMO clusters at the ciliary tip in an UbK63-dependent manner, and optogenetic induction of SMO clustering is sufficient to trigger EV budding from cilia. Together, our findings suggest that ESCRT-0 condensation and SMO clustering cooperate to drive reverse-topology budding of ciliary EVs independently of ESCRT-III.

MS1707

Matrix stiffness induces heritable changes in chromosome numbers, aligning with solid tumor heterogeneity

*Markus Spranger[1], Alisya Anlas[1], Dennis Discher[1]; University of Pennsylvania[1]

Extracellular matrix often accumulates in and around solid tumors, and such tumors also evolve diverse mutations that drive cancers and confound therapies. Across cancer types, we observe chromosome number changes associate with collagen-I levels, and our experiments show rare heritable chromosome losses are induced by a stiff 3D matrix around spheroids. Chromosome reporters (ChReporters) reveal losses in as few as ~0.1% of cells, with a mechanism in spheroids based on distortion of mitotic spindles – which increases with myosin-II suppression in 3D stiff matrix. Chromosomes mis-segregate into micronuclei that increase with matrix stiffness despite suppressed cell division. Drugs that increase micronuclei in 2D and that rely on an unperturbed spindle show no effect in 3D where the spindle is perturbed. Tumors in vivo that are surrounded by stiff collagen likewise show more but varied chromosome loss and slower growth than 2D cultures. High variance of ChReporter-negative colonies further illustrate increased heterogeneity with 3D matrix stiffness and heritable mutations per Luria-Delbruck theory. Physical learning models of evolving chromosome numbers in proliferating cells are developed and fit key statistical trends. Overall, stiff 3D matrix fuels mechano-evolution that diversifies solid tumors and complicates treatments.

MS1706

Occludin Links Endosomal Trafficking to Mitotic Spindle Assembly and Epithelial Morphogenesis

*Pengfei Lu[1]; University of South China[1]

The emergence of vertebrates is thought to depend in part on lineage-specific innovations in cell division and trafficking, yet the genetic underpinnings remain poorly understood. We identify the vertebrate-specific tight junction protein Occludin (OCLN) as a critical regulator of epithelial morphogenesis through its unexpected role in coordinating vesicle trafficking and spindle function. Mammary glands lacking Ocln exhibit impaired epithelial branching due to reduced cell proliferation and surface expansion. Mechanistically, Ocln binds the Rab11 adaptor FIP5 to recruit recycling endosomes to the centrosome, thereby ensuring proper spindle assembly and orientation. Loss of either Ocln or FIP5 leads to prolonged prophase, spindle dysfunction, and

failed nuclear and cytoplasmic division. These findings establish OCLN as a central link between endosomal trafficking and mitotic spindle dynamics. Together with our prior work showing roles for OCLN in SNARE-dependent secretion and lipid droplet release, this study reveals how a vertebrate-specific gene integrates trafficking and division machinery to control tissue growth and highlights an evolutionarily conserved vesicle-based mechanism essential for epithelial development.

MS1704

The Actomyosin Cortex Controls T-tubule Remodeling In Skeletal Muscle

*Silvia Di Francescantonio[1], Ana Pereira[1], Ana Soares[1], Tianyang Liu[3], Filomena Carvalho[1], Josie Ferreira[3], Graciano Leal[1], Inês Faleiro[1], Naoko Kogata[2], Nuno Santos[1], Michael Way[2], Carolyn Moores[3], Edgar Gomes[1]; GIMM - Gulbenkian Institute for Molecular Medicine, Avenida Prof. Egas Moniz, 1649-028 Lisboa, Portugal[1], Cellular signalling and cytoskeletal function laboratory, The Francis Crick Institute, 1 Midland Road[2], Institute of Structural and Molecular Biology

The actomyosin cortex is essential for the mechanical properties of the cell membrane and its ability to reshape. During development, muscle cells need to grow an intricate network of plasma membrane invaginations called t-tubules that are coupled to the sarcoplasmic reticulum forming triads. Although the importance of t-tubules for controlling calcium and muscle contraction is well established, and abnormalities are found in multiple muscle disorders, the mechanisms that regulate t-tubule growth during skeletal muscle development remain elusive. In this work we found that t-tubule growth requires membrane availability by disruption of the actomyosin cortex. We had previously implicated the cortical actin nucleator Arp2/3 complex, through its Arpc5 subunit, in the organization of triads by an unknown mechanism in vitro. By depleting Arpc5 in primary myofibers, we now found that Arpc5 limits t-tubule overgrowth by organizing the proper distribution of the actomyosin cortex. Loss of Arpc5 leads to an increase in membrane roughness, suggesting an excess of membrane availability for t-tubule growth. This Arpc5-dependent t-tubule overgrowth leads to clusters of enlarged t-tubules at the triad and to asynchronous myofiber contraction and irregular calcium dynamics, emphasizing the role of controlling the actomyosin cortex and membrane availability in forming functional triads. Finally, we employed a mouse model with targeted Arpc5 depletion during postnatal muscle development and observed that depleting Arpc5 leads to significant muscle fatigue alongside increased membrane roughness and enlarged t-tubules at the triads, reinforcing the critical role of Arpc5-driven actomyosin cortex establishment in triad formation and muscle function. We propose that the actomyosin cortex through Arpc5 gatekeeps membrane availability for t-tubule growth and that t-tubule overgrowth leads to dysfunction of triads and muscle cell contraction. These insights offer a potential pathophysiological mechanism and open avenues for therapeutic strategies aimed at normalizing t-tubule morphology and membrane dynamics in muscle disorders.

MS1701

The role of polarized vesicular trafficking in intestinal tissue homeostasis and ageing

*Sandra Scharaw[1]; Max Planck Institute of Molecular Cell Biol & Genet[1]

Gastrointestinal function commonly declines with age. Intestinal stem cells (ISCs) reside at the bottom of intestinal crypts, laterally aligned with their supportive niche Paneth cells. ISCs rely on crosstalk with Paneth

cells to maintain tissue homeostasis, but with age, this intercellular communication becomes altered. Understanding how stem cell–niche communication changes over time is crucial for optimizing intestinal function in later life. To elicit cell communication, cells must traffic newly synthesized signaling proteins to their site of action—for example, an ISC receptor to the membrane for ligand exposure from Paneth cells. The identity of critical stem cell signals has been intensively characterized. However, how the ligands and receptors are trafficked to the cell surface in a spatially and temporally coordinated manner remains poorly understood. We find that ISCs contain split Golgi complex stacks that are laterally oriented toward neighboring Paneth cells. The number of Golgi stacks correlates with the ISC position between Paneth cells, and Golgi splitting enhances receptor trafficking toward Paneth cells, an advantage that declines with age. These findings suggest a stem cell–specific Golgi complex configuration that facilitates polarized trafficking between ISCs and Paneth cells to control tissue function. To further probe this mechanism, we developed a novel optically controlled tool to dissect trafficking dynamics between ISCs and Paneth cells. This system enables spatially and temporally precise tracking of cargo transport from split Golgi complexes to the plasma membrane in intestinal organoids. Together, our study aims to clarify how Golgi positioning and architecture regulate vesicular trafficking for stem cell–niche communication, thereby influencing ISC fate decisions during homeostasis and aging.

Evolutionary Cell Biology - Minisymposia

MS2605
A Rapidly Evolving Protein Domain of Flightin Influences Flight Muscle Myofilament Lattice Structure, Power Output, Courtship Song Quality, and Mating Success in Drosophila
*Jim Vigoreaux[1], Emily Price[1], Harshal Athalye[1], Samya Chakravorty[1]; University of Vermont[1]

The N-terminal domain (NTD) of flightin (fln), a myosin-binding protein essential for indirect flight muscle (IFM) function in Drosophila melanogaster, is evolving at a faster rate than other domains of the protein. The IFM is responsible for generating flight, subject to natural selection, and a species-specific courtship song, subject to female choice. Here, we test the hypothesis that NTD influences courtship song properties and mating success. We compared courtship song characteristics and mating outcomes of D. melanogaster males from three transgenic lines: a wild-type rescued control line (fln+), an NTD-deleted line (flnΔ62), and a chimeric line with the NTD from D. virilis (flnvir), a distantly related species with distinct courtship song properties. Song analysis revealed that flnΔ62 and flnvir males produced songs with altered parameters. Foremost among these is an increase in the interpulse interval (IPI), a discriminatory signal used by females in recognizing and selecting males from their species. Pairwise mating competition assays showed that wild-type females preferred fln+ males over flnΔ62 or flnvir males when both males sang, as manifested by a decrease in the courtship index. In single-male trials or pairwise trials when only one male sang, females did not discriminate, indicating that altered courtship song, not other courtship behaviors, drove female choice. Compared to fln+, flnΔ62 males showed a 33% decrease in flight ability despite no difference in wing beat frequency, while flnvir males showed slightly improved flight ability with no change in wing beat frequency. Fourier Transform analysis of IFM electron micrographs showed an ~11% decrease in myofilament lattice spacing (d1,0) in flnΔ62 males and an ~9% increase in flnvir males. These findings show that NTD influences IFM myofibrillar structure and production of courtship acoustic signals, with consequences in flight ability and mating success. We conclude that flightin is under dual selection pressure resulting in protein domains evolving at different rates. Our results support the hypothesis that the faster rate of NTD evolution contributes to behavioral isolation and speciation through its impact on mating song characteristics.

MS2601
Assembly state of human phosphofructokinase-1 regulates localization and activity in cells
*Heather Hansen[1], Bradley Webb[1]; West Virginia University[1]

Glycolysis is an evolutionarily conserved metabolic pathway that breaks down glucose to generate energy and biosynthetic precursors to sustain cellular functions. Pathway flux is controlled by rate-limiting enzymes, one of the most important being phosphofructokinase-1 (PFK1), which catalyzes the step committing glucose to breakdown. While PFK1 has been extensively studied in vitro, its regulation in cells remains incompletely understood, in part because most cultured cells express all three human isoforms which complicates structure-function analysis. To overcome this limitation, we generated a CRISPR PFK1 liver isoform (PFKL) knockout HepG2 cell line with <5% of normal PFK1 activity. Proteomic and metabolomic analyses revealed that loss of PFKL induced severe energetic stress, which was rescued by re-expression of FLAG-PFKL. Using this cell line, we asked how alternative assembly states of PFKL regulate activity and function. PFK1 activity is regulated by allosteric ligands and assembly state. While tetramers have long been viewed as the canonical active form, recent studies suggest that higher-order filaments regulate spatial localization and that dimers may retain catalytic activity in cells. Both wild type (WT)- and filament-incompetent PFKL (FI-PFKL) rescued glycolytic flux, indicating filaments are not required for basal activity. However, WT-PFKL assembled into biomolecular condensates in a filament-dependent manner. Further, WT-PFKL localized to lamellipodia, while FI-PFKL remained cytoplasmic. To investigate the role of this filament-dependent localization, we expressed WT- and FI-PFKL in migratory MDA-MB-231 human breast cancer cells and observed that FI-PFKL impaired chemotaxis, consistent with a model in which higher-order assemblies supply ATP to regions of high energetic demand. We next asked whether PFKL dimers are catalytically active in the cellular environment. Re-expression of tetramer-incompetent PFKL (TI-PFKL) partially restored glycolytic flux in knockout cells. Native PAGE showed TI-PFKL exists as monomers and dimers and was unable to form higher-order assemblies like the WT enzyme. In vitro activity assays showed TI-PFKL had reduced activity, but moderate activity under activating levels of AMP and fructose-1,6-bisphosphate. In conclusion, we identified a novel function of PFKL filaments in spatial regulation, and provide evidence for a catalytically active PFK1 dimer in cells. Additionally, our results establish a new cell platform to perform structure-function analysis of PFK1 and investigate isoform-specific roles of PFK1 in cells.

MS2603
Differential regulation of actin paralogs as a conserved mechanism of stress response
*Ananya Nidamangala Srinivasa[1], Ravi Ranjan[1], Lilian Fritz-Laylin[1]; University of Massachusetts Amherst[1]

Actin is essential to eukaryotic life, and understanding how it has evolved and diversified is crucial to understanding the evolution and diversification of eukaryotes themselves. While some organisms encode a single actin gene, others possess multiple, sometimes highly divergent actin paralogs. Since the actin cytoskeleton is dynamic, functional differences in the actin paralogs can influence cellular processes, including cytokinesis and cell motility, and/or response to environmental changes. While the roles and regulation of a few intraspecies actin paralogs in mammals and plants have been studied, divergent actin paralogs in most eukaryotic species remain unexplored. Additionally, though many eukaryotes can sense and respond to actin cytoskeletal perturbations, it is unclear if and how actin paralogs are involved in response to perturbations.

The amoeba Naegleria gruberi has 30 actin paralogs with pairwise sequence identities ranging from 30-100%, making it an excellent system to study the functional consequences of actin paralog diversity. Despite this sequence variation, the N. gruberi actin paralogs phylogenetically group with actins rather than actin-related proteins (Arps). Under normal conditions, N. gruberi amoebae cells primarily express conventional actin, which is ~85% identical to Rabbit muscle actin, but the function and regulation of the divergent paralogs are unknown. To test if N. gruberi actin paralogs are differentially regulated in response to actin disruption, we performed RNA sequencing upon actin depolymerization by the drug Latrunculin B (LatB). We identified eight divergent actin paralogs that were upregulated in response to LatB, while other conventional actins or stress response genes (e.g., HSPs) were not. The gene with the highest fold change was a divergent actin that was 92% identical to the conventional actin. A similar mechanism exists in Chlamydomonas reinhardtii, which upregulates a divergent actin gene (~65% identical to its conventional actin) in response to LatB treatment. These findings suggest a common mechanism of actin stress response through the differential regulation of actin paralogs. Testing whether this occurs across eukaryotes will reveal if actin paralog diversification enhances resilience to chemical and genetic perturbations of the actin cytoskeleton. To evaluate this, I will extend these experiments to other actin-targeting drugs and species, including Dictyostelium discoideum. Identifying conserved strategies of actin paralog regulation will uncover new principles of actin cytoskeleton adaptability and could reveal novel cellular strategies used for responding to actin disruption.

MS2606

Intraprotein domain competition dictates pulsatile response of photoreceptors shaped by temperature and evolution

*Zikang (Dennis) Huang[1], Malvin Forson[2], William Benman[1], Kevin Gardner[2], Lukasz Bugaj[1]; University of Pennsylvania[1], City University of New York[2]

Stimulation of sensory systems commonly shows pulsatory responses that are generated by networks of molecular interactions. Here we find that pulsatory activation patterns can also be encoded by individual proteins in response to multiple concurrent environmental inputs, and that such unimolecular dynamics have been conserved over 100s of millions of years of evolution. BcLOV4 is a fungal photoreceptor that can generate a temperature-dependent pulse of activity in response to light in mammalian cells, though the molecular nature of pulse generation and thermosensitivity were not understood. We combined live cell imaging, in vitro biochemistry, and simulations to find that activity pulses result from intraprotein competition between distinct light- and temperature-sensitive protein fragments. This competition generates 3 temperature-dependent classes of activity in response to a constant light input: constant (low T), pulsatile (intermediate T), or silent (high T). These schemes occur because light stimulation exposes an activation region that is subsequently caged by a C-terminal temperature-sensing domain (TSD) whose caging strength increases with increasing temperature. The TSD implements thermosensing through conformational changes in two co-evolved loop regions , and mutations or deletions within these loops tuned the amplitude and dynamics of BcLOV4 activity. Finally, we found that the 3-class photo-thermal response has been conserved in fungi that diverged over 300 million years ago, and that the temperature of switching between classes adapted to match the ecological niche of the host, including in extreme environments like Antarctica and in thermal ponds. Collectively our study finds 8 novel proteins that can be actuated with light and temperature in heterologous cells, enabling development of new light- or temperature-responsive molecular probes and deeper understanding of how proteins can sense small changes in temperature. More broadly our study uncovers how time-varying

activation dynamics can be compactly encoded within individual proteins and implies the functional importance of such response dynamics throughout evolution.

MS2602
Losses of Deeply Conserved Genes in the Mitotic Exit Network Led to Multiple Rewiring Events in Budding Yeasts
*Xiaoxue Zhou[1], John Wolters[1], Smrthi Krishnamurthi[1], Rayhana Sow[1], Xiaofeng Zhou[1], Johnathan Crandall[2], Antonis Rokas[3], Chris Hittinger[2]; New York University[1], University of Wisconsin-Madison[2], Vanderbilt University[3]

The mitotic exit network (MEN) is a signaling pathway first discovered in the budding yeast Saccharomyces cerevisiae where it regulates an essential cell-cycle checkpoint. Intriguingly, components of this pathway are widespread in eukaryotes, but the function has diverged to varying degrees even within fungi. To understand how the MEN has evolved, we identified orthologs of all MEN components across over 1,000 species of Saccharomycotina yeasts and found that the S. cerevisiae pathway composition is derived as a key intermediate step in the kinase cascade was lost in the common ancestor of Saccharomycetales and Saccharomycodales order. Furthermore, we discovered that a critical regulatory component has been lost in Phaffomycetales yeasts. This loss led to rapid evolution of the downstream gene with striking functional consequences as revealed by complementation analysis in S. cerevisiae. Our results demonstrated that both the composition and function of essential cellular signaling pathways can change drastically during evolution.

MS2608
Organelle inheritance and scaling in a multi-budding yeast
*Alison Wirshing[1], Daniel Lew[1]; Massachusetts institute of technology[1]

Cells typically divide in half producing two daughters, but there are diverse examples of more elaborate division strategies that give rise to multiple daughters. These include multiple fission of some green algae and bacteria, cellularization during embryogenesis of plants and insects, and similar strategies in Ichthyosporeans, Chytrids, and Apicomplexans. In some multinucleate yeast species, large mother cells can produce multiple buds (daughters) in a single cell cycle. Here we investigate whether mother cells confer an equal inheritance to each bud or whether sibling buds receive unequal contents during multibudding yeast proliferation. Using live cell probes, we imaged the inheritance of cytoplasm, nuclei, mitochondria, endoplasmic reticulum (ER), vacuoles, and peroxisomes in multibudded Aureobasidium pullulans. Mother cells grew sibling buds at similar rates, and delivered organelles to each bud at stereotyped times. On rare occasions when buds grew at different rates, organelle inheritance generally correlated with growth rate. While cortical ER and mitochondria were evenly partitioned across daughters, nuclei, vacuoles, and peroxisomes could vary >2-fold across sibling buds. Across a population of yeast cells, cell volume varied up to ~100-fold, and we found that ER and mitochondria content was directly proportional to volume, while vacuole and peroxisome content was more weakly correlated with volume. Nevertheless, daughter cells inheriting unusually high or low numbers of vacuoles or peroxisomes appeared to adjust organelle content during subsequent growth. Our findings suggest that this unconventional fungus has developed accurate mechanisms to evenly partition cortical ER and mitochondria among sibling buds, and compensatory pathways to adjust the content of other organelles so as to maintain an optimal content across an impressive range of cell sizes.

MS2607

Signaling pathways governing the trigger of regeneration in Stentor coeruleus

*Connie Yan[1], Wallace Marshall[2]; University of California, San Francisco[1], University of California San Francisco[2]

A key question in the biology of regeneration is what triggers the process? While multicellular organisms often rely on reactivation of developmental gene networks, it remains unclear whether single cells employ similar strategies. Regeneration in the giant ciliate Stentor coeruleus, not only involves the reassembly of complex structures such as the oral apparatus but also recapitulates many of the morphogenetic events typically associated with cell division. Previously, it has been suggested that Stentor cells are either in a state of activation or inhibition depending on its stage of development, which may correlate to cell cycle stages. Transcriptional profiling has identified an E2F homolog (SteCoe_12750) that is upregulated early in regeneration, even without new protein synthesis, indicating it is a primary response gene in the regenerative signaling cascade. Pharmacological inhibition of CDK4 with Palbociclib impairs regeneration and downregulates expression of E2F pathway components, including Rb, cyclins, CDKs, and downstream targets. Phosphoproteomic analyses further implicate MAPK signaling in even earlier signaling regeneration events, where MAPK inhibition during regeneration produces abnormal morphologies and ectopic structures, suggesting that the MAPK signaling may be part of the cell's inhibitory state. These results indicate that canonical cell cycle regulators function as molecular switches linking external injury cues to transcriptional programs that control regeneration. Thus, Stentor demonstrates how cell cycle machinery can be repurposed for non-canonical roles, highlighting that regeneration depends on the strategic redeployment of developmental pathways in a spatiotemporally regulated manner.

MS2609

Subcellular localization and expression patterns of de novo emerged microproteins

*Carly Houghton[1], Nelson Castilho Coelho[1], Annette Chiang[1], Aaron Wacholder[1], Allyson O'Donnell[1], Anne-Ruxandra Carvunis[1]; University of Pittsburgh[1]

Recent genomic analyses have revealed thousands of previously overlooked small open-reading frames that undergo active translation, giving rise to evolutionarily novel microproteins. Despite emerging evidence that some of these microproteins form structured domains and influence cellular fitness, their subcellular localization, expression stability, and cell biology remain largely unknown. Are these novel microproteins rapidly degraded or selectively shuttled to specific cellular compartments? Do their localization patterns differ fundamentally from canonical proteins? To systematically address these questions, we engineered approximately 260 fluorescently-tagged de novo emerged microproteins for inducible expression in the model organism Saccharomyces cerevisiae. Using live-cell fluorescence microscopy, we comprehensively mapped the subcellular localization and derived protein abundance profiles of these novel microproteins. Our data reveal a remarkable diversity of localization and abundance patterns. These novel microproteins display subcellular localization distributions distinct from canonical proteins, with notable enrichment in mitochondria and ER and reduced presence in the nucleus. Moreover, we identify specific sequence features associated with distinct subcellular localizations, suggesting intrinsic cellular recognition mechanisms for these evolutionarily naive

sequences. This comprehensive cellular atlas provides foundational insights into the cell biology of de novo emerged microproteins, as well as their potential impact on cellular homeostasis and evolutionary innovation.

MS2604
Testis-Specific Actl8 Is a Novel Nuclear Actin Related Protein That Alters Gene Expression
*Nuria Cortes-Silva[1], Kristen Amarillo[1], Sahiti Peddibhotla[1], Courtney Schroeder[1]; University of Texas Southwestern Medical Center[1]

Cytoskeletal proteins, including actin-related proteins (Arps), are evolutionarily ancient, expressed across all tissue types, and perform essential cellular functions. However, a subset of Arps has recently evolved in mammals and acquired testis-specific expression with unknown roles. Here, we investigated the testis-specific Arp “Actin-like 8” (Actl8), which is expressed exclusively in early sperm development. Actl8, a cancer/testis antigen, is also aberrantly expressed in multiple cancer types and is correlated with poor patient prognosis. Despite its unique appearance in Arp evolution and potential impact in cancer, the molecular functions and cellular localization of Actl8 remain largely unexplored. We discovered that Actl8 surprisingly localizes to the nucleus of spermatogonia and in cancer cells and is partly chromatin-bound, making Aclt8 a novel nuclear Arp. Due to its localization, we investigated the impact of Actl8 on gene expression and leveraged its misexpression in triple-negative breast cancer cell lines. We found that numerous genes involved in cell proliferation and migration are differentially expressed as a result of Actl8 misexpression. In support of this finding, we found that breast cancer cells migrate faster and exhibit increased proliferation upon reduction of Actl8 expression. We further discovered that Actl8 interacts with the histone modifier KDM5B—a histone lysine demethylase that is also a cancer/testis antigen. Given this interaction, we compared histone marks between cancer cells expressing Actl8 and those with reduced Actl8 expression and found alterations in several histone marks. This work is the first to identify a molecular role for Actl8 and reveals that it is a nuclear Arp involved in histone biology and gene expression. These findings provide insight into how Actl8 impacts breast cancer, while also suggesting Actl8 may have evolved a specialized nuclear function in the testis.

Evolutionary Cell Biology - Symposia

SY202
Cytoskeleton, Evolution, and Cells
*Lilian Fritz-Laylin[1]; University of Massachusetts Amherst[1]

Organisms from across the eukaryotic tree use the cytoskeleton for fundamental activities including cell division, cell motility, and morphogenesis. Although the core cytoskeletal polymers—actin filaments and microtubules—are usually built from highly conserved proteins, the networks they build vary wildly both within individual cells and between cell types. Small changes in actin, tubulin, and their regulators can alter the emergent behaviors of entire networks, cells, and multicellular organisms. These systems, however, are involved in almost every essential cell function. To understand how cytoskeletal systems diversify over evolutionary timescales and understand the molecular mechanisms that drive functional specialization, we combine cell biology, comparative genomics, and phylogenetics to investigate the composition and function of the cytoskeletons of species from understudied eukaryotic lineages. Our research using the amoeboflagellate

Naegleria reveals two key principles governing cytoskeletal diversification. First, we find that microtubule networks can evolve independently within a single organism. We show that Naegleria uses different tubulins for different networks: conserved tubulins build flagella while divergent tubulins build mitotic spindles, each paired with specialized binding proteins. Critically, divergent residues in mitotic tubulins cluster within binding sites of differentiation-specific regulators, suggesting co-evolutionary constraints between tubulins and their binding partners. Second, we use Naegleria to change our understanding of the evolution of contractile actin networks. It was previously unknown if Myosin-2 dependent actin contractility—a key force that powers cell division and migration in animals, fungi, Dictyostelium, and other species in the group Amorphea—occurred outside this single group of eukaryotes. Our discovery of functional Myosin 2 in Naegleria, which diverged from Amorphea approximately two billion years ago, shows that contractile actin networks may be widespread amongst eukaryotic phyla, and not limited to a single phylogenetic group of eukaryotes. Together, these findings illuminate how cytoskeletal networks diversify through coordinated evolution of multiple network components and reveal the broader evolutionary origins of fundamental cellular processes.

SY201
The Secret Sex Lives of Choanoflagellates
*Nicole King[1]; UC, Berkeley[1]

[PG-13] Birds do it, bees do it, even choanoflagellates do it. I will share how exogenous and endogenous cues influence mating in the closest living relatives of animals, the choanoflagellates. We recently found that a G-protein coupled receptor controls the switch between asexual and sexual reproduction in the choanoflagellate S. rosetta. By studying the regulation of gametogenesis in choanoflagellates in mechanistic detail, we aim to reconstruct the evolution of gametes in animals.

From Mechanisms to Therapeutic Insight in Neurodegeneration

MS1402
Designed Ankyrin Repeat Proteins as tools to investigate the role of Leucine Rich Repeat Kinase 2 autoinhibition in Parkinson's Disease linked mutants
*Kathryn Hatch[1], Katherine Surridge[4], Eva Karasmanis[1], Marta Sanz Murillo[1], Sebastian Mathea[3], Verena Dederer[3], Stefan Knapp[2], Andres Leschziner[4], Samara Reck-Peterson[5]; University of California, San Diego[1], Goethe University[2], Institute of Pharmaceutical Chemistry, Goethe-Universität[3], Weill Cornell Medicine[4], Weill Medical College/Cornell University[5]

Leucine Rich Repeat Kinase 2 (LRRK2) is one of the most commonly mutated genes that leads to Parkinson’s Disease (PD). PD-linked LRRK2 mutations cause increased phosphorylation of LRRK2’s Rab GTPase substrates. In the transient autoinhibited conformation, where the N-terminal domains of LRRK2 drape across the kinase domain, Rab substrate cannot access the kinase active site to be phosphorylated. Due to a lack of tools to study LRRK2 conformation in cells, little is known about the mechanism of LRRK2 autoinhibition or the effects of PD-associated mutations on conformational autoinhibition. This study uses novel Designed Ankyrin Repeat Proteins (DARPins) to investigate how LRRK2’s conformation affects activation and substrate phosphorylation, and how PD-associated mutations affect autoinhibition. DARPins are small proteins selected for high binding

affinity to target proteins. Here we use the DARPin C12 to test how conformation can affect LRRK2 behavior. Using cryogenic electron microscopy, we found that DARPin C12 promotes the activated conformation of LRRK2 by interfering with the autoinhibitory interaction interface. In cells, DARPin C12 promotes increased substrate phosphorylation similarly to PD associated LRRK2 mutants. This suggests that promoting the active conformation of LRRK2 results in increased substrate phosphorylation by increasing the accessibility of the kinase domain. We hypothesize that this mimics how PD linked mutations outside the kinase domain increase substrate phosphorylation. When co-expressed with kinase domain mutant Gly2019Ser LRRK2, DARPin C12 induces an increase in LRRK2 Rab phosphorylation over baseline, but Arg1441His LRRK2, a mutation outside of the kinase domain, does not show altered Rab phosphorylation in the presence of C12. This suggests that mutations in the kinase domain affect substrate phosphorylation via a different mechanism than mutations outside of the kinase domain. LRRK2 double mutants containing both mutations show additive increases in Rab phosphorylation over baseline of either individual mutant. Our work shows that DARPin C12 induces conformational changes in LRRK2 that lead to relief of autoinhibition. Our initial analysis of LRRK2 PD-linked mutations shows that our assay could be expanded to profile any PD-linked LRRK2 mutation to determine if Rab phosphorylation is increased by relief of autoinhibition.

MS1407

Harnessing PINK1 Kinase Molecular Glues to Trigger Mitophagy in Parkinson's Therapy

*Kumar Suresh[1], Christopher Rainville[1], Muhammad Shahzad[1], Hehe Wang[1], David Sterner[1], Dominick Rendina[1], Michael Mattern[1], Tauseef Butt[1]; Progenra Inc[1]

Progenra has identified a new class of small molecules that act as molecular glues for PINK1. By stabilizing and activating PINK1, these compounds enhance phosphorylation of ubiquitin and activate the parkin E3 ligase. This PINK1–parkin activation cascade drives robust mitophagy at nanomolar concentrations in both cell-based and animal models. Beyond promoting mitochondrial clearance, the PINK1 glues stimulate mitochondrial biogenesis. Multi-omics approaches—including proteomics, metabolomics, and Seahorse bioenergetic profiling—demonstrate that treatment restores and augments mitochondrial performance under disease-relevant conditions. To monitor PINK1 activity in cells and tissues, Progenra employed Tandem Ubiquitin Binding Entity (TUBE) affinity platforms. A next-generation tool, the phospho-ubiquitin–specific TUBE (pTUBE), enabled mapping of the phospho-ubiquitome induced by PINK1 glues. While the role of PINK1 and phospho-ubiquitin signaling is well established in Parkinson’s disease, emerging evidence also points to broader functions in skeletal muscle physiology and other systemic processes, which will be highlighted.

MS1403

LASER senses lysosomal damage and initiates ESCRT assembly for membrane repair

*Aakriti Jain[1], Roberto Zoncu[1]; University of California, Berkeley[1]

Maintaining lysosomal membrane integrity is essential for cellular homeostasis, and its disruption contributes to neurodegenerative diseases. While the Endosomal Sorting Complex Required for Trafficking (ESCRT) machinery plays a key role in lysosomal repair, how ESCRTs first detect damage remains unclear. Through a genome-wide CRISPRi screen in damage-sensitized genetic background, we discovered a multimeric protein, renamed LC3-Assisted Sensor for ESCRT Recruitment (LASER), that bridges membrane damage sensing with

repair. LASER, which normally resides at ER exit sites, binds to ATG8 (LC3/GABARAP) proteins conjugated to damaged lysosomal membranes. At the lysosome, ATG8-bound LASER forms multimeric assemblies that directly recruit the ESCRT-I subunit, Tumor Suppressor Gene 101 (TSG101), via its PSAP motif and avidity-driven interactions, triggering downstream ESCRT I-II-III polymerization to promote rapid membrane repair. Importantly, mutations in LASER, known to cause hereditary spastic paraplegia, disrupt its assembly and impair ESCRT recruitment, linking defective repair to TFG-associated neurodegeneration. Thus, LASER mediates spatiotemporal control of ESCRT polymerization and couples lysosomal damage to repair.

MS1405

Lysosomal PI(4)P Signaling Links PIKfyve Dysfunction to Organelle Repair and Mitochondrial Homeostasis

*Candice Kutchukian[1], Maria Casas[1], Rose Dixon[1], Eamonn Dickson[1]; University of California, Davis[1]

Lysosomes are vital organelles that regulate cellular catabolism, autophagy, and lipid metabolism, and their dysfunction contributes to a wide spectrum of diseases, including neurodegeneration. At the lysosomal surface, the phosphoinositide kinase PIKfyve, the phosphatase Fig4, and the scaffold protein Vac14 form a regulatory complex that plays a central role in phosphoinositide metabolism. However, a paradox has emerged: while loss-of-function mutations in this complex cause severe neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Yunis-Varón syndrome and Charcot-Marie-Tooth disease, pharmacological inhibition of PIKfyve has shown therapeutic benefits in models of neurodegeneration. In this study, we seek to resolve this paradox by investigating how disruption of the PIKfyve complex reshapes lysosomal lipid signaling and organelle homeostasis. Using genetic and pharmacological perturbations of the PIKfyve complex, combined with advanced imaging and molecular biology approaches, we demonstrate that disruption of the PIKfyve complex leads to the accumulation of phosphatidylinositol 4-phosphate (PI(4)P) on lysosomal membranes. This occurs through ULK1-dependent trafficking of the PI(4)P-synthesizing enzyme PI4KIIα from the trans-Golgi network to lysosomes following reduced mTORC1 activity. Elevated lysosomal PI(4)P mediates two distinct adaptive responses: (i) it promotes lysosomal membrane repair by recruiting lipid transfer proteins to deliver cholesterol and phosphatidylserine to lysosomes, and (ii) it enhances ER-lysosome-mitochondria contact formation, driving mitochondrial fragmentation and enhancing respiratory capacity. Importantly, we show that pharmacological inhibition of PI4KIIα and/or ULK1 reverses these phenotypes, underscoring the central role of this pathway in stress adaptation. In conclusion, our study identifies a novel mechanism by which PIKfyve complex dysfunction triggers adaptive PI(4)P remodeling to coordinate lysosomal membrane repair and enhanced mitochondrial function. These findings provide mechanistic insight into the paradoxical effects of PIKfyve disruption and highlight new avenues for targeting phosphoinositide signaling in neurodegenerative disorders.

MS1404

Neuronal Aß generation is elevated by Swedish APP bypassing a lysosomal trafficking pathway that degrades wild-type APP

*Cameron Plowinske[1], Alec Nabb[1], Sarah Tedesco[1], Geraldine Quinones[1], Marvin Bentley[1]; Rensselaer Polytechnic Institute[1]

Cleavage of amyloid precursor protein (APPwt) by BACE-1 is the determinative step for generating amyloid β (Aβ), the primary component of amyloid plaques in Alzheimer's disease (AD). BACE-1-mediated APP cleavage is thought to occur mostly in neuronal endosomes. The Swedish variant of APP has two point mutations (KN670/671ML, APPSwe) that cause early-onset AD in patients and up to ten-fold more Aβ production than wild type. This difference in the rate of Aβ formation has been primarily attributed to the efficiency with which BACE-1 cleaves APP. It is unclear if APP mutations associated with increased Aβ formation change the neuronal trafficking of APP and increase its exposure to BACE-1. We used live-cell microscopy to characterize APPwt and APPSwe trafficking and their targeting to endosomes, the compartment where BACE-1-mediated cleavage and Aβ generation are thought to occur. In non-neuronal cells, APPwt and APPSwe were both trafficked to endosomes with high efficiency. Trafficking of APPwt in cultured hippocampal neurons differed substantially from non-neuronal trafficking: only a small fraction of the protein localized to dendritic endosomes. Of the APPwt that was endocytosed in dendrites, most was targeted to lysosomes for degradation. Such degradation would prevent Aβ formation and may be neuroprotective. APPSwe differed in its neuronal trafficking from APPwt. APPSwe was targeted to neuronal endosomes more frequently than APPwt. The trafficking of endocytosed APPSwe to dendritic lysosomes was less efficient, potentially enhancing exposure to BACE-1 in endosomes. Specific staining for Aβ revealed that APPSwe expression resulted in more vesicles containing Aβ than expression of APPwt. Surprisingly, a large number of Aβ-positive vesicles were not endosomes. These vesicles were Golgi-derived and contained Aβ, likely derived from APP that had not trafficked to the plasma membrane. This study shows that APP trafficking is a key determinant in Aβ generation and that trafficking differences between APPwt and APPSwe contribute to the rate of Aβ formation. The trafficking of endocytosed APP to lysosomes is a potential neuroprotective mechanism that avoids Aβ formation. The ability of APPSwe to avoid lysosomal degradation may contribute to the increase in Aβ formation associated with this mutation. Finally, the differences in APP trafficking between neurons and non-neuronal cells emphasize that neuron-specific studies are essential to understand the function and processing of APP in the nervous system.

MS1406

Novel crosstalk between TDP-43 oxidation and the GADD34-PP1 complex at RNA granule-mitochondria contact sites in ALS

*Hannah Ball[1], Abby Woods[1], Yvette Wong[1]; Northwestern University[1]

Inter-organelle contact sites are key hubs for organelle bidirectional crosstalk which are critical for cellular homeostasis and implicated in human disease etiology. However, whether mitochondria and RNA granules directly interact at contact sites, and the role of reactive oxygen species (ROS) generated from mitochondrial oxidative phosphorylation (OXPHOS) in mechanistically regulating this pathway is still not well understood. Here, using Super-Resolution live microscopy, we identify the dynamic formation of RNA granule-mitochondria contact sites in OXPHOS conditions. We find that ROS generated by mitochondrial OXPHOS is sufficient to recruit RNA granules containing TDP-43 to the cytoplasm and is dependent on oxidation of TDP-43 at cysteine residues. Mechanistically, RNA granule-mitochondria contact site tethering is mediated by TDP-43 binding to GADD34 on mitochondria, while contact untethering is driven by release of its binding from GADD34 and regulated by TDP-43's oxidation status. Functionally, this allows for the bidirectional crosstalk between TDP-43 RNA granules and a GADD34-PP1 complex on the mitochondria, whereby GADD34-PP1 regulates TDP-43 RNA granule dynamics, and conversely, TDP-43 oxidation status regulates PP1 granule phase separation dynamics. Furthermore, we demonstrate that this pathway is misregulated by disease-associated mutant TDP-43. Thus,

RNA granule-mitochondria contacts represent an important site for the molecular crosstalk between TDP-43 oxidation and GADD34-PP1 and highlight a key role for inter-organelle contacts in cellular homeostasis.

Immune Cell Biology

MS702
A time limit for phagocytosis: FcRIIB triggers delayed inhibition of phagocytic cup extension
*Erika Snyder[1], Annalise Bond[1], Meghan Morrissey[1]; University of California, Santa Barbara[1]

Whole cell engulfment, or phagocytosis, is regulated by activating and inhibitory signals. Fc Receptors on the surface of innate immune cells like macrophages bind the Fc region of IgG on pathogens or diseased cells to trigger phagocytosis. While Fc Receptor activity is critical for pathogen clearance, overactive Fc Receptor signaling is associated with autoimmune conditions. The Fc Receptor family contains several activating receptors and one inhibitory receptor: FcRIIB. All Fc Receptors respond to the same ligand of IgG. From a systems-level perspective, FcRIIB is puzzling – why express a dedicated inhibitory receptor paired to activating receptor engagement, when macrophages could more simply modulate activating FcR expression? To isolate FcRIIB signaling, I designed a synthetic DNA receptor system that allows selective stimulation of FcRIIB and activating Fc Receptors. Using this system, I examined how FcRIIB activation affected the kinetics of phagocytosis. I found that FcRIIB activation did not affect the early stages of phagocytosis, but caused phagocytosis to abruptly fail near the end of the process, when the macrophage membrane was covering >75% of the target. We confirmed that this effect required the recruitment of SHIP1, an inositol phosphatase known to complex with FcRIIB's intracellular signaling motif in B cells. SHIP-1 dephosphorylates the key signaling molecule PIP3, which is required for phagocytosis. Interestingly, I found that blocking PIP3 synthesis reduced initiation of phagocytosis instead of affecting completion of phagocytosis like FcRIIB. I then developed an in silico model of phospholipid conversion during concurrent activating and inhibitory FcR signaling over the course of a single phagocytic event. This model suggests that the rapid generation of PIP3 at the phagocytic cup by the activating Fc Receptors and the slow depletion of PIP3 by FcRIIB generates an incoherent feedforward loop that limits the duration of activating Fc Receptor signaling. To test this model, I challenged macrophages with targets of various sizes. I found that with increasing target size, the percentage of the target's surface engulfed at failure decreased, while the total surface area engulfed and the duration of the phagocytic attempt were consistent between sizes. Collectively, this work establishes FcRIIB signaling as a molecular timer that limits phagocytic persistence. We postulate that this molecular fail-safe could prevent long, unproductive interactions with a target.

MS707
Chimeric Antigen Receptor T Cells generate antigen-specific mechanical forces
*Rachel Fitzgerald[1], Gianna Branella[1], Areeba Hashmi[1], Anna Davis[1], Jhordan Rogers[1], Sunil Raikar[1], Khalid Salaita[2]; Emory University[1], Emory[2]

Chimeric Antigen Receptors (CAR) are engineered to bind to cancer-associated antigens, with this interaction leading to cancer cytotoxicity. Recently, the T cell field has come to recognize the role of small, piconewton level forces in establishing specificity and cytotoxicity in naïve T cells with the αβ TCR, raising the possibility

that these forces could be present in CAR T cells despite the structural differences between CARs and TCRs. We developed single-receptor cancer-antigen DNA tension probes and utilized them to reveal the presence of F>18 pN mechanical forces in healthy, active anti-CD19 CAR T Cells directed against target protein CD19. Remarkably, these forces occur at levels similar to the known mechanoreceptor CD3ε in human T cells. We found that CAR forces are spatially and temporally dynamic and appear more disordered compared to that of the TCR-pMHC forces likely reflecting the structural differences between the CAR and TCR synapse. We further find that CD33 CARs also transmit pN forces to CD33, indicating that is a general feature of CARs. The magnitude of CAR forces display donor-to-donor variability reflecting the heterogenous activity of human T cells. These forces are highly dependent on the CAR T cell's ability to restructure the local cytoskeletal network and recruitment of the actin cytoskeleton, and can be inhibited with pharmacological inhibitors. We show that factors that modulate the cytotoxicity of CAR T cells such as exhaustion and immunomodulatory agents like dasatinib also modulate the mechanical forces exerted by CAR T cells on CD19 antigens, showing that these forces are strongly correlated with their cytotoxicity. Taken together, our work identifies a previously ignored aspect of CAR signaling and suggests potential applications in screening and as novel biophysical marker of CAR T cell health and efficacy.

MS704

Glycosylation inhibition enhances natural killer cell cytotoxicity through nanoscale reorganization of CD16a signaling and effector domains

*Hijab Fatima[1], Richard Ren[1], Maria Benavente[3], Adam Barb[3], Charles Murin[2], Emily Mace[1]; Columbia University Irving Medical Center[1], SanDiego BioMed[2], University of Georgia[3]

Natural killer (NK) cells mediate cytotoxicity through antibody-dependent cellular cytotoxicity (ADCC). NK cells express the Fc receptor CD16a (FcγRIIIa), which binds to antibodies' Fc regions to facilitate ADCC. Engagement of CD16a at the immune synapse triggers phosphorylation of CD3ζ and Syk, leading to target cell lysis. Post-translational glycosylation modulates ADCC and inhibiting glycan processing enhances NK cell cytotoxicity. We investigated the effects of kifunensine (kif.), a Golgi α-mannosidase I inhibitor, on NK cell killing kinetics and CD16a activation. We previously showed that detection of phospho-CD3ζ (pCD3ζ) via confocal microscopy serves as a proximal marker for CD16a activation and that following kif. treatment there is a significant increase in the size and number of pCD3ζ-containing foci. In parallel, downstream signaling is enhanced, as reflected by elevated pSyk levels. This led us to the hypothesis that inhibiting the formation of branched glycans enables the formation of larger signaling clusters. To determine how inhibiting glycan processing translates into functional gains, we performed quantitative live-cell imaging which revealed that kif. treated NK cells lyse target cells in approximately half the time and display increased serial killing efficiency compared to their untreated counterparts. To uncover the structural basis of this enhancement, we employed high resolution imaging. SIM-TIRF microscopy showed the formation of discrete actin foci at the NK cell-target interface that consistently colocalized with pCD3ζ, indicating cytoskeleton-mediated reorganization of signaling machinery during Fc receptor engagement. Single-molecule imaging further revealed that pCD3ζ assembles into tightly organized microclusters in response to Fc receptor binding to ligand, highlighting a nanoscale architecture that underpins signal amplification. Super-resolution imaging confirmed that CD16, like pCD3ζ, formed larger and more numerous microclusters under these conditions. Together, these data underscore a coordinated reorganization of signaling and effector domains and suggest that inhibiting complex glycan formation permits more effective microcluster formation. In summary, kif. treated NK cells exhibit faster and more efficient ADCC, driven by enhanced proximal signaling and nanoscale reorganization at the immune synapse.

MS703

Human NLRP3 inflammasome activation leads to formation of condensate at the microtubule organizing center

*Jue Wang[1], Man Wu[3], Le Xiao[3], Gang Du[3], Muyuan Chen[1], Venkat Magupalli[4], Peter Dahlberg[1], Hao Wu[3], Grant Jensen[2]; SLAC National Accelerator Laboratory[1], Brigham Young University[2], Harvard Medical School[3], New York Blood Center Enterprises[4]

Over the past decade, cryogenic focused ion beam milling combined with scanning electron microscopy (Cryo-FIB-SEM) has been established as the leading approach for preparing thin sections (lamellae) for in situ visualization of cellular structures by cryogenic electron tomography (Cryo-ET). When specific sub-cellular structures are of interest, fluorescence microscopy (FM) is often integrated into Cryo-FIB-SEM to target fluorescently labelled regions during milling. However, guiding milling along the optical axis of the microscope, which coincides closely with the thin axis of the lamella, remains highly challenging for small and rare structures. To overcome this limitation, here we employed a new, tri-coincident Cryo-FM-FIB-SEM instrument, called the ENZEL, which enables real-time, simultaneous cryogenic fluorescent imaging and FIB milling for precise targeting along the optical axis. Utilizing the ENZEL in combination with Cryo-ET, we investigated the NLRP3 inflammasome in complex with the microtubule organizing center (MTOC) in human macrophages at various stages of activation. The NLRP3 inflammasome has been known as a master regulator of innate immunity, mediating proinflammatory cytokine maturation and pyroptotic cell death, and its dysregulation has been linked to diverse human diseases. Our cryo-tomograms did not reveal the expected large, well organized macromolecular assembly identified from in vitro studies. Instead they showed dense condensates within and around the microtubule organizing center (MTOC). We also found that following activation, the Golgi cisternae expand and disperse while 50-nm NLRP3-associated vesicles emerged, likely ferrying NLRP3 to the MTOC. At later stages, these electron-dense condensates progressively solidified, coinciding with pyroptosis, widespread mitochondrial damage, and autophagy. Our study provides the first in situ visualization of the NLRP3 inflammasome and uncovers coordinated organelle remodeling events during inflammation, offering structural and mechanistic insights that could guide therapeutic strategies for modulating overactive immune pathways.

MS705

Role of citrullinated vimentin in the pathogenesis and progression of multiple sclerosis

*Lukasz Suprewicz[1], Paul Janmey[1], Robert Bucki[2]; University of Pennsylvania[1], Medical University of Bialystok[2]

Multiple sclerosis (MS) involves blood–brain barrier (BBB) dysfunction, leukocyte trafficking into the CNS, and ongoing neuroinflammation. Citrullination—the process of converting arginine to citrulline by peptidylarginine deiminases—produces neoepitopes that can intensify inflammation. Vimentin, a type III intermediate filament, appears outside cells during inflammation and is often citrullinated (CitVim). We hypothesized that CitVim acts as a damage-associated molecular pattern (DAMP) that weakens BBB integrity and promotes leukocyte extravasation. We measured CitVim levels in cerebrospinal fluid (CSF) and serum from MS patients and non-inflammatory neurological controls. To model barrier function, we used 2D and 3D human BBB platforms consisting of channels within extracellular matrices in which brain microvascular endothelial cells are interfaced with astrocytes. Barrier integrity was evaluated through fluorescent dextran permeability and tight-junction morphology, while neutrophil transmigration into the matrix was quantified during exposure to

CitVim. Pharmacologic pathway analysis involved Toll-like receptor 4 (TLR4). CitVim levels were higher in MS CSF and serum compared to controls. Exogenous CitVim disrupted endothelial tight junctions, increased paracellular permeability, and boosted neutrophil extravasation into hydrogel matrices. CitVim is elevated in MS biofluids and alone can impair BBB function and promote leukocyte trafficking in a human 3D model. These results support CitVim as a mechanistic link between peripheral inflammatory signals and the entry of innate immune cells into the CNS in MS.

MS708

The functional role of localized plasma membrane scrambling in immune cell activation

*Daryna Sputay[1], Sze Ham Chan[1], Kandice Levental[1], Ilya Levental[1]; University of Virginia[1]

Membrane asymmetry is a carefully controlled and energetically costly differential in lipid composition between the inner and outer plasma membrane (PM) leaflets. Asymmetry is created and maintained by lipid translocases (flippases) that force anionic and amino-phospholipids into the cytosolic leaflet, and released by lipid channels (scramblases) that shuttle phospholipids down their concentration gradient to disrupt PM asymmetry. The release of lipid asymmetry exposes the charged lipid phosphatidylserine (PS) to the external leaflet, where it is essential for apoptosis and blood clotting. Additionally, exposure of inner leaflet phospholipids has been observed during the activation of several immune cell types (mast cells, basophils, leukocytes). We confirmed these observations, showing exposure of PS and other inner leaflet phospholipids on the PM outer leaflet following antigen-mediated stimulation of T cells and mast cells. Importantly, in contrast to wholesale release of PM asymmetry by apoptosis, PS exposure in activated immune cells is focal, transient, reversible, and not associated with cell death. This lipid scrambling appears to be involved in immune cell function, as inhibition of the scramblase TMEM16F attenuates antigen-activated mast cell degranulation. To investigate the mechanisms underlying these effects, we knocked out (KO) TMEM16F, the ubiquitous Ca2+-dependent scramblase / lipid channel, which is putatively responsible for PS and PE translocation during immune cell signaling. Indeed, we find that TMEM16F is essential for activation-associated lipid scrambling. Finally, we combine theory and cell experiments to explore the molecular nature and functional role of localized, transient membrane scrambling during mast cell activation and degranulation. Altogether, our findings suggest that localized scrambling is an essential component of signal transduction downstream of immune receptor activation.

MS706

Tropomyosin and integrin engagement cooperate to promote actomyosin arc-dependent force generation at the T cell immune synapse required for efficient target cell killing

*Dillon Schrock[1], Dominic Golec[2], Pamela Schwartzberg[2], Marco Heydecker[3], Roberto Weigert[3], Mikayla Greiner[4], Arpita Upadhyaya[4], Anjelika Gasilina[1], Edna Hardeman[5], Peter Gunning[5], John Hammer[1]; Cell and Developmental Biology Center, NHLBI, NIH, Bethesda MD[1], Cell Signaling and Immunity Section, NIAID, NIH, Bethesda MD[2], Laboratory of Cellular and Molecular Biology, NCI, NIH, Bethesda MD[3], University of Maryland, College Park[4], University of New

Efficient target cell killing by CD8+ T cells requires the exertion of force on the target cell. This force may be generated by a contractile actomyosin arc network that comprises the medial, pSMAC portion of the T cell's

immune synapse. The pSMAC is also where the T cell's integrin LFA-1 binds to ICAM-1 on the target cell to drive T cell: target cell adhesion. Here we show that ligating LFA-1 with ICAM-1 promotes the formation of the actomyosin arc network, and that a tropomyosin (Tpm3.1/3.2) decorates this network. Abrogating Tpm3.1/3.2 function using either a small molecule inhibitor or CRISPR-mediated gene deletion disrupts the concentric organization of the arc network, reduces its content of myosin 2A, reduces T cell traction force, reduces T cell: target cell adhesion, and reduces the ability of effector CD8+ T cells to kill antigen-pulsed target cells without attenuating signaling. These and other results argue that Tpm3.1 and LFA-1 ligation cooperate to promote the generation of actomyosin arc-dependent force at the T cell immune synapse required for efficient target cell killing, and they add to a growing appreciation for the role that positive feedback between adhesion and myosin force plays in promoting T and B cell effector functions.

Integrative Cell and Developmental Systems Biology

MS401
Aggregation and DNA binding of Dorsal/NF-?B in early embryos
*Gregory Reeves[1], Sadia Dima[1]; Texas A&M University[1]

The mechanism by which transcription factors (TFs) regulate gene expression, a process crucial for the development and maintenance of an organism, remains puzzling despite decades of research. Recent advances propose that TFs form dynamic, possibly phase-separated hubs that contribute to transcriptional regulation. However, the mechanisms connecting hub formation with transcriptional regulation remain poorly understood. To address this gap, we measured biophysical properties of the TF Dorsal (Dl), which forms a gradient to pattern the dorsal-ventral (DV) axis of the early Drosophila embryo. Previous work using fluorescent imaging of live and fixed embryos indicated that the Dl gradient has a Gaussian-like shape with the highest level of Dl present on the ventral side of the embryo. The level of Dl oscillates in a progressively increasing saw-tooth pattern during blastoderm nuclear cycles (ncs) 10 to 14. The spatiotemporal variation of Dl levels ensures the accurate spatial borders and dynamic pattern of target gene expression. Here we use Raster Image Correlation Spectroscopy (RICS), a type of scanning Fluorescence Correlation Spectroscopy, and single particle tracking in live embryos to quantify absolute concentrations of three sub-populations of Dl, each with distinct diffusivities and binding properties, along the DV axis from nc 10 to 14. We show that, while the absolute nuclear concentration of Dl increases over time, the fraction of Dl bound to DNA decreases over time. The DNA binding cannot be explained by a simple dose response relationship between free and bound concentrations, implying the existence of a mechanism beyond simple TF/DNA binding. The results from all the experiments encompassing different length and time scales suggest the presence of slowly moving clusters of Dl in addition to the expected populations of freely mobile Dl and DNA bound Dl. The data suggest that the clusters form only above a threshold concentration, a phenomenon typically associated with phase-separation. The formation of similar mobile clusters has been previously observed in other TFs, such as Bicoid, and has been proposed to provide an efficient search strategy for regulatory regions. We also found that the clusters bind the DNA transiently. These findings enhance our understanding of the mechanism of transcriptional interpretation of the Dl gradient and are expected to generalize to other TF/DNA interactions.

MS406
AI-POWERED ACCELERATION AND PARAMETER TUNING FOR COMPUTATIONALMODELING
*Linlin Li[1]; Purdue University[1]

Computational models of biological systems provide critical insights into complex processes, yet high computational costs and challenges in parameter estimation often limit their predictive power. To address these limitations, we developed AI-powered frameworks that accelerate simulation and improve parameter tuning. By leveraging neural network metamodels, we achieved rapid approximations of PDE-based models of morphogen signaling, enabling efficient exploration of biophysical hypotheses such as advection-driven gradient formation in zebrafish embryos in both 1D and 3D models. Furthermore, we introduced invertible neural networks (INNs) through the Inverse Problem Antidote (IPA) framework to tackle parameter identifiability, offering a bidirectional mapping between model parameters and biological outcomes. Together, these advances demonstrate how AI can reduce computational burden, enhance model interpretability, and enable systematic investigation of signaling mechanisms across developmental systems.

MS405
Investigating cytoskeletal and cellular dynamics during morphogenesis in the zebrafish
*Bakary Samasa[1], Linlin Li[3], Sharon Minsuk[2], Joe Zinski[4], James Glazier[2], David M. Umulis[3], Mary Mullins[4]; University of Pennsylvania-Biomedical Graduate Studies - Philadelphia, PA[1], Indiana University[2], Purdue Univerisity[3], University of Pennsylvania[4]

The first coordinated morphogenetic cell movement during zebrafish development is a process called epiboly, wherein the cells of the embryo spread over and envelop the yolk. These morphogenetic movements are essential to the transition from a group of individual cells to the formation of a body plan during development. Thus, the dynamics and regulation of epiboly remains a fundamental question in biology. Epiboly is initiated by an expansion of an epithelial enveloping cell layer (EVL) coupled with EVL planar cell divisions that leads to yolk cell doming and progression to 50% epiboly. However, the mechanism/s of epiboly progression beyond 50% remain less clear. Here we use live and fixed imaging of actin to investigate cytoskeletal and cellular dynamics in mediating epiboly progression, together with computational modeling. Specifically, we investigated straightening of the leading edge of the EVL margin, which is an emergent property of our mathematical model of epiboly. We show that the embryo EVL margin starts uneven, but by the end of epiboly, the margin straightens. Additionally, we investigate cell shape changes using CellPose and a custom algorithm to track cells during the morphogenetic process. We show that cells initially do not elongate as epiboly progresses and rather cells rearrange at the EVL leading edge margin, while nearer the end of epiboly, cell elongation dominates. We quantified these changes by an increasing cell shape index as epiboly progresses. Additionally, we show that the number of junctions per cell does not change dramatically as epiboly progresses. These and other results set the stage for further investigation of epiboly dynamics in mutants and fluorescent reporter lines of the cytoskeleton and adhesion factors, as well as comparison to our mathematical models.

MS403
Reactive Oxygen Species Counteract Zebrafish Wound Contraction and Promote Wound Healing
*Chang Ding[1], Linlin Li[3], Yueyang Wang[2], Hong-Anh A. Nguyen[3], Deva Chan[3], David M. Umulis[3], Adrian T. Buganza[3], Qing Deng[1]; Purdue University[1], Harvard Medical School[2], Purdue Univerisity[3]

Reactive oxygen species (ROS) are second messengers that drive wound closure. However, the mechanism by which ROS regulate wound contraction to facilitate wound healing remains unclear. Here, we report that ROS counteract wound contraction by inhibiting the phosphorylation of myosin regulatory light chain. Acute ROS inhibition, through pharmacological perturbations, disturbs wound relaxation, delays wound closure, and impairs regrowth following amputation. Moreover, actomyosin inhibition relaxes tailfin contraction without impairing wound closure or regrowth. Over-contraction, on the other hand, impedes wound closure. Meanwhile, chronic depletion of epithelial ROS during embryonic development, achieved through morpholino-mediated knockdown of the duox gene, alters tissue stiffness, as measured using atomic force microscopy-based nanoindentation. Despite a reduced contraction force, the wound also appears to be over-contracted, with delayed healing and regrowth. An in silico linear elasticity simulation to calculate the second principal stress based on node-wise prescribed displacement recapitulated the contraction dynamics during acute and chronic ROS inhibition. Together, our results provide a novel understanding of how reactive oxygen species (ROS) facilitate wound closure, a process instrumental in restoring tissue integrity and maintaining homeostasis.

MS404
Reverse engineering organ size and shape control through the integration of quantitative experiments and multi-scale computational modeling
*Jeremiah Zartman[1]; University of Notre Dame[1]

Predicting how an organ’s size and shape emerge from complex biomolecular interactions remains one of the most significant challenges in systems biology and tissue engineering. Our group’s research integrates experimental and computational approaches to elucidate the underlying principles of morphogenesis. To do so, we use the larval wing disc of the fruit fly (Drosophila melanogaster) as a powerful model system for discovering the mechanisms regulating a growing organ’s shape and size. I describe the formulation and experimental validation of a multi-scale computational model that has generated new mechanistic insights into the control logic of morphogenesis. This work relies on the integrative efforts of multiple collaborative projects. Using tools that create spatially defined perturbations to growth or mechanotransduction pathways, we demonstrated that increasing cell proliferation through different pathways leads to distinct outcomes in the regulation of organ shape. For example, increased insulin signaling enhances the tissue’s basal curvature while stimulating growth through Bone morphogenetic protein (BMP/Dpp) signaling or elevated Myc expression has the opposite effect and flattens the central tissue region. These variations in outcomes arise from differences in how each growth pathway regulates actomyosin contractility, cell-ECM adhesion, and ECM stiffness. In summary, we propose that organ size control relies on orchestrating tissue morphogenesis through the balancing of the differential effects of intrinsic, extrinsic, and mechanotransduction regulators of growth and cell mechanics.

MS402
Signal processing and robustness of the BMP network during zebrafish embryogenesis
*Nissa Larson[1], Bobby Madamanchi[2], Linlin Li[1], David Umulis[1]; Purdue University[1], University of Michigan[2]

In developing tissues, signal transduction from morphogen gradients conveys positional information to cells, resulting in cell specification and differentiation. One such morphogen is bone morphogenetic protein (BMP), of the TGF-β superfamily, whose network is highly conserved across many species. In zebrafish species Danio rerio, this signaling pathway directs dorsoventral axis formation during early embryogenesis. Many of the molecules that play a role in this network are known; however, the mechanisms through which they achieve noise attenuation and gradient robustness have not been defined. Specifically, the heterodimer-heterotetramer complex has been shown to be required for signal transduction, but deterministic modeling of the BMP membrane receptors at this stage has not given any insight into evolutionary drivers of the requirement. Building and developing a stochastic, multiscale model of this process allows us to mechanistically assess zebrafish phenotype variability related to the distributions of noise and stochasticity. We can also analyze time-dependent signaling and frequency metrics that are not available in traditional, deterministic modeling. The stochastic receptor model has been constructed using the open-source python wrapper GillesPy2 and the framework StochKit2. This framework employs tau-leaping and Gillespie's stochastic simulation algorithm to rapidly simulate stochastic trajectories in biochemical systems with facilitated cluster-implementation. Fast Fourier Transform and cumulative energy spectral density visualization show that the heterodimer-heterotetramer complex functions as part of a low-pass mechanism in the dorsal-ventral axis formation process. Reliance on the heterodimer-heterotetramer complex for signal transduction through the few hours of embryogenesis may drive higher accuracy in downstream cell differentiation by way of fewer low frequency fluctuations. To further probe this system, we are working to develop an experimental optogenetics protocol to genetically manipulate dorsoventral signal transduction in vivo and collect and analyze fluorescence intensity of the downstream pSmad gradient. Through these multiscale modeling efforts and experimental coupling, we hope to further understand the noise origins and signal processing of this network. As the BMP signaling pathway is highly conserved and has been implicated in human bone growth and wound healing, its study in simpler systems stands to accelerate our comprehension of BMP network structure and molecular mechanisms with application in regenerative medical studies.

Interplay Between Nuclear Pore Complex and Cell Physiology

MS2201
A conserved mechanism of membrane fusion in nuclear pore complex assembly
*Jonas Fischer[1], Matthias Wojtynek[1], Ashutosh Kumar[3], Harry Baird[1], Kateřina Radilová[1], Daria Maslennikova[1], Kaustubh Ramachandran[2], Anna Becker[1], Arantxa Agote-Aran[1], Alessia Loffreda[4], Annemarie Kralt[1], Madhav Jagannathan[1], Gautam Dey[2], Ulrike Kutay[1], S; Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich CH-8093, Switzerland[1], Cell Biology and Biophysics, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany[2], Department of Biology, University of Fribourg, Fribourg CH-1700, S

The nuclear pore complex (NPC) is the central gateway between the nucleus and cytoplasm in all eukaryotes, and its biogenesis requires fusion of the inner and outer nuclear membranes. Although this membrane fusion

step is essential for eukaryotic life, its molecular mechanism has long remained mysterious. In our work, we elucidate the mechanism by which two paralogous transmembrane proteins, Brl1 and Brr6, mediate membrane fusion in S. cerevisiae. Both proteins form ring-like multimeric complexes that remodel membranes and interact with one another across the nuclear envelope lumen through conserved hydrophobic loop motifs. Disrupting either multimerization or Brl1-Brr6 interaction blocks fusion and halts NPC assembly. Remarkably, phylogenetic analyses reveal that Brl1/Brr6 homologues are broadly distributed across eukaryotes, and functional experiments in human cells and D. melanogaster establish CLCC1 as an NPC fusogen in metazoans. Together, our results uncover a novel, conserved mechanism for membrane fusion in eukaryotes.

MS2208

Determining the order of import of nuclear proteins post-mitosis required for the gradual activation of mRNA transcription, processing and export

*Yaron Shav-Tal[1], Alon Boocholez[1]; Bar-Ilan University[1]

Gene expression must be re-established in the nucleus after every cell division, to reinstate transcription, RNA processing, and export. In addition, nuclear bodies, such as nuclear speckles that contain many mRNA-processing factors, reassemble. Prior studies have demonstrated the assembly of the nucleolus by the gradual return of rRNA transcription and nucleolar proteins following mitosis. However, it is unknown whether the timing of return of the many factors reactivating mRNA transcription, splicing, and export into the nucleus follows an ordered return or whether this is a random occurrence. Our study investigates the re-entry of nuclear proteins essential for re-initiating gene expression in conjunction with the formation of nuclear speckles during early interphase. The time-frame of this flux of molecules into the newly forming nucleus is short, making it challenging to follow temporally. We found that by targeting the nuclear pore complex it is possible to prolong the phase of nuclear entry of numerous RNA-binding proteins. Since many of these proteins concentrate in cytoplasmic granules that form during mitosis, we can use live-cell microscopy to follow their import dynamics after mitosis. We find that certain RBPs return in distinct waves and that their re-entry is tightly linked to nuclear speckle formation and the reactivation of gene expression. The absence of these processing factors in the nucleus led to impaired RNA splicing and export, which were restored upon the re-entry of these factors. We are determining which import mechanisms may be employed to regulate the timing of protein entry into the nucleus. Altogether, this study suggests a regulated hierarchy of nuclear import that is required for proper mRNA processing and export.

MS2205

Early Inner Nuclear Membrane Deformations Induced by Progerin Are Modulated by Cell Division

*Ayse Turkmen[1], Katharine Ullman[1]; University of Utah[1]

The nuclear envelope (NE) undergoes dynamic remodeling during both physiological and pathological processes, yet the regulatory mechanisms governing these changes are not fully understood. To investigate distinct stages of NE remodeling, we utilized inducible expression of Progerin, a mutant form of Lamin A bearing a persistently farnesylated tail that causes Hutchinson-Gilford Progeria Syndrome (HGPS), a premature aging disorder marked by progressive nuclear dysmorphism and early onset of musculoskeletal and cardiovascular symptoms. Live-cell imaging revealed that as Progerin accumulates, cells develop NE-associated

foci without significant changes in nuclear shape until after mitosis, when NE reassembly produces a lobulated nuclear architecture characteristic of HGPS. Electron microscopy of interphase-arrested cells showed that prior to cell division, Progerin primarily affects the inner nuclear membrane (INM), inducing focal expansion, invagination, and the formation of spherical, multi-membranous structures, while the outer nuclear membrane remains largely unaffected. Expression of the farnesylated tail domain of Lamin B2 (LaminB2tail) recapitulated these findings. To identify modulators of this early NE phenotype, we screened NE proteins and found a subset enriched at Progerin-induced NE foci in arrested cells, including the nucleoporin Nup153. Although Nup153 is a component of the nuclear pore complex (NPC), it also interacts with INM proteins such as lamins and can associate directly with membranes. Nup153 enrichment at LaminB2tail-induced NE foci, along with the absence of other nucleoporins like Nup98 and Nup62, suggests that Nup153 recruitment is driven by INM invagination rather than interactions with a specific lamin or NPCs. Depletion of Nup153 reduces NE foci formation in arrested cells, suggesting it promotes or stabilizes INM invagination. These findings provide new insights into how cell cycle state influences NE remodeling, raising the possibility that proliferative status plays a role in tissue-specific manifestations of HGPS. Additionally, the identification of Nup153 as a facilitator of early Progerin-induced INM invaginations provides an inroad to understanding cell division-independent NE remodeling and reveals a factor to test for contribution to other physiological or pathological processes involving NE invagination.

MS2206

ESCRT III-Driven Repair of Nuclear Rupture Reverses Transcriptional Arrest in Lamin-Related Cardiomyopathy

*Kohta Ikegami[1], Atsuki En[1], Melanie Gucwa[1]; Cincinnati Children's Hospital Medical Center[1]

Loss-of-function mutations in the nuclear lamina protein Lamin A/C (LMNA) cause heart arrhythmia, chamber dilation, and fatal cardiac arrest in LMNA-related cardiomyopathy. A lack of understanding of its pathogenesis has hampered the development of treatment strategies. Previously, we generated a mouse model of this disease with inducible, cardiomyocyte-specific Lmna knockout (LmnaCKO). Nearly half of all cardiomyocytes in LmnaCKO hearts developed localized rupture of the nuclear envelope before any other deficits, suggesting that nuclear rupture is a disease-initiating event (En et al., 2024; PMID: 38814785). Here, we report that the ESCRT-III membrane remodeling complex repairs nuclear ruptures, restores rupture-associated transcriptional defects, and counteracts disease progression in LmnaCKO mice. We found that LmnaCKO cardiomyocytes lost RNA Polymerase II (Pol II) from ruptured nuclei and globally downregulated transcriptional activity. However, nearly half of ruptured nuclei eventually restored nuclear compartmentalization, indicating endogenous repair. Repaired nuclei resumed transcriptional initiation but did not fully restore transcriptional elongation, likely due to frequent re-rupture. We found that ESCRT-III complex proteins accumulated at nuclear envelope rupture sites and that this accumulation was driven by BANF1 binding to the rupture sites. Inhibiting the ESCRT-III ATPase VPS4 increased rupture frequency, reduced repair, and accelerated progression of cardiomyopathy in LmnaCKO mice, suggesting that endogenous rupture repair counteracts disease progression. A heart from an LMNA-DCM patient similarly exhibited frequent nuclear envelope rupture and its association with BANF1, suggesting rupture repair activity in this disease. Collectively, our data implicate rupture-associated transcriptional arrest in the pathogenesis of LMNA-related cardiomyopathy and suggest that enhancing rupture repair may be a therapeutic strategy. More broadly, this study sheds light on the etiology of other conditions associated with nuclear envelope rupture, including myocardial infarction, neurodegenerative disorders, cancer, and senescence.

MS2207

Fission yeast SPB proteins establish an alternative proteinaceous diffusion barrier at the nuclear envelope during mitosis

*William Chadwick[1], Nicholas Ader[2], Ivan Surovstev[3], Megan King[3], Patrick Lusk[3]; Yale University[1], University of North Carolina Greensboro[2], Yale School of Medicine[3]

The nuclear compartment is defined by the nuclear envelope and nuclear pore complexes (NPCs) that regulate traffic across this boundary by imposing a size-selective diffusion barrier. In our recent work studying the mitotic remodeling of the nuclear envelope, we uncovered a diffusion barrier that acts alongside NPCs to maintain the nuclear compartment. During the closed mitosis of Schizosaccharomyces pombe, the mitotic spindle is organized by the spindle pole body (SPB), which undergoes a cycle of insertion and extrusion in and out of the nuclear envelope. Despite the generation of a nuclear envelope fenestra to facilitate this SPB cycle, the nuclear compartment remains intact throughout cell division, even in contexts when this fenestra becomes enlarged, for example in late anaphase upon disruption of Cmp7, the nuclear envelope adapter for the endosomal sorting complexes required for transport (ESCRT) machinery that exerts a grommet-like function upon SPB extrusion. Thus, elements of the SPB likely form a remarkably plastic diffusion barrier that can expand to fill nuclear envelope fenestrations as large as 500 nm. Indeed, the integrity of the nuclear compartment is lost after conditional depletion of several SPB proteins, particularly the key structural components Pcp1 and Pcp89. Consistent with the ability of these proteins to respond to nuclear envelope hole size, they are both found at higher copy number at the SPB in cmp7Δ cells through a mechanism that we tie to the activity of polo like kinase (Plo1). This pool of Pcp1 occludes a fluorescent diffusion barrier reporter through a mechanism that is disrupted by treatment with the aliphatic alcohol, 1,6 hexanediol. Thus, we propose that mitotic nuclear envelope holes are plugged by the Plo1-dependent expansion of at least Pcp1, which may form a biomolecular condensate that can be tailored to nuclear envelope hole size and restricts the diffusion of large macromolecules.

MS2202

GlcNAcylation of NPCs modulates the diffusion barrier in response to cell type and mechanical state

*Sunandini Chandra[1], Kimberly Morgan[1], Megan King[1], Patrick Lusk[1]; Yale School of Medicine[1]

Nuclear pore complexes (NPC) are embedded in the nuclear envelope and control molecular exchange between the cytoplasm and nucleoplasm. There is emerging evidence of plasticity in the molecular composition and dilation state of NPCs observed in different cell types and mechanical environments, but how such changes ultimately alter an NPC's selective sorting properties remains uncertain. In contrast, there is evidence that changing the levels of the post-translational O-linked N-acetylglucosamine (GlcNAc) modification of nucleoporins impacts NPC permeability. Here, we demonstrate that GlcNAcylation of NPCs is variable across cell types including in cultured neurons, which are depleted of GlcNAc and maintain a more stringent NPC diffusion barrier. These data suggest that cell-type specific NPC GlcNAcylation is a previously unappreciated mechanism that may tailor the selective permeability properties of NPCs to meet cell-type specific nuclear transport demands. Further, like NPC dilation, NPC GlcNAcylation is responsive to mechanical cues: we find that culturing cells on "stiff" or "soft" substrates either increases or decreases NPC GlcNAcylation, respectively,

through a mechanism that is independent of altering the levels or localization of the enzymes that control global GlcNAcylation. Using pan-expansion microscopy and new conditional tools that specifically alter the GlcNAcylation of NPCs, we demonstrate that modifying NPC GlcNAcylation not only modulates the stringency of the diffusion barrier but is also sufficient to directly impact NPC diameter. These changes may also underlie our observation that nuclear volume equilibration after hyper-osmotic treatment is slower when NPC GlcNAcylation is inhibited. Together, our data support that the GlcNAcylation of nucleoporins is a driver of NPC dilation state that may help to explain changes to NPC permeability in different mechanical environments.

MS2204

Reconstitution of lamin assembly on nuclear pore complex-containing membranes

*Ross Pedersen[1], Yinyin Zhuang[2], Andres Reyes[1], Shou-Ling Xu[1], Xiaoyu Shi[2], Yixian Zheng[1]; Carnegie Institution for Science[1], University of California, Irvine[2]

Intermediate filaments called lamins line the metazoan nuclear envelope and organize the nucleus and genome. Unlike actin and microtubules, purified intermediate filament proteins assemble into non-physiological structures, making it difficult to connect lamin functions to their assembly and regulation. To overcome this challenge, we reconstituted lamin assembly without nuclear assembly using interphase Xenopus laevis egg extracts that recapitulate physiological context. Mimicking nucleoplasm conditions triggers dispersed lamin assembly in egg extracts. Such ectopic lamin assembly occurs on nuclear pore complex-containing membranes, but does not recruit known nuclear lamina components, demonstrating that lamin assembly is separable from the rest of the nuclear lamina and nucleus. This assembly assay in the physiological context of cellular components opens the door to mechanistically dissecting nuclear lamina function in nuclear organization.

MS2203

The role of Spindle Assembly Checkpoint proteins at Nuclear Pore Complexes: Implications beyond Chromosome Segregation

*Sanjana Sundararajan[1], Fatimatou Diouf[1], Vasilisa Aksenova[1], Caroline Esnault[2], Lijin Dong[3], Alexei Arnaoutov[1], Mary Dasso[1]; National Institute of Child Health and Human Development, National Institutes of Health[1], Bioinformatics and Scientific Programming Core, National Institute of Child Health and Human Development, National Institutes of Health[2], Genetic Engineering Core, N

Accurate chromosome segregation is critical for genomic stability. This process relies on proper chromosome attachment to the mitotic spindle and alignment at the metaphase plate before segregation in anaphase. The Spindle Assembly Checkpoint (SAC) safeguards segregation fidelity by delaying anaphase onset through generation of Mitotic Checkpoint Complex (MCC) from unattached kinetochores. While SAC components MAD1, MAD2, and the silencing factor p31 are well characterized at kinetochores, they also localize to interphase Nuclear Pore Complexes (NPCs), the portals of nucleocytoplasmic transport. Despite the fact that NPC localization is conserved across eukaryotes, the functional roles of these proteins at NPCs remain largely unclear. This study investigates the association and functional significance of SAC proteins at interphase NPCs. Using an auxin-inducible degron system in DLD1 cells, we found that the nucleoporin TPR mediates the localization of MAD1, MAD2, and p31 at NPCs. We further observed that SAC proteins assemble at NPCs in the

same hierarchical order as at kinetochores: TPR → MAD1 → MAD2 → p31. For the first time in live human cells, we measured the dynamics of SAC proteins at NPCs and found that MAD1 is very stably associated while the turnover of other SAC components is more rapid. Given its stable association and MAD1's essential role in recruiting other SAC proteins, we tested several hypotheses for its function at NPCs. First, we tested a model wherein MAD1 may facilitate interphase MCC formation at NPCs to reinforce the mitotic checkpoint. We found that MCC assembly is restricted to mitotic onset, arguing against this model. Second, although MAD1 has been demonstrated to control nucleocytoplasmic transport in yeast, we found no evidence that MAD1 regulates transport in human cells. Finally, MAD1 depletion did not result in altered gene expression, arguing against a role in RNA homeostasis. We are currently investigating whether NPC-associated MAD1 plays a developmental role using a mouse model that exclusively expresses a MAD1 mutant localized to the cytoplasm during interphase.

Keith Porter Legacy Awards Session

AW401
Dysregulated subcellular localization and alternative translation in rare human diseases
*jimmy ly[1], Matteo Di Bernardo[1], Yi Fei Tao[1], Ekaterina Khalizeva[1], Christopher Giuliano[1], Sebastian Lourido[1], Mark Fleming[2], Iain Cheeseman[1]; Whitehead Institute and MIT[1], Boston Children's Hospital[2]

Mitochondrial endosymbiosis was a pivotal event in eukaryotic evolution, requiring core proteins to adapt to function both within the mitochondria and in the host cell. Alternative start codon selection of a single mRNA generates alternative N-terminal protein isoforms with shared core sequences but distinct N-termini. These alternative N-terminal isoforms of a gene may localize to distinct subcellar locations to enable the same function in different organelles. Here, we systematically profile the localization of alternative protein isoforms generated by alternate start codon selection during translation. Using protein language models, we identify 498 of pairs of differentially-localized protein isoforms, many of which affect mitochondrial targeting and are essential for mitochondrial function. By tracing the evolution of alternative start codons, we find that the emergence of dual-localized mitochondrial isoforms coincides with mitochondrial acquisition in early eukaryotes. We uncover multiple different mechanisms by which alternative translation controls N-terminal localization, including signal sequence masking, a novel strategy that conceals targeting signals to prevent organelle import. Finally, we leverage the identification of these alternative N-terminal isoforms to reshape our interpretation of disease alleles, understand clinical manifestations, and potentially treat rare human diseases. Pathogenic nonsense and frameshift mutations are typically assumed to completely eliminate a gene's function. We demonstrate that alternative start codon selection can bypass these genetic disruptions, selectively eliminating specific isoforms of a gene while preserving others—a phenomenon we term Isoform-Specific Alleles (ISAs). We found that ISAs in the gene TRNT1 eliminate a specific alternative N-terminal isoform of TRNT1, explaining the distinct clinical symptoms among patients with TRNT1-associated diseases. We further identify thousands of ISAs in rare human diseases and demonstrate that these alleles result in isoform-specific effects on the protein. Together, our findings illuminate the evolutionary and pathological relevance of alternative translation initiation, offering new insights into organelle biology and the molecular basis of rare disease.

AW402
Satellite DNA shapes dictate pericentromere packaging in female meiosis
*Damian Dudka[1], Jennine Dawicki-McKenna[3], Xueqi Sun[1], Keagan Beeravolu[1], Takashi Akera[2], Michael Lampson[1], Ben Black[3]; Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA[1], Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.[2], Department of Bio

The abundance and sequence of satellite DNA at and around centromeres is evolving rapidly despite the highly conserved and essential process through which the centromere directs chromosome inheritance. The impact of such rapid evolution is unclear. Here we find that sequence-dependent DNA shape dictates packaging of pericentromeric satellites in female meiosis through a conserved DNA-shape-recognizing chromatin architectural protein, high mobility group AT-hook 1 (HMGA1). Pericentromeric heterochromatin in two closely related mouse species, M. musculus and M. spretus, forms on divergent satellites that differ by both density of narrow DNA minor grooves and HMGA1 recruitment. HMGA1 binds preferentially to M. musculus satellites, and depletion in M. musculus oocytes causes massive stretching of pericentromeric satellites, disruption of kinetochore organization and delays in bipolar spindle assembly. In M. musculus × spretus hybrid oocytes, HMGA1 depletion disproportionately impairs M. musculus pericentromeres and microtubule attachment to their kinetochores. Thus, DNA shape affects both pericentromere packaging and the segregation machinery. We propose that rapid evolution of centromere and pericentromere DNA does not disrupt these essential processes when the satellites adopt DNA shapes recognized by conserved architectural proteins (such as HMGA1). By packaging these satellites, architectural proteins become part of the centromeric and pericentromeric chromatin, suggesting an evolutionary strategy that lowers the cost of megabase-scale satellite expansion.

Life in 3D

SY601
Generation of Urinary Tract Organoids from Human Pluripotent Stem Cells
*Minoru Takasato[1]; RIKEN Center for Biosystems Dynamics Research[1]

The mammalian urinary system constitutes an integrated physiological unit comprising the kidney, ureter, bladder, and urethra. Its developmental origins are remarkably intricate, as multiple distinct cellular lineages cooperate to establish the urinary tract. In our previous studies, we established three-dimensional kidney tissues (“kidney organoids”) from human induced pluripotent stem (hiPSCs) cells via intermediate mesoderm, employing an autonomous tissue-construction strategy. However, kidney organoids lack a ureter—the essential outflow tract for urine—rendering them unsuitable for direct transplantation. During normal development, the ureter not only elaborates within the kidney but also extends toward the bladder, thereby connecting the two structures. This indicates that the presence of the bladder is indispensable for the formation of a kidney equipped with a ureter. Accordingly, in addition to our established kidney organoids, we have undertaken the challenge of generating bladder organoids from human iPS cells. The developmental process of the bladder is unique to mammals, with the ventral side of the cloaca separating from the future intestinal tract and developing into the urothelium. Here, we show how hiPSCs can be differentiated stepwise into hindgut, cloaca

and then bladder organoids with structural and functional characteristics specific for the bladder. During initial differentiation steps, we differentiated definitive endoderm into Mid/Hindgut-like spheroids using medium with FGF4, which posteriorized gut spheroids. We next optimized specific culture conditions that promote ventralization to selectively induce urothelium. Therefore, we screened for growth factors to induce ventral hindgut-like spheroids and succeeded in inducing bladder-like spheroids expressing urothelium makers such as UPK2, P63, and KRT5 in vitro. These sac-like structures consist of a highly specialized, stratified epithelial layer that include all urothelium cell types, can stretch and have a barrier function. Bladder organoids can thus provide a powerful model for understanding human bladder development, serve as disease models and provide therapeutic cells in the future.

SY602
Not Just for Gas Exchange, a 3D view of the Many Curiosities of the Lung
*Xin Sun[1]; University of California, San Diego[1]

The lung, best known as the vital gas exchange organ, harbors ~50 cell types, each with specialized function, some not directly linked to gas exchange. These include mesothelial cells that “shrink wrap” not just the lung, but also internal organs. They also include rare neuroendocrine cells that senses signals in lung, either directly, or in collaboration with lung-innervating nerves originating from peripheral neural ganglia and also the brain. In this talk, I will showcase the lung as a representative of the intricacies of our internal organs in 3D. I will also use the lung as starting point to discuss studies of organ-brain crosstalk in the context of considering the lung as a sensory organ.

MBoC Highlights: The Future of Cell Biology

SG1102
A human interpretable cell behavior grammar to democratize simulations in virtual multicellular laboratories
*Paul Macklin[1], Jeanette Johnson[4], Daniel Bergman[4], Heber Rocha[1], Randy Heiland[1], Young Hwan Chang[3], Laura Heiser[3], Genevieve Stein-O'Brien[2], Elana Fertig[4]; Indiana University[1], Johns Hopkins University[2], Oregon Health and Science University[3], University of Maryland School of Medicine[4]

Cells interact as dynamically evolving ecosystems. While exciting new single-cell and spatial multi-omics technologies are quantifying individual cell characteristics with snapshots at unprecedented levels of detail, they cannot unlock the dynamics of these systems, nor can they readily explore the impact of chemical and physical perturbations without running many experimental combinations. Agent-based models (ABMs), which simulate individual cells as software agents that interact in simulated tissue environments, can serve as “virtual laboratories” to test and refine our understanding of the “rules” of multicellular biology. However, ABMs have traditionally required extensive programming knowledge, limiting their use. In this talk, we present a new conceptual framework—a cell behavior hypothesis grammar—that uses natural language statements (cell rules) to create mathematical and simulation models in real time without writing code. This enables systematic integration of biological knowledge and multi-omics data to generate in silico models with user-friendly online tools, enabling virtual “thought experiments” that interactively test and expand our understanding of multicellular systems and generate new testable hypotheses. We demonstrate its use in developing both de

novo mechanistic models and those informed (and initialized!) by multi-omics data, with examples in cancer biology, cancer immunology, and neurodevelopment. This approach bridges biological, clinical, and systems biology research for mathematical modeling at scale, allowing the community to predict emergent multicellular behavior. Moreover, the grammar transforms mathematical modeling into an AI-ready language problem, suggesting new possibilities to integrate human and machine intelligence.

SG1103

A human interpretable cell behavior grammar to democratize simulations in virtual multicellular laboratories

*Elana Fertig[1]; University of Maryland School of Medicine, Institute for Genome Sciences[1]

Cells interact as dynamically evolving ecosystems. While exciting new single-cell and spatial multi-omics technologies are quantifying individual cell characteristics with snapshots at unprecedented levels of detail, they cannot unlock the dynamics of these systems, nor can they readily explore the impact of chemical and physical perturbations without running many experimental combinations. Agent-based models (ABMs), which simulate individual cells as software agents that interact in simulated tissue environments, can serve as “virtual laboratories” to test and refine our understanding of the “rules” of multicellular biology. However, ABMs have traditionally required extensive programming knowledge, limiting their use. In this talk, we present a new conceptual framework—a cell behavior hypothesis grammar—that uses natural language statements (cell rules) to create mathematical and simulation models in real time without writing code. This enables systematic integration of biological knowledge and multi-omics data to generate in silico models with user-friendly online tools, enabling virtual “thought experiments” that interactively test and expand our understanding of multicellular systems and generate new testable hypotheses. We demonstrate its use in developing both de novo mechanistic models and those informed (and initialized!) by multi-omics data, with examples in cancer biology, cancer immunology, and neurodevelopment. This approach bridges biological, clinical, and systems biology research for mathematical modeling at scale, allowing the community to predict emergent multicellular behavior. Moreover, the grammar transforms mathematical modeling into an AI-ready language problem, suggesting new possibilities to integrate human and machine intelligence.

SG1104

Biological imaging across scales with reconfigurable reactive microscopes

*Wesley Legant[1]; University of North Carolina[1]

Comprehensive biological imaging requires tools that can visualize objects ranging from molecules to organisms over milliseconds to days. Over the past decades, different microscopy modalities have been developed to visualize different biological systems at specific spatiotemporal scales. However, optimization for one task or scale often sacrifices versability and performance in other contexts. In this seminar, I will describe our efforts to develop a Multimodal Adaptive Optical Microscope for In vivo Imaging from Molecules to Organisms (MOSAIC). This instrument can dynamically reconfigure itself to integrate multiple advanced imaging techniques including light-sheet, label-free, super-resolution, and multi-photon, all equipped with adaptive optics to compensate for sample-induced aberrations. Applications include non-invasive imaging of subcellular dynamics in both cultured cells and live multicellular organisms, nanoscale mapping of molecular architectures across millimeter-scale expanded tissues, and structural/functional neural imaging within live

mice. I will also describe computer vision-based approaches to implement real-time switching between modalities based on dynamic changes in sample structure. These automated methods efficiently detect rare events within heterogeneous cell populations and record these processes with high spatiotemporal four-dimensional imaging over statistically significant replicates. I will highlight recent applications to study chromosome segregation and immune synapse structure.

SG1107
Bridging the gap between molecular and cellular structure with cryoEM and cryoET
*Justin Kollman[1]; University of Washington[1]

Recent advances in technology and image processing approaches in cryo-electron microscopy have revolutionized our understanding of cell biology at the level of molecular mechanism, and at placing those molecular insights in the context of intact cells. New single particle cryo-EM approaches that enable deconvolution of complex protein movements and heterogeneous assemblies are providing unprecedented insight into fundamental mechanisms of allostery, force generation, transport, and other essential cellular functions. Advances in cryo-electron tomograph are enabling investigation of molecular function in their native cellular context. Here, we focus on large assemblies of regulatory metabolic enzymes in nucleotide biosynthesis, revealing the structural basis of catalysis and enzyme regulation, the mechanisms and functional consequences of higher-order assembly, and the functional role of enzyme assemblies in cells. We find in several systems that enzyme assembly serves as a mechanism of allosteric control of metabolic flux, with implications for mechanisms of hereditary diseases and therapeutic approaches.

SG1105
Patterns of Mitochondrial ATP Predict Actomyosin contraction and Apical Constriction
*Bezia Lemma[1], Megan Rothstein[1], Pengfei Zhang[1], Bridget Waas[1], Marcus Kilwein[1], Safiya Topiwala[1], Sherry Zhang[1], Anvitha Sudhakar[1], Katharine Goodwin[1], Elizabeth Gavis[1], Ricardo Mallarino[1], Andrej Kosmrlj[1], Celeste Nelson[1]; Princeton University[1]

Contraction of the actomyosin network is used to construct tissue shapes during embryonic development and wound healing, generating mechanical forces that are fueled by chemical energy from ATP hydrolysis. Here we find that this chemical energy is spatially patterned prior to and during actomyosin contraction events. Specifically, mitochondria are locally enriched at the apical sides of epithelial cells during apical constriction, which is widely used across the animal kingdom to fold epithelial tissues. Timelapse imaging, spatial transcriptomics, and measurements of oxygen consumption rate reveal that mitochondrial density, potential, and ATP increase in epithelial cells prior to actomyosin contraction and tissue folding, which is prevented by inhibiting oxidative phosphorylation. Mitochondrial enrichment and apicobasal patterning are conserved during apical constriction of epithelial tissues in flies, chicks, and mice, and these subcellular patterns can be used to predict computationally patterns of epithelial folding. These findings highlight a spatial dimension of bioenergetics in the cell biological processes that drive embryonic development.

SG1106

Photoactivatable proximity labeling reveals local protein synthesis and metabolic regulation by ECM stiffness

*Congying Wu[1]; Peking University[1]

Cellular metabolism, a central biochemical process influencing numerous cellular processes, has intimately link with mechano-transduction. Uncovering the mechano-metabolomic crosstalk will provide promising new perspective in understanding cell physiology and disease. Focal adhesions (FAs) are mechano-transduction hubs, that regulates cell motility and fate. Recent studies have highlighted the role of ECM rigidity in lipid and glucose metabolisms via FA signaling. It has also been identified that protein synthesis can take place at FAs. However, the molecular components, functional roles, and regulatory mechanisms of this mechano-metabolic interplay of proteins are still poorly characterized. Furthermore, current research predominantly focuses on intracellular mechanosensitive responses, whereas knowledge of the secretory phenotype and intercellular communication driven by mechanical stimuli to influence metabolism is limited. Proximity labeling methods use enzymes to catalyze the covalent binding of small molecules to biomolecules near the target protein, offering an antibody-free approach that can detect weak or transient interactions. To overcome the limitations of background labeling and improve temporal resolution, optogenetic approaches have been employed to control labeling enzyme activity by tuning exposure time and region. Total internal reflection fluorescence (TIRF) excites fluorophores in a thin layer (~100 nm) at the interface between the substates and cell ventral surface, reducing background fluorescence in the cytosol and improving axial resolution. Thus, TIRF microscopy is widely used for FAs imaging. However, commercial objective-based TIRF microscopy has a limited field of view (~2.5×10^{-5} cm^2), which is insufficient for large-scale proteomics sample collection if combined with optogenetic control of proximity labeling. Here, by combining an optimized optogenetic proximity labeling (PA-ultra)-mass spectrometry with customized pTIRF excitation, we reveal the interactome of FAs and look for transient interactions that may potentially unveil new roles of FA-mediated mechano-transduction. Our study uncovers a new mechano-metabolic axis linking extracellular matrix (ECM) stiffness to selenium (Se) homeostasis. We identify the dynamic interaction of FAs with SerRS which is critical for SELENOP synthesis. FA-localized SELENOP mRNA and newly-synthesized SELENOP have been revealed by biochemical and imaging assays. Functionally, increased ECM stiffness enhances FA assembly and upregulates intracellular SELENOP level.

SG1108

Tubulin glutamylation increases tau condensate diffusivity and enables kinesin motility

*Antonina Roll-Mecak[1]; National Institutes of Health[1]

Motors carrying intracellular cargo encounter on the microtubule surface a brush of intrinsically disordered tubulin C-terminal tails that are abundantly posttranslationally modified, and a dense mesh of microtubule-associated proteins (MAPs). tau, a MAP associated with Alzheimer's and frontotemporal dementia, is highly abundant in neurons and forms on microtubules condensates which block kinesin motors. Here we show that microtubule glutamylation, a modification highly abundant on neuronal microtubules enables kinesin-3 KIF1A processive motion through tau condensates, but not that of kinesin-1 KIF5B. Increased permeability of tau condensates to KIF1A is facilitated by a monotonic increase in tau diffusivity within condensates as a function

of tubulin glutamylation and requires the motor-specific K-loop. Our work reveals that the tubulin code modulates a key property of microtubule-associated condensates and shows how motility through the crowded MAP-covered microtubule surface is an emergent property arising from tubulin glutamylation.

Mechanisms of Protein Turnover and Roles in Terminally Differentiated Cells

MS2501
A novel ribosome assembly surveillance pathway (RASP) ensures continuous ribosome production and maintains cellular proteostasis
*Kamena Kostova[1]; Stowers Institute for Medical Research[1]

Ribosome biogenesis is a complex process that requires more than 200 protein factors, many non-coding RNAs, and hundreds of interdependent steps. 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. However, how cells cope with defective biogenesis intermediates and what are the quality control factors necessary for their detection and clearance is poorly understood. Here, we present a novel ribosome assembly surveillance pathway (RASP) that targets faulty pre-ribosomes. We developed a tissue culture-based system to model stochastic failure during the assembly of the ribosomal large subunit. We discovered that defective biogenesis intermediates are rapidly detected and degraded through the ubiquitin proteasome system. We used CRISPR-Cas9-based screening to identify factors necessary for the clearance of these defective ribosomes and characterize how they interact with faulty ribosomes. Importantly, inhibition of RASP leads to accumulation of defective ribosomes that ultimately inhibits global ribosome production. To understand the role of RASP on the organismal level, we obtained zebrafish lines harboring mutations in key RASP factors. Loss of ribosome quality control caused defects in various tissues and organs, reminiscent of ribosomopathy phenotypes. To date, this group of disorders has been associated with mutations in ribosome biogenesis factors and core ribosomal components. Here, we show that loss of RASP can initiate similar pathological changes. Therefore, identifying and characterizing factors involved in ribosome biogenesis and quality control is critical for improving patient diagnostics and may lead to novel therapeutic interventions.

MS2505
Analysis in vivo using a new method, ARGO (Analysis of Red-Green-Offset), reveals complexity, cell-type specificity, and requirement for TFEB/HLH-30 in presynaptic turnover of synaptic vesicle protein Synaptogyrin/SNG-1
*Claire Richardson[1], Nikita Shilieav[1], Ruiling Zhong[1], Sophie Baumberger[1]; University of Wisconsin, Madison[1]

The maintenance of proper synaptic vesicle (SV) cycling throughout a neuron’s extended lifespan requires SV protein turnover – the production and delivery of newly synthesized proteins balanced with the removal and degradation of damaged or excess proteins. How SV protein degradation is regulated in vivo remains incompletely understood, largely due to the prior lack of robust methods to quantify protein turnover with subcellular spatial resolution. We developed the ARGO method, a genetically encoded, ratiometric fluorescence imaging approach that visualizes and quantifies protein turnover in vivo with subcellular

resolution. With ARGO, the protein of interest is cell-specifically, co-translationally labeled with both RFP and GFP. Steady-state imaging reveals whether and where the protein is degraded within lysosomal compartments. Next, a Cre/Lox 'pulse' removes the gfp gene from the argo cassette, and periodic ratiometric imaging quantifies the amount of old (RFP+GFP) versus total (RFP) protein during the 'chase'. The approach is modular, inexpensive, and scalable for use in genetically tractable experimental organisms. Using ARGO, we examine the turnover of a SV protein, Synaptogyrin/SNG-1, in adult C. elegans neurons. Our data support the model that SV proteins are sorted for degradation at the synapse and then trafficked to the neuron cell body to complete degradation within lysosomal compartments. We find that Transcription factor EB (TFEB)/HLH-30 is required to generate proper neuronal lysosomal capacity and promote presynaptic protein turnover. Specifically, loss of HLH-30 leads to slowed SNG-1::ARGO turnover in the presynapses without increased steady-state SNG-1 abundance or accumulations in somatic lysosomal compartments. These findings suggest that the neuron may possess feedback mechanisms to accommodate, at least initially, defects in protein turnover. In addition, we show that SNG-1 turnover rate is consistent across synapses within an individual neuron but varies between neuron classes. Notably, in at least one neuron type SNG-1 exhibits biphasic turnover within each presynaptic compartment. This result suggests that the presynapse contains two chronically distinct, non-intermixing populations of SNG-1. This separation could be an intrinsic property of the SNG-1 protein or indicative of two distinct, non-intermixing pools of SVs. In summary, ARGO enables routine, spatially resolved protein turnover quantification and reveals previously hidden complexity in SV protein maintenance.

MS2504

Enhanced recruitment of lysosomal repair pathways in response to lysosomal damage in astrocytes versus neurons

*Erin Smith[1], Natali Chanaday[1], Sandra Maday[1]; Perelman School of Medicine, University of Pennsylvania[1]

Lysosomal damage impairs proteostasis and contributes to many neurodegenerative diseases. In response to lysosomal membrane damage, cells can employ several mechanisms to restore lysosomal integrity. Recent proteomic profiling has revealed that lysosomal protein composition varies across cell types, including those in the brain, suggesting that lysosomes may have specialized properties depending on the cell type. However, cell-type-specific properties of lysosomes, particularly in their resilience to membrane damage, in neurons versus astrocytes is poorly understood. Here, we compare the response of neurons versus astrocytes to lysosomal injury induced by a lysosomotropic methyl ester (e.g., LLOMe), with a focus on three key pathways: ESCRT-mediated membrane repair, TBC1D15-mediated membrane reformation, and the PITT pathway to replenish lipids. To elucidate cell-type-specific responses, we use a neuron-astrocyte coculture system that recapitulates several morphological, proteomic, and functional signatures of neuron-astrocyte interactions in vivo. We first established functional evidence that LLOMe effectively induces lysosome damage in both neurons and astrocytes based on galectin-3 recruitment to luminal lysosomal b-galactosides, elevated lysosomal pH, and engagement of lysophagy receptors TAX1BP1 and p62. Next, we investigated the ESCRT-mediated repair pathway which utilizes membrane scission to repair small perforations in the lysosome membrane. Despite lysosomal damage occurring in both cell types, astrocytes showed a preferential recruitment of ESCRT repair machinery to damaged lysosomes, as compared to neurons. This enhanced ESCRT recruitment in astrocytes was observed with multiple components of the ESCRT pathway (e.g., CHMP2B, CHMP2A, ALIX and IST1). Moreover, enhanced ESCRT recruitment was conserved in astrocytes isolated from different brain regions (e.g., the hippocampus or cortex). Additionally, the lysosomal membrane reformation pathway regulated by the RAB7-GAP, TBC1D15, was also more robustly activated in astrocytes as compared to

neurons. By contrast, the PITT pathway, detected with PI4K2A and ORP9, which mediates lipid shuttling between the endoplasmic reticulum and damaged lysosomes, was engaged in both cell types. Our data reveal a divergence in how neurons and astrocytes mobilize repair pathways to manage lysosomal damage. These data may reflect differences in lysosomal resilience between astrocytes and neurons and inform therapeutic strategies to correct lysosomal dysfunction in neurodegenerative diseases.

MS2502

PP2A and CDK16 regulate WIPI2B phosphorylation and neuronal autophagy

*Heather Tsong[1], Andrea Stavoe[2]; The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences[1], University of Texas Health Science Center at Houston McGovern Medical School[2]

With today's aging population, the incidence of age-related diseases, such as neurodegenerative diseases (NDDs), is increasing. As such, there is a growing need to understand the cellular mechanisms contributing to neuronal aging. A hallmark of aging is the aggregation of misfolded proteins. Autophagy is a highly conserved degradation pathway that neurons utilize to clear protein aggregates and is essential for maintaining neuronal health. However, autophagy function decreases with age, and dysregulated autophagy is implicated in many NDDs. Previously, we restored autophagy function in aged primary neurons by overexpressing WIPI2B, a key autophagy component. Interestingly, this restoration was contingent upon the phosphorylation state of WIPI2B. These data suggest that WIPI2B phosphorylation regulates its function in neuronal autophagy. Thus, identifying the kinase and phosphatase that regulate WIPI2B phosphorylation will provide an avenue to modulate WIPI2B function and control rates of neuronal autophagy. To identify these WIPI2B regulators, we utilized Caenorhabditis elegans as a screening system. The autophagy pathway is highly conserved between mammals and C. elegans and the WIPI2B ortholog in C. elegans (CeWIPI2B) also has a conserved phosphorylation site. Using a simple, visual readout of neuronal autophagy function, we determined that phosphorylation of CeWIPI2B regulates neuronal autophagy in vivo. With this same phenotype, we screened for regulators of neuronal autophagy and identified PP2A and CDK16 as candidates. Epistatic analysis suggested that PP2A, CDK16, and CeWIPI2B function in the same pathway to modulate neuronal autophagy. Given the genetic evidence, we verified PP2A and CDK16 enzymatic activity on mammalian WIPI2B through in vitro phosphorylation assays and showed localization of PP2A and CDK16 to autophagosomes in cultured primary neurons. Altogether, these data support PP2A and CDK16 as regulators of WIPI2B phosphorylation and neuronal autophagy. Ultimately, a better understanding of how neurons regulate autophagy will uncover novel targets for modulating neuronal autophagy, providing potential therapeutic strategies for NDDs.

MS2503

SNX-RGS proteins are conserved phospholipid transporters which loss causes lysosome membrane lipid disruption and neurodegeneration

*Vanessa Sanchez[1], Kaitlynn Gov[2], Emma Reynolds[2], Mike Henne[2], Naiara Akizu[1]; University of Pennsylvania - Children's Hospital of Philadelphia[1], University of Texas Southwestern Medical School[2]

Recent advances in genomics, cell and structural biology suggest the involvement of inter-organelle lipid transport proteins (LTPs) in the pathogenesis of neurodegenerative disorders. LTPs bind and transport lipids

between organelles influencing membrane integrity and aiding in membrane repair following damage. Such membrane repair is important for neurons, yet the mechanisms and machinery that maintain organelle membrane integrity remain elusive. Here, we reveal that members of the SNX-RGS protein family linked to spinocerebellar ataxia recessive 20 (SCAR20) encode a novel LT domain that binds to multiple phospholipids with a preference for phosphatidic acid (PA), a non-bilayer lipid which perturbs membranes when accumulated. In line with this, SNX-RGS protein depletion leads to pathological accumulation of PA and diacylglycerol on lysosomes, disrupting lysosome lipid composition. Following lysosome membrane perturbation, we find SNX-RGS proteins, SNX13 or SNX14 are recruited to damaged lysosomes, and their loss perturbs lysosome membrane damage repair in both HeLa cells and hPSC-derived neurons. Furthermore, our recent genetic studies have uncovered SNX13 mutations in a cohort of patients diagnosed with a novel SCAR20-like neurodegenerative disorder, supporting an overlapping role for SNX-RGS proteins in neuronal function and survival. These findings suggest that SNX-RGS proteins mediate multi-organelle lipid transport to act as neuronal lysosome quality control factors to safeguard lysosome membrane composition and aid membrane repair following lysosome membrane permeabilization (LMP). On-going work is focused on mechanistically defining how SNX-RGS proteins sense and repair LMP, including how their loss leads to neurodegeneration.

MS2506

The Autophagy Protein BECN1 Regulates AMPA Receptor Stability To Promote Social Behavior

*Congcong He[1], Kenta Kuramoto[1], Idalis Ramirez[1]; Northwestern University Feinberg School of Medicine[1]

Social interaction is a sophisticated behavior that is impaired in individuals with autism spectrum disorders (ASD). Because of its complexity, the mechanisms underlying typical social interactions, as well as the development of ASD pathology, remain poorly understood, and effective treatments are still lacking. Autophagy is a lysosomal degradation pathway important for intracellular protein homeostasis and is controlled by ~40 autophagy-related proteins, including the Beclin family member BECN1. Biallelic variants or reduced expression of autophagy genes, including Becn1, Atg7 and Atg8, are recently discovered in complex neurodevelopmental syndromes in humans; however, the underlying pathogenic mechanism is mysterious. While autophagy proteins are generally thought to participate in lysosome-mediated degradation, emerging evidence indicates that they may have more diverse non-canonical roles beyond this function. Here we discover that the autophagy protein BECN1 is a regulator of social behavior by stabilizing cell-surface AMPA receptor (AMPAR) neurotransmission, via a crosstalk with the ubiquitin-proteasomal machinery. Proteomic and biochemical studies identify BECN1 as a binding partner of both an E3 ubiquitin ligase UBE3A and its substrate Arc. Accumulation of Arc is associated with excessive endocytosis and reduced levels of AMPARs in ASD. We reveal that BECN1 serves as an adaptor for UBE3A-mediated Arc polyubiquitination and proteasomal degradation, thereby maintaining postsynaptic AMPAR levels. We further found that loss of BECN1 in hippocampal CA3 neurons decreases excitatory AMPAR neurotransmission and impairs social interaction, resembling ASD-associated phenotypes. Thus, our study demonstrates that BECN1 is a novel regulator of social behavior by promoting UBE3A-mediated Arc degradation and ensuring postsynaptic AMPAR stability and neurotransmission in CA3 neurons.

MS2507
Uncovering mechanisms underlying the trans-cellular spreading of tau and a-synuclein in neurodegenerative diseases
*Eloïse Néel[1], Morgane Denus[1], William Fargues[1], Philippe Marin[1], Marie-Laure Parmentier[1], Julien Villeneuve[1]; Institute of Functional Genomics (IGF)[1]

Neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, share two common pathogenic mechanisms: the toxic accumulation and aggregation of specific proteins (Tau and alpha-synuclein (aSNC), respectively), and the sequential dissemination of these aggregation-prone proteins within the central nervous system. Emerging evidence indicate that these proteins propagate from cell to cell via unconventional protein secretion (UcPS), a process that remains poorly understood. Hence, to uncover mechanisms underlying UcPS is of outmost importance. Toward this objective, we developed a new cell-based assay using the SH-SY5Y neuroblastoma cell line and the Split Luciferase technology to precisely quantify the secretion of multiple cargo proteins released via UcPS. Through targeted genetic screens, we identified the central role played by secretory lysosomes in the UcPS of both Tau and aSNC. Confocal microscopy, biochemical assays, and purified lysosome analyses, revealed the presence of these proteins within lysosomal compartments. Furthermore, by combining proteomic analysis and functional tests on lysosome properties, we identified factors that regulate Tau and aSNC UcPS, particularly prosaposin (PSAP), which appears to play a key role in directing lysosomes toward secretory functions. At the crossroad of critical challenges, this work could shed light on fundamental mechanisms governing lysosome biology that could ultimately contribute to identify new therapeutic targets to prevent or slow the progression of neurodegenerative diseases.

Mentoring Keynote

AW101
The Importance of Imagination, a Superpower, in Survival, Science, and Our Lives.
*Margaret Werner Washburne[1], Alex Washburne[2]; University of New Mexico[1], Selva, LLC[2]

As life has sped up and information comes from more directions, our ability to access, grow, and use our imaginations has suffered. While not a new phenomenon, with the development of AI, our ability to avoid reactivity and find time to use our imaginations has diminished. We think we are imaginative because we see creative worlds on large and small screens and integrate more new information daily. But this is generally passive activity that doesn't help us be imaginative. Imagination is a crucial skill throughout our lives. Einstein knew this when he said, "Imagination is more important than knowledge". It starts with young children, who require creative play for empathy, problem-solving, analytical and symbolic reasoning, and more. But, too soon, creative play is seen as dispensable. We forget that sea change discovery in every domain requires great imagination. Two decades ago, I realized students in my upper-level genomics class weren't using their imaginations to learn science. It limited their ability to have new ideas and interesting questions. They didn't realize the magic that was inside them. Over a year, I developed approaches for accessing, restoring, and growing their imaginations. I will share these approaches and resources with you. Ultimately, imagination work led to a set of 4 principles, retrospectively tested by my path in life, that are key to resilience, emotional intelligence, and leadership – and a set of 4 characteristics for organizing and sustaining great teams. This

foundation has proved valuable to hundreds of students and academic staff over the years, in paths as diverse as academia, medicine, law, sales, and stand-up comedy. Resources for using the Principles and Teams characteristics will be provided. Finally, working on Imagination and the Principles as well as my research, led to what we hope is an important approach in cancer. A year ago, Christmas eve of 2024, I was surprised to receive a diagnosis of pancreatic cancer. But what surprised me most was feeling excited and almost honored. "At my age", such a profound challenge felt strangely like a gift. I immediately began looking for agency. It appeared when I saw the PET scan, which uses radioactive glucose and active cancer cells light up like a spotlight! My research at UNM was on yeast responses to glucose starvation, so these cancer cells immediately felt familiar. My son, Alex, and I developed a, paradoxically, novel approach we hypothesized would make cell-cycle-targeting chemotherapy more effective. The success of this n=1 approach has been remarkable, but more research will be needed. We have identified communication issues and assumptions that may have slowed progress. But it has made me most grateful for my path and, especially, for you and your potential to be the most imaginative generation ever.

Metabolism and Cell Size in Tissue Regeneration

MS1804

A Novel Doxorubicin-Adaptive hiPSC Model Links Early Stem Cell Stress to Impaired Cardiomyocyte Maturation

*Rosy Joshi-Mukherjee[1], Sooriyan Sahay[1], Mobolanle Badejo[1], Anvesha Mukherjee[4], Luis Mandl[2], Somdatta Goswami[3]; Notre Dame of Maryland University[1], Institute of Structural Mechanics and Dynamics in Aerospace Engineering, University of Stuttgart[2], Johns Hopkins University[3], University of Wisconsin[4]

Doxorubicin (Dox), a highly effective anthracycline chemotherapy, is limited by its cardiotoxicity, which is caused in part by the depletion of cardiac progenitor cells (CPCs). However, the mechanisms by which chemotherapy stress induces these maladaptive states in stem cells, thereby impairing cardiac regeneration, are not well-defined. We hypothesized that human induced pluripotent stem cells (hiPSCs) could serve as a renewable model to track how early stress persists into differentiated states. We developed a novel Dox-adaptive hiPSC (D50-hiPSC) model by treating hiPSCs with 50 nM Dox for 2 hours, followed by five passages to stabilize adaptive responses. This system recapitulates acquired adaptation—defined as the ability to survive sub-cytotoxic stress while retaining pluripotency and differentiation potential—and enables direct tracking of how stem cell stress impacts lineage decisions. Morphometric analysis by deep-learning segmentation revealed a maladaptive 'Size-Structure Signature,' consistent with a failure to achieve a critical cell size (a 'Sizer' defect) required for normal maturation. Compared to controls, Dox-treated cells displayed significantly smaller area (1384 ± 477 vs. 1963 ± 657 μm^2, p = 0.0001) and higher nuclear-to-cytoplasmic ratio (0.66 ± 0.24 vs. 0.50 ± 0.17, p = 0.0001), indicating remodeling into smaller, rounder cells with a relatively enlarged nuclear compartment. Despite these changes, D50-hiPSCs retained pluripotency, with upregulation of stemness markers including SSEA4 (~2.8-fold), Nanog (~1.8-fold), and Oct4 (~1.6-fold). Functionally, cardiomyocytes derived from D50-hiPSCs at day 7 post-differentiation exhibited impaired contractile kinetics, with significantly prolonged time-to-peak contraction (1.06 ± 0.20 vs. 0.85 ± 0.05 s, p = 0.0001) and slowed relaxation, with delayed 30% decay (1.51 ± 0.13 vs. 1.17 ± 0.06 s, p = 0.0001) and 90% decay (2.03 ± 0.21 vs. 1.81 ± 0.27 s, p = 0.0001), while beat frequency remained unchanged. In conclusion, we discovered that Dox-adapted hiPSCs exhibit a distinct 'Size-Structure Signature' characterized by: (1) a reduction in cell size and altered nuclear morphology; (2) a concomitant reinforcement of the pluripotent state; which (3) functionally transmits to derived cardiomyocytes as impaired contractile maturation. Our novel Dox-adaptive hiPSC model provides a powerful platform to mechanistically dissect how chemotherapy stress reshapes stem cell fate and propagates

functional deficits across lineages. This work advances our understanding of cardiotoxicity and provides a new paradigm for studying chemotherapy-induced tissue toxicities.

MS1805

Cytoskeletal Control of Rosette Remodeling and Lumen Formation in the Zebrafish Left-Right Organizer

*Yan Wu[1], Yiling Lan[2], Miriam Allred[3], Heidi Hehnly[1]; syracuse university[1], Syracuse Unviersity[2], University of Arizona[3]

Left-right asymmetry in vertebrate embryos is initiated by the Left-Right Organizer (LRO), with the zebrafish Kupffer's Vesicle (KV) serving as a model to dissect the cellular mechanisms of symmetry breaking. During early KV formation, dorsal forerunner cells (DFCs) interface with the enveloping layer (EVL) via transient tight junctions and organize into small rosettes. These DFC-EVL contacts are lost as DFCs transition to forming a contiguous cyst of cells, coinciding with the onset of lumenogenesis. Prior to this transition, we observe that microtubule (MT) bundles that arise from non-mitotic origins at sites preceding nascent ZO-1 puncta at DFC-EVL interfaces. Live imaging and targeted ablation reveal that LRO cells divide and orient their cytokinetic bridges, with associated MT bundles, toward ZO-1–positive sites that promote actin recruitment and rosette expansion. Disruption of cell division or spindle orientation impairs rosette coalescence and lumen formation. These findings highlight a temporally restricted role for MT architecture in spatially patterning ZO-1 and actin-enriched rosettes, which ultimately fuse to form the KV lumen. Our work reveals how cytoskeletal organization and division history govern epithelial morphogenesis at the earliest stages of LRO development.

MS1806

Gene Expression Changes Induced by MAHS Expression in Human Cells Include Stress-Associated Genes

*Sara Nafar[1], Daniel Bustamante[1], Keyanna Pinzon[1], Joshua Morgan[1]; University of California, Riverside[1]

Tardigrades are microscopic animals known for their ability to survive extreme conditions, including but not limited to extreme temperatures, radiation, and desiccation. This resilience is incompletely understood but has been attributed in part to genes encoding intrinsically disordered proteins, such as the mitochondrial abundant heat-soluble (MAHS) protein. Although transgene expression of MAHS has been shown to increase stress tolerance of HEp-2 cells and adipose-derived stem cells following various stressors, the mechanisms by which it reprograms cellular states to confer resilience remain unclear. To address this, we used lentiviral transduction to generate U87 cells stably expressing MAHS and performed triplicate RNA sequencing followed by gene ontology (GO) analysis. MAHS expressing cells had 754 upregulated and 221 downregulated genes with respect to control cells (expressing AcGFP). Key findings of the enrichment analysis include differentially expressed genes clustered in transcriptional regulation, including GO:0000978. Notably, this enrichment was largely driven by changes in Cys2(C2) His2(H2) zinc finger transcription factors. Given zinc fingers are implicated in neurological disorders in humans, upregulation of neurogenesis-related genes such as LRFN5 (log2FC: +2.4) and NEGR1 (log2FC: +2.8), suggests that MAHS-induced transcriptional changes may intersect with neuronal pathways. In addition to transcriptional regulators, we observed differential expressions of genes involved in mechanotransduction such as upregulation of integrins (ITGA1, ITGA2, ITGA6, ITGA10, ITGA11) and collagens (COL7A1, COL13A1, COL4A3, COL12A1). Data also shows upregulation of several G protein–coupled receptors (GPR1, GPR37, GPR135, GPR155), PIK3AP1, a catalyzer for PIP3 production as a lipid

second messenger, and several genes encoding calcium voltage-gated ion channels (CACNG7, CACNG8); Pointing to MAHS interfering with signal transduction pathways. Expectedly, several stress associated genes (E2F7, GAS1, SENS3) were also regulated in MAHS group. These findings suggest that MAHS expression, potentially mediated by zinc finger transcription factors, broadly reprograms cellular signaling and stress response in the mammalian context.

MS1803

HDL-bound S1P affects the subventricular niche and early neuropathological features of Alzheimer's disease

*Hee Kyung Jin[1], Jae-sung Bae[2]; Kyungpook Natl Unive[1], School of Medicine, Kyungpook National University[2]

Circulating blood factors are critical for homeostasis of the adult ventricular-subventricular (V-SVZ) and subgranular zones, which contain neural stem cells (NSCs) crucial for sustained neurogenesis. Circulating sphingosine-1-phosphate (S1P) bound to apolipoprotein M (ApoM), a principal component of high-density lipoproteins, is involved in various biological processes, but its role in neurogenic niches is poorly understood. Herein, using Apom-/- mice, we show that blood ApoM-S1P deficiency impairs the SVZ-NSC pool, neurogenesis, ependymal cell polarity, and cerebrospinal fluid flow, leading to olfactory dysfunction and ventricular enlargement, early neuropathological features of Alzheimer’s disease (AD). Enhancing the complex significantly rescues these defects by activating S1P1 receptor signaling in SVZ-NSCs. Consistently, blood ApoM-S1P levels are reduced in early AD patients and correlate with olfactory deficits and ventricular enlargement. Similar abnormalities are recapitulated in young APP/PS1 mice and reversed by restoring blood ApoM-S1P levels. Thus, these data reveal pathogenic mechanisms underlying early neuropathological features of AD and identify the blood ApoM-S1P complex as a potential diagnostic and therapeutic target.

MS1801

The fate of pyruvate: metabolic regulation of cellular homeostasis

*Ashish Toshniwal[1]; University of Utah[1]

Cell growth requires a delicate balance between energy production, biosynthesis, and redox homeostasis. Our study identifies pyruvate metabolism as a central decision point that integrates these demands. We demonstrate that the fate of pyruvate—whether directed toward mitochondrial oxidation or alternative metabolic pathways—directly influences the cellular redox potential. This control over $NADH/NAD^+$ balance, in turn, dictates the capacity of cells to sustain growth. By establishing a mechanistic link between pyruvate utilization and proliferation, our work advances understanding of how metabolic networks are wired to support cellular fitness. These findings unify previously separate views of pyruvate as a fuel, biosynthetic precursor, and redox regulator, thereby reframing it as a pivotal node in controlling size and growth. Because dysregulated growth underlies cancer, regenerative responses, and tissue degeneration, our results open new avenues to manipulate pyruvate metabolism therapeutically. More broadly, this work illustrates how altering the flow of central metabolites can reprogram fundamental cell behaviors.

Motility, Mechanics and Migration

SY402
Self-steering in Chemotaxis
*Robert Insall[1]; University College London[1]

Cells are remarkably good at navigating accurately. Immune cells chase pathogens and find wounds with great precision, and cell movements in embryogenesis are of necessity accurate and reproducible. However, most cells' steering needs and local environments are too complex to be explainable by simple chemotaxis up pre-existing gradients. Our work shows that many of these cells — including immune cells like macrophages and dendritic cells, and cancers — generate the very gradients they follow. They consume or degrade attractants such as lipids, complement components and chemokines, creating local asymmetries that they then steer up. This self-generated chemotaxis allows cell populations to distribute efficiently through tissues, avoid overcrowding, and adapt to changing environments. More recently we have shown that steering by secreting self-attractants - chemoattractants that attract similar neighbours - can enhance the accuracy and range of chemotaxis. Using a combination of computational modelling, live imaging, and microfluidics, we reveal how feedback between receptor signalling, attractant production and destruction, and the motility machiney enables cells to define their own direction. Self-steering emerges as a general principle that unifies immune and cancer cell migration, explaining how cells remain oriented even when external gradients collapse or reverse. Understanding this self-driven steering reframes chemotaxis from a passive response to an emergent, self-organized process — one that offers new explanations for cell migration in immunity, inflammation, and metastasis.

SY401
The Spatiotemporal Coordination of Bacterial Cell Wall Constriction During Division
*Jie Xiao[1]; Johns Hopkins University[1]

My laboratory focuses on developing novel single-molecule imaging tools in live cells to probe various aspects of microbial cellular processes. We are broadly interested in understanding how the molecular constituents of bacterial cellular processes are spatially organized and what essential functions such an organization conveys. In this talk, I will discuss our recent work on the structure, function, and dynamics of bacterial cell division machinery. Using single-molecule imaging in live E. coli cells, we illustrated the organization, function, and dynamics of the bacterial cytokinesis apparatus. We show that during bacterial cell division, three spatial tracks, formed by the cytoskeletal protein FtsZ, active cell wall enzymes, and a special cell wall intermediates, coordinate the spatiotemporal progression of cell wall constriction, enabling robust septum morphogenesis and cell division. Our work opens new directions to study the precise spatial coordination and regulation of the large ensemble of cell division proteins.

Multimodal Imaging and AI in Cell Biology

SY102
Structure and Dynamics in Neuronal Synapse
*Guoqiang Bi[1]; University of Science and Technology of China[1]

Synaptic transmission, the fundamental operation of neural information processing, depends on the coordinated action of organelles such as synaptic vesicles and protein complexes such as neurotransmitter receptors and scaffolding molecules. Yet, exactly how these components are organized in situ and how their configurations change during synaptic transmission remains unclear. Using cryo-electron tomography (cryoET) and correlative light-electron microscopy (CLEM), we visualize the three-dimensional architecture of organelles and macromolecular assemblies in intact synapses of cultured hippocampal neurons in near-native states, revealing a “mesophasic” organization of GABAA receptors and scaffolding molecules in inhibitory synapses. We further develop a “flash-plunge-freeze” method coupled with time-resolved optogenetic stimulation to capture the dynamic process of synaptic vesicle exocytosis, and resolve a series of rapid immediate states of vesicle-plasma membrane interaction that point to a “kiss-shrink-run” mechanism of vesicle exocytosis and recycling.

SY101
Tau-Mediated Microtubule Organization Impacts Neurite Outgrowth and Axonal Transport in Dorsal Root Ganglion (DRG) Neurons
*Lisha Singh[1], Michael Holtsmannpötter[1], Laryssa Alves Borba[1], Roland Brandt[1]; Osnabrueck University[1]

In axons from neurons, microtubules form a dynamic and spatially regulated network that is crucial for structural integrity, regeneration, and long-distance transport. In peripheral neurons such as DRGs, the microtubule-associated protein - tau is expressed in different isoforms, including a particularly long isoform called “big tau”. However, how tau affects microtubule organization, supports neurite outgrowth, and influences axonal transport remains unclear. We used DNA-PAINT, a super-resolution microscopy technique, followed by algorithmic image analysis to investigate microtubule organization in cultured DRG neurons from one and two-year-old control and tau-knockout (TauKO) mice. We found that in TauKO neurons, mean microtubule length was decreased in an age-dependent manner by 30 – 50% compared to controls. In addition, we observed that microtubule straightness was significantly reduced in DRG neurons from TauKO mice at both ages. To determine the functional consequences of these structural alterations, we examined axonal regeneration and mitochondrial transport in TauKO and control DRG cultures. Surprisingly, TauKO neurons exhibited significantly longer axonal processes, indicating increased axonal sprouting and less dynamic mitochondrial movement. To investigate the proteome changes caused by the absence of tau, we performed mass spectrometry of cultures prepared from one-year old mice. We did not observe differential expression of other microtubule-associated proteins, but identified the kinesin motor Kif1b as significantly upregulated in TauKO DRG neurons. Our study demonstrates that DNA-PAINT followed by algorithmic image analysis is a powerful approach for quantitatively assessing the organization of densely packed microtubule in cultured peripheral neurons. The data suggests that big tau is a regulator of microtubule architecture and axonal regeneration in DRG neurons and influences mitochondrial transport.

Opening keynote: The Evolving Cell: From Ancient Origins to Modern Defenses
Buzz Baum and Zhijian Chen

K101
53BP1 condensates function as bioreactors for NHEJ directed DNA repair and insulators to determine pathway choice
*Mikael Garabedian[1]; University of Pennsylvania[1]

Genomic integrity requires efficient resolution of DNA damage. Non-homologous end joining (NHEJ) is the primary mechanism of DNA double strand break (DSB) repair in mammalian cells and is mediated by 53BP1, a tumor suppressor involved in preventing DSB end-resection and homologous recombination. NHEJ repair foci form following DSB formation, however how mesoscale assembly occurs and whether 53BP1 is the driver of this process are unknown. Also, despite knowledge of the identify of key pathway molecules, the specific functions of mesoscale repair condensates in DNA repair and pathway selectivity are unknown. To address these gaps, we determined the minimal domain of 53BP1 sufficient for phase separation in vitro and identified the key residues that governing its condensation. Utilizing a separation-of-function mutant, we demonstrate that 53BP1and its protein condensation is the core driver of NHEJ foci formation. 53BP1 condensates function as bioreactor compartments, increasing the effective concentration of substrates near double strand breaks and are essential for efficient DNA damage resolution. Additionally, we show that 53BP1 condensates function as insulators around DSB sites to prevent broken end processing and direct repair pathway selectivity. Collectively our work reveals a specialized compartment for DNA repair through the spatial clustering of 53BP1 molecules into repair foci essential to maintain genome integrity.

K102
The Lipid Chaperone FABP5 is a Druggable Driver of Metastatic Prostate Cancer
*Manojit Swamynathan[1], Eileen Cheng[1], Siyang Long[1], Diya Ghosh[1], Samantha Sanchez[1], Lloyd Trotman[1]; Cold Spring Harbor Laboratory[1]

Large scale genome sequencing efforts have demonstrated that lethal, therapy-resistant, metastatic prostate cancer (PC) is enriched for loss of function alterations in PTEN and TP53 tumor suppressor genes. Unfortunately, patients with metastatic PC do not respond to PI 3-Kinase pathway inhibitors and invariably present with lethal disease that is resistant to androgen receptor blockade and taxol-based chemotherapy. This underscores the impending need for new targetable avenues in the metastatic setting. Altered metabolism is a cancer vulnerability, and several metabolic pathways have been shown to promote PC. Through a cross-species cancer genomics and transcriptomics analysis, we nominate the lipid carrier protein FABP5 as an actionable target in PC. Oncogenic signaling pathways that drive prostate tumor progression independently converge on FABP5 via transcriptional activation and post-translational regulation. CRISPR-based genetic perturbation of FABP5 hampers proliferation of human and mouse-derived prostate cancer cell lines that highly express the gene. Pharmacological screening in isogenic Pten/Trp53-deficient cells that have or lack FABP5 revealed marked sensitization to ferroptosis inducers upon Fabp5 loss, thus highlighting a gatekeeping role for this lipid carrier in limiting ROS in membrane compartments. This led us to further validate this dependency in

RapidCaP, our autochthonous mouse model of metastatic prostate cancer. We delivered Cre-expressing lentivirus into the prostates of Ptenfl/fl;Trp53fl/fl or Fabp5fl/fl;Ptenfl/fl;Trp53fl/fl mice to evaluate the functional consequences of Fabp5 ablation on Pten/Trp53 deficient PC initiation, progression, and overall survival of tumor-bearing animals. Fabp5 loss significantly delays prostate tumor progression, blunts metastatic colonization, and extends the lifespan of prostate tumor-bearing mice. Together, our findings reveal FABP5 as a druggable vulnerability that prostate cancer cells rely on to evade ferroptosis during metastatic tumor progression.

Physical Biology of Infection and Immunity

SG904
An automated imaging platform for quantitative antibody-dependent cellular phagocytosis
*Valentin Jaumouillé[1], Abdolrasol Khaef Panah[1], Damon Poburko[1]; Simon Fraser University[1]

Targeted therapies, such as monoclonal antibodies (mAbs) that bind to tumour-associated antigens have become a staple of many cancer treatment regiments. Although several therapeutic mechanisms have been proposed, compelling evidence supports the notion that treatment efficacy involves antibody-dependent cellular phagocytosis (ADCP) by macrophages. Phagocytosis is a specialized process reserved to “professional phagocytes” such as macrophages, whereby such cells ingest large particles (≥0.5 µm), aiding in tissue development, remodelling, and for immunity to infection. Combined with fluorescent markers, microscopy and flow cytometry have become hallmark techniques to visualize spatiotemporal signalling and quantify rates of phagocytosis. However, these methods have inherent limitations – microscopy experiments require lengthy data processing and are prone to observer bias, while flow cytometry does not reliably distinguish between fully engulfed target cells and mere cell-cell contacts with macrophages. To overcome these challenges, I developed an automated imaging platform on an open-source framework that uses a machine-learning-based segmentation model to quantify ADCP through long-term time-lapse imaging. This high-throughput, unbiased acquisition-to-analysis pipeline not only enables robust quantification of total phagocytic efficiency but also provides temporal insights into engulfment speed and phagocytosis rates over several hours. Furthermore, the platform can produce visual annotations of quantified data directly on imaging acquisitions for better visual interpretation. As such, this robust and quantitative image-based assay accelerates data analysis relative to manual quantification methods, providing a powerful tool to investigate how various signalling cascades, mutations and treatment conditions influence ADCP.

SG906
Antibodies disrupt bacterial adhesion by ligand mimicry and allosteric interference
*Kelli Hvorecny[1], Gianluca Interlandi[1], Tim Veth[1], Pavel Aprikian[1], Anna Manchenko[1], Veronika Tchesnokova[1], Miles Dickinson[1], Joel Quispe[1], Nicholas Riley[1], Rachel Klevit[1], Pearl Magala[1], Evgeni Sokurenko[1], Justin Kollman[1]; University of Washington[1]

A critical step in infections is the attachment of microorganisms to host cells using lectins that bind surface glycans, making lectins promising antimicrobial targets. Upon binding mannosylated glycans, FimH, an adhesin in E. coli, undergoes an allosteric transition from an inactive to an active conformation that under tension acts

as a catch-bond. Distinct monoclonal antibodies that alter FimH glycan binding are available, but the mechanisms of action remain unclear. Here, we use cryoEM paired with mass spectrometry, adhesion assays, and molecular dynamics simulations to determine the structure-function relationships underlying antibody-FimH binding. Our studies show four mechanisms of action, including an antibody containing a variable-loop glycan that mimics a FimH ligand and binds directly in the binding pocket of FimH. Hypervariable loop glycosylations occur in 15% of circulating immunoglobulin G antibodies, and while several hypotheses have been proposed to explain why they exist, none included the direct, specific engagement of the glycans with the target antigen. The antibody mAb475 presents a previously unreported, yet likely common, mechanism of interaction between antibodies and lectin domains via a glycan. Several B-cell malignancies are characterized by high-mannose or mixed type glycosylations on the hypervariable loops of Fabs produced from IgGs or B-cell receptors of these cells. Specifically, the glycosylated position on mAb475 has previously been identified on a Fab from the B-cell receptor of lymphocytes from a patient with diffuse large B-cell lymphoma. N-linked glycosylations on hypervariable loops have also been described in follicular lymphoma and Burkitt's lymphoma. Our data directly visualize the interaction between a glycosylated Fab and a bacterial lectin, supporting the hypothesis that microbial lectins may contribute to the progression of a subset of B-cell lymphomas. In addition, this work shows that high-mannose glycans on B-cell receptors may be selected during an infection by a microbe that uses mannose-binding lectins as part of its repertoire. This finding supports the hypothesis that FimH and other mannose-binding lectins trigger initial steps in the development of B-cell malignancies like diffuse large B-cell lymphomas that present with high-mannose glycans on B-cell receptors.

SG908
Bacteria Distinguish Between Local and Global Envelope Stress
*Enrique Rojas[1]; New York University[1]

Sensing of solid surfaces, like epithelia, is critical for bacterial pathogenesis. We discovered that a canonical Escherichia coli envelope stress pathway that is known to respond to various envelope-targeting antibiotics is also triggered by growth on surfaces. However, the transcriptional response of this pathway depends on the type of perturbation. We found that this dependence is mediated by a bistable genetic circuit that is tuned to distinguish between local stress (surface-sensing) and global stress (antibiotics). This result provides a new paradigm for surface-sensing during pathogenesis, and demonstrates how the smallest organisms on earth can specifically respond to local perturbations via a quintessentially prokaryotic mechanism.

SG901
Chromatin organization regulates plasma membrane tension and cell integrity independently of gene expression
*Aidan Cabral[1], Manasi Sawant[1], Minwoo Kang[1], Xie Liangqi[2], Hawa Thiam[1]; Stanford University[1], Cleveland Clinic Lerner Research Institute[2]

Primarily studied for its role in gene expression, chromatin organization is emerging as an important regulator of nuclear mechanics. Although the nucleus is in mechanical equilibrium with the cell, we do not know whether and how chromatin reorganization actively regulates the mechanical properties and downstream behaviors of cells. Here, we tested the hypothesis that as a dynamic crosslinked polymer, chromatin directly

impacts cell mechanics independently of transcription by studying NETosis: a transcription-independent immune process where chromatin decompacts and the plasma membrane (PM) ruptures. Using high resolution microscopy and ATAC-see, we found that chromatin accessibility progressively increases during NETosis suggesting that chromatin binding proteins (CBPs) dissociate from chromatin during NETosis. To determine the identity and dynamics of these dissociated CBPs, we used fluorescent-recovery after photobleaching to measure the mobility and localization of the linker histone H1, the nucleosomal histone H3 and the heterochromatin binding protein HP1α. We found that the mobile fraction of H1 increases during NETosis while HP1α diffuses faster outside of the nucleus suggesting that it becomes a cytosolic osmolyte. Using optical tweezers, we found that chromatin decompaction during NETosis increases PM tension, independently of the cytoskeleton. In non-NETing U2OS cells, we found a similar PM tension increase upon induced chromatin decompaction and disrupted cytoskeleton, suggesting that chromatin is a global mechanical integrator. We theorized that chromatin might regulate PM tension by generating cellular osmotic pressure. Lattice light sheet microcopy experiments indicate a 10% increase in volume during NETosis, supporting our theory. Our findings reveal a novel non-genetic role of chromatin in cellular biophysics: regulating cell volume, PM tension, and thus, overall cell mechanics. Considering the critical role of cell mechanics in biological processes such as cell migration, proliferation and pathogen killing, our work broadens our understanding of how chromatin regulates cell physiology and pathology.

SG902

In Vitro Generated Neutrophils Reveal High Deformability is an Emergent Property

*Allen Yesin[1], Regina Sanchez-Flores[1], Hawa Thiam[1]; Stanford University[1]

Neutrophils must deform their nuclei up to < 10% their cross-sectional area to migrate in vivo, yet when this ability emerges remains unresolved. Cancer-derived models fail to reproduce the multilobulated nuclear morphology and envelope composition of primary neutrophils. Here, we developed a platform to differentiate human neutrophils from CD34+ stem cells and investigate when high nuclear deformability emerges in neutrophils. We cultured CD34+ stem cells with growth factors and cytokines to differentiate them into functional neutrophils (dCD34-neutrophils) in 14 days with 76.5 ± 6.3-fold expansion. We showed that dCD34-neutrophils expressed canonical human neutrophil markers (99% CD15+, 78% CD11b+), generated reactive oxygen species, and underwent NETosis comparable to primary human neutrophils (41.3%). Using quantitative microscopy, we showed that nuclear circularity, an indicator of multilobulation, decreased from 0.84 to 0.77 as cells transition from early progenitors (D3) to dCD34-neutrophils (D16). Coincidently, nuclear envelope composition changed: Lamin A/C and B1 decreased by 1.6- and 2.7-fold, respectively; Lamin B Receptor (LBR) increased by 7.3-fold, inversely correlating with nuclear circularity in dCD34-neutrophils. To determine if nuclear remodeling correlates with deformability, we measured the migration speed of progenitor and dCD34-neutrophils using microfabricated channels with and without constrictions. We found that migration speed increases from 7.7 ± 3.1 μm/min in early progenitor to 14.1 ± 4.9 μm/min in late progenitor cells. In 2 μm constrictions, progenitors slowed markedly (9.9 ± 3.9 before vs 5.5 ± 2.6 inside), whereas dCD34-neutrophils maintained high speed (11.4 ± 6.2 before vs 14.4 ± 7.6 inside). After constriction, progenitors remained slow (5.9 ± 2.6), while dCD34-neutrophils kept high speed (14.3 ± 6.2). Together, our data show that high nuclear deformability emerges late in human neutrophil differentiation and correlates with LBR upregulation and Lamin A/C and B1 reduction. dCD34-neutrophils provides an accessible platform to dissect how nuclear envelope composition regulates the biophysical mechanisms of human neutrophil functions in health and disease.

SG907
Molecular thresholds of CAR T cell activation
*Shalini Low-Nam[1], Kevin Scrudders[2], Suriya Selvarajan[2], Philip Low[2]; Purdue[1], Purdue University[2]

T cells expressing engineered chimeric antigen receptors (CARs) consolidate key components of T cell receptor (TCR) triggering machinery into a single transmembrane receptor capable of recognizing tumor markers and initiating signals for target elimination. These programmable therapeutics, while potent, have had limited success and often exhibit toxicity from hyperactivation of the immune response. Whereas activation of native T cells has been extensively studied, the mechanistic determinants for threshold setting in CAR T cells are still poorly understood. Signaling onset, at the junction between the CAR T cell and the tumor surface, may be tuned by spatiotemporal, mechanical, and chemical parameters. Here, we focus on a CAR T cell that recognizes tumors overexpressing the folate receptor (FOLR1) via a bivalent bridging ligand. An in vitro cell:cell killing assay using low CAR T cell:target cell ratios showed that small numbers of binding interactions by CAR T cells are sufficient to induce target cell death. Additionally, CAR T cells experiencing high environmental stiffness are most efficient in generating killing outcomes. To measure single cell activation, we use in vitro reconstitution-based assays to map CAR T cell inputs to cellular activation and cytotoxic responses with single molecule resolution. We monitor the collection of binding interactions that culminate in polarization of lytic granules that are trafficked to the intercellular junction and may undergo exocytosis to release cytolytic factors. We find that, surprisingly, CAR T cells can mobilize cytotoxic responses to a small number of antigenic binding events, suggesting a different molecular activation threshold than previously appreciated. Taken together, our data argue that CAR T cell activation is tuned by mechanochemical features of antigen binding and may suggest actionable insights to improve the efficacy of these therapies.

SG905
Role of listeriolysin O and phospholipases C in Listeria monocytogenes intercellular protrusion dynamics, resolution, and autophagy avoidance
*Stéphanie Seveau[1]; Ohio State University[1]

Listeria monocytogenes is a facultative intracellular bacterium that infects a large diversity of host cells and directly spreads from cell to cell to avoid the extracellular immune defenses while disseminating within infected tissues and causing a systemic infection. In this work, we characterized in real time by fluorescence microscopy the host cell plasma membrane dynamics during L. monocytogenes cell-to-cell spread. The plasma membranes of donor and recipient cells were labeled with distinct fluorochromes and 3 dimensional movies were acquired for 7 hours to monitor fluorescent L. monocytogenes and establish the role its major secreted virulence factors listeriolysin O (LLO) and phospholipases C (PLCs) at each cell-to-cell spread stage. We characterized, for the first time, the dynamics of the canonical cell-to-cell spread pathway in which intercellular protrusions resolve into double-membrane vacuoles, followed by the successive disruption of donor and recipient membranes. We found that LLO controls protrusion resolution into a double-membrane vacuole and the disruption of both donor and recipient membranes, whereas the phospholipases C (PLCs) control donor membrane disruption and prevent bacterial entrapment into autophagosomes. Furthermore, we identified a second, more efficient “non-canonical” cell-to-cell spread pathway strictly requiring LLO/PLCs cooperation,

which resolves into a single-membrane vacuole that is rapidly disrupted. This study provides the first detailed analysis of membrane dynamics within intercellular protrusions and vacuoles, establishing an advanced model for L. monocytogenes cell-to-cell spread.

SG903
Target cell motility dictates the phagocytic outcome by initiating a mechanical tug-of-war
*Brandon Scott[1]; South Dakota School of Mines and Technology[1]

The integration of mechanical cues is critical for phagocytic decision-making. A key unresolved question is how macrophages choose between full engulfment (phagocytosis) and partial membrane removal (trogocytosis), a process that allows target cells to evade immune clearance by shedding therapeutic antibodies. We tested the hypothesis that this outcome is dictated by mechanical resistance arising from the target's own motility. To investigate this, we established a controllable model system using undifferentiated, non-motile HL-60 cells and their differentiated, motile (dHL60) counterparts, employing high-resolution 4D Lattice Lightsheet Microscopy to visualize their interactions with macrophages in real-time. Our 4D imaging revealed that macrophages successfully phagocytosed non-motile HL-60s by forming stable, symmetric phagocytic cups that progressed to complete engulfment. In stark contrast, engaging motile dHL60s led to disorganized and asymmetric membrane protrusions as macrophages failed to establish a stable foothold. Consequently, the outcome switched to trogocytosis, stripping the opsonizing antibody from the target surface while allowing the cell to survive and escape. Critically, pharmacologically arresting target cell motility with Latrunculin B was sufficient to completely restore the formation of stable phagocytic cups and rescue the engulfment phenotype. In conclusion, this work begins to establish a new mechanical framework for understanding immune evasion. Our results suggest that target cell motility can function as a physical defense against immune clearance, as the resulting mechanical disruption is sufficient to alter the phagocytic outcome. Understanding this physical 'tug-of-war' may provide new therapeutic avenues aimed at preventing immune escape in cancer and chronic infections.

Physical Biology of the Cytoplasm

SG506
Beyond Brownian motion: Statistical complexity in cytoplasmic diffusion
*Alexandros Papagiannakis[1], Oded Agam[2], Christine Jacobs-Wagner[1]; Stanford University[1], The Hebrew University of Jerusalem[2]

Despite lacking membrane-bound compartments, the cytoplasm of Escherichia coli (E. coli) is not homogeneous. It is shaped by phase separations between chromosomal DNA, actively translated mRNAs, and storage carbohydrates. This spatial heterogeneity is maintained by active processes such as genome-wide transcription and glycogen accumulation, which create cytoplasmic order out of equilibrium. Microrheology is commonly used for studying the physical properties of the cytoplasm and its complexity. The diffusion statistics of tracer particles are confounded by geometric confinement and cytoplasmic flows, but they are also prone to errors associated with the finite sampling of particle positions and their localization accuracy. In our study, we use the μNS particles to quantify cytoplasmic complexity in E. coli. By leveraging the variable particle sizes

(~50-150nm), restricting our analysis to long trajectories (>199 positions), examining multiple time scales, and comparing with simulated particle traces, we disentangle the biologically meaningful variability in cytoplasmic diffusion. This variability, which cannot be solely attributed to geometric confinement and methodological errors, increases during growth in transient environments. Currently we are studying the physiological relevance of this tracer particle diffusion variability, focusing on cellular adaptation and growth.

SG503

Determining mechanisms underlying nuclear size control in fission yeast

*Joël Lemière[1], Zhidong Tan[1], Fred Chang[1]; University of California, San Francisco[1]

The objective of this study was to test a physical model of nuclear size control. Precise regulation of nuclear size relative to cell size is a robust feature of cellular biology, yet the mechanisms governing nuclear size control remain poorly understood. We and others recently proposed a physical model in which the nuclear-to-cell volume ratio (N/C ratio) at steady state is set by a balance between colloid osmotic pressures generated by the numbers of osmotically active macromolecules within the nucleus and cytoplasm, and by nuclear envelope tension. In fission yeast, where envelope tension is minimal, the nucleus can be regarded as an osmometer whose size is primarily determined by intracellular osmotic forces. As a key test of this model, we increased osmotic pressure in a compartment-specific manner by massively expressing exogenous proteins targeted to either the nucleus or cytoplasm. Loading the nucleus with millions of copies of mCherry-GST-NLS proteins caused a striking, dose-dependent expansion of nuclear volume, elevating the N/C ratio up to four-fold. Conversely, expression of mCherry-GST-NES proteins in the cytoplasm reduced the N/C ratio in a dose-dependent fashion. As one control, equivalent high expression of mCherry in both compartments did not affect cell growth or nuclear size. These experimental data, which closely fit model predictions, provide significant quantitative support for this osmotic model for nuclear size and yield fundamental parameters for osmotic theory in living cells. Additionally, these perturbations altered the physical properties of the nucleoplasm. Expression of mCherry-GST-NLS in the nucleus significantly increased diffusion of 40nm-GEMs nanoparticles, consistent with nuclearexpansion and dilution of endogenous nuclear components. Conversely, mCherry-GST-NES expression in the cytoplasm reduced nuclear diffusivity. To test whether changes in macromolecular crowding affect nuclear condensate assembly, we observed consistent effects on Swi6/ HP1 heterochromatin loci and on the nucleolus. Together, this work establishes a tunable experimental strategy to manipulate the N/C ratio and physical properties of the nucleoplasm, providing a powerful tool to study how osmotic forces influence nuclear size control, molecular crowding, and nuclear organization.

SG505

Hag accumulation in the Bacillus subtilis cytoplasm slows Mesoscale Diffusion and limits Z-ring Condensation

*Katherine Andre[1], Jane Joncha[2], Abigail King[1], Hui Qiu[1], Reid Oshiro[2], Stephen Jacobson[2], Daniel Kearns[2], Alexandre Bisson[1]; Brandeis University[1], Indiana University[2]

Metabolically active bacterial cells have limits on macromolecule concentrations in the cytoplasm to avoid excessive crowding and impacts on diffusion. However, there is still little direct evidence to support the functional influence of cytoplasm homeostasis on large scale processes such as cell division. Oshiro et al., 2019 (PMID: 31113895) show that accumulation of the flagellin protein (hag) in the cytoplasm of Bacillus subtilis by

multiple orders of magnitude results in the misplacement of cell division away from the midcell position, rescued by hag deletion. Here, this system is utilized to show that intracellular accumulation of hag is a major contributor to asymmetric cell division using single-particle tracking of genetically encoded multimeric nanoparticles (GEMs) in live cells. In the hag accumulation mutant (++hag), diffusion of 20 nm GEMs is slowed, with significant rescue of mesoscale diffusion upon hag deletion. Hag was tagged with mNeonGreen (mNG) to show that it does not form localized aggregates in the cytoplasm, indicating that the cell experiences relatively homogeneous crowding throughout the cytoplasm. Furthermore, the crowding effect is shown to be non-specific to hag, where replacement of hag with mNG in ++hag also results in disruption of the precision of division site placement at the midcell position. ++hag shows significantly lower fluorescence of FtsZ localized at the Z-ring using super resolution microscopy. Additionally, speeds of FtsZ treadmilling are significantly lower, suggesting a lack of Z-ring condensation is primarily responsible for inefficiencies in cell division. Current work is exploring the observed positive correlation between cell size and the average GEM diffusion coefficient, which is lost in the hag accumulation mutant. Experiments using GEMs in a cell division incompetent strain which forms long multi-nucleoid filaments are underway. Preliminary results show that GEM diffusion in filaments remains similar to what is seen in divided cells. Since cytoplasm contents remain equivalent in each single cell sized section of the filament, this hints at a correlation between cell cycle and cytoplasm fluidity, where larger cells that tend to be closer to division have more fluid cytoplasms. This work reveals that hyper accumulation of a single protein can slow mesoscale diffusion in bacteria, connecting this to the efficiency of cell division and highlighting the importance of the biophysical properties of the cytoplasm to basic cell function.

SG507

Nuclear morphogenesis reflects both cell lineage and environmental context in 2D and 3D hiPS cell models

*Leigh Harris[1], Gokhan Dalgin[1], Julie Dixon[1], Christopher Frick[1], Chantelle Leveille[1], Antoine Borensztejn[1], Philip Garrison[1], Caroline Hookway[1], Peyton Lee[1], Saurabh Mogre[1], Benjamin Morris[1], Nivedita Nivedita[1], Ritvik Vasan[1], Tiffany Barszczewski[1], Sara; Allen Institute for Cell Science[1]

Nuclear size and shape are emergent properties shaped by many factors, including cell size and geometry, nuclear import and export, cytoskeletal and nuclear mechanics, and chromatin state. Additionally, because most cells have one nucleus, this structure is also a useful window into single cell behaviors such as movement and cell cycle duration. Because it is such a powerful readout of these diverse processes, we analyzed nuclear morphogenesis across multiple length- and timescales. Using spinning-disk confocal microscopy imaging and deep-learning 3D segmentation of the nuclear envelope in proliferating human iPSC colonies, we tracked nuclear morphology in >1000 cells for multiple days. Focusing on size and growth, we found that nuclei expanded rapidly at the beginning of the cell cycle, likely due to an import-mediated post-mitosis expansion, and then entered a slower growth phase. Across generations, nuclear starting size and growth duration were inherited, with nuclei born larger growing for less time before dividing, contributing to an average “adder” behavior at the population level. Growth rate, however, was not related to lineage or starting size. Rather, nuclear growth rate fluctuations were coordinated across multiple nuclei within local neighborhoods, implying a cell-extrinsic, context-dependent control of growth rate. To extend these quantitative approaches to a more physiologically relevant 3D context, we developed adherent hiPSC lumenoids. We found that overlaying 2D hiPSC colonies with dilute extracellular matrix causes colonies to close via a purse-string-like mechanism and form hollow, monolayered, pluripotent epithelial structures that remain anchored to the substrate. Lumenoids are often spherical, with a single central lumen, apical in/basal out polarity, and are surrounded by an hiPSC-

derived basement membrane shell. In contrast to the flattened nuclei observed when hiPS cells are grown as 2D colonies, lumenoid nuclei are rounded, and cell movements that are limited in 2D become faster and locally coherent as nuclei circulate around the edgeless epithelium. We are currently developing methods to track nuclear and cellular morphogenesis in 2D and 3D while also measuring the physical properties of cells to disentangle intrinsic and extrinsic influences and understand how single-cell processes give rise to emergent multicellular phenomena.

SG502

PKA Activity Modulates Cytoplasmic Density and Stress Resistance Through Storage Carbohydrate Production

*Marina Kunzi[1], Lorena Kronig[1], Martina Bonassera[1], Matthias Peter[1], Karsten Weis[1], Gabriel Neurohr[1]; ETH Zuerich - 35222255[1]

Cytoplasmic density and crowding conditions are tightly controlled and narrowly distributed within a given cell type. Deviations from this set point occur in response to various stresses, suggesting that cytoplasmic density is actively adjusted to protect the cell from stress. In budding yeast, different types of stress induce a canonical transcriptional response (environmental stress response, ESR), but how the ESR affects cell density is unclear because different types of stress can have opposite effects on cell density. A central ESR regulator is the cAMP activated kinase (PKA). Using refractive index tomography we show that inhibition of PKA in budding yeast leads to a 70% increase in dry mass density. This increase was not caused by increased protein concentration and depends on the presence of glucose in the growth medium. Mutating the stress-induced transcription factors Msn2/4, which are normally repressed by PKA, prevents cell density increase upon PKA inhibition. PKA inhibition activates the ESR and induces the production and accumulation of storage carbohydrates glycogen and trehalose, however abrogating their synthesis cannot entirely prevent the density increase. The increased density is accompanied by cytoplasmic restructuring, organelle deformation, increased cell wall thickness and decreased diffusion of genetically encoded nanoparticles. The dry mass density we observe in PKA inhibited cells rivals that of spores, which are highly resistant to starvation and heat stress. PKA inhibited cells exhibit improved resistance to extreme heat stress in an ESR and glucose dependent manner. Similarly, PKA inhibition confers glucose starvation resistance in an ESR and partially glycogen synthesis dependent manner. Curiously, interfering with glycogen degradation also increases starvation resistance, suggesting that glycogen does not only act as energy source but also promotes survival through a different mechanism. We hypothesize that the cytoprotective effect of glycogen and possibly trehalose is conferred by altered cytoplasmic properties. In summary, we show that PKA and the ESR play a central role in regulating cytoplasmic properties and survival during stress - at least in part by accumulating storage carbohydrates. In addition, we developed a reproducible and reversible method of creating extreme crowding conditions in living cells.

SG504

Single-molecule and super-resolution diffusivity quantification elucidates physical properties of the cytoplasm

*Ke Xu[1]; University of California, Berkeley[1]

The living cell creates a unique internal molecular environment that is challenging to characterize. We discuss our recent efforts to elucidate the physical properties of the cytoplasm via single-molecule and super-resolution diffusivity quantification. With the development of single-molecule displacement/diffusivity

mapping (SMdM), a novel super-resolution method, we map molecular diffusivity in living mammalian cells, extracted cytoplasm, and the solution phase with unprecedented spatial resolution and fidelity. We thus resolve nanoscale spatial heterogeneity in intracellular diffusion and elucidate their origins due to macromolecular crowding effects from cytoskeletal and chromatin ultrastructures. Moreover, we identify the protein net charge as an overlooked key determinant of intracellular diffusion, unveiling a striking trend in which the diffusion of positively charged proteins is biasedly impeded and recapitulating this behavior in vitro. Together, these results point to an overwhelmingly negatively charged macromolecular environment in the crowded cell that differentially interacts with molecules carrying different net charges and of different sizes.

SG501
The role of cell ionic homeostasis in regulating cell mass density and cell membrane voltage
*Sean Sun[1], Qin Ni[1], Zhuoxu Ge[1], Jinyu Fu[1]; Johns Hopkins University[1]

Cells utilize ion channels and exchangers to maintain the ionic composition of the cytoplasm, and in the process, regulate cytoplasmic osmolarity, water content, and membrane voltage. The water content of the cytoplasm, which is cell volume, also defines the concentration of all proteins, or the cell mass density (CMD). To investigate this active control mechanism that impacts major physical properties of the cytoplasm, we apply perturbations such as osmotic shock. We find that while the cell volume and cell mass can show complex dynamics post shock, the CMD regulation is prioritized through a straightforward monotonic recovery in 48 hrs. The sodium/hydrogen exchanger (NHE) plays a critical role in CMD maintenance by simultaneously affecting the rate of cell volume increase and the rate of cell protein synthesis. Nucleoplasm–cytoplasm transport is also affected by CMD and is potentially a negative feedback on CMD and protein synthesis. Beyond CMD, ionic homeostasis also influences the electrical state of the cell. We find that the regulation of transmembrane voltage involves a broader set of mechanisms, where ion exchangers alone cannot account for the observed dynamics. Anionic lipids, whose transport is influenced by NHE and the cytoskeleton, play a major role in determining the membrane voltage and cell polarization. Taken together, we find that fundamental cell properties are shaped by ionic homeostasis, enabling cells to preserve growth and proliferation under environmental perturbations.

Physical Cell Biology from Molecules to Organisms

MS209
A self-limiting positive feedback loop ensures robust inner ear canal morphogenesis
*Yusuke Mori[1], Anne Belle Cheney[1], Akankshi Munjal[1]; Duke University, School of Medicine[1]

Successful organ development requires tissues to undergo reproducible shape changes while buffering developmental noise. The mechanisms that ensure developmental robustness remain poorly understood. During zebrafish inner ear development, the semicircular canals form through sequential steps of epithelial budding, extension, and fusion, which must occur with precise timing and positioning. Our recent work demonstrated that a few patterned epithelial cells initiate local hyaluronic acid (HA) synthesis, creating an ECM that osmotically swells to drive initial bud formation. However, how this small initial signal is amplified and coordinated across the tissue to generate the complex three-dimensional canal architecture remains unclear.

Using quantitative imaging of a Yap knock-in reporter, we revealed that HA-driven ECM expansion mechanically activates Yap in budding cells, which is required for proper bud extension. Single-cell RNA sequencing identified the growth factor ccn1l1, a Yap target, in canal-forming cells. Using quantitative fluorescent in situ hybridization (HCR-FISH), we found HA pressure significantly amplifies ccn1l1 expression via Yap mechanotransduction during bud extension. Functionally, ccn1l1 promoted local HA synthesis for ECM expansion, establishing a self-amplifying feedback loop. In addition, live imaging and theoretical modeling further showed that this feedback drives non-linear ECM expansion and sustains bud growth by mechanically recruiting and stretching neighboring cells. These recruited cells upregulated the Yap–ccn1l1 circuit, further reinforcing the loop. Significantly, disrupting this feedback by inhibiting Yap or Ccn1l1 linearized growth kinetics, slowed morphogenesis, and prevented fusion, whereas mild perturbations were buffered, allowing buds to ultimately fuse successfully. Subsequently, bud fusion itself terminates the positive loop through Gpr126–cAMP–PKA–CREB signaling, which suppresses ccn1l1 expression. Supporting this model, gpr126 mutants that fail to fuse showed persistent ccn1l1 expression and runaway bud growth with continued non-linear ECM expansion. Our findings reveal a general principle in which positive feedback drives morphogenetic transitions, while the morphological outcome provides the termination signal. This mechanochemical circuit represents a fundamental solution to the paradox of using potentially unstable positive feedback for reliable developmental outcomes.

MS203

Adhesion-Cortical Flow Coupling Drives Spinning Cytokinesis to Establish Embryonic Chirality

*Yuxuan Rain Xiong[1], Marcus Lai[1], Eric Cytrynbaum[1], Kenji Sugioka[1]; The University of British Columbia[1]

Chirality is one of the fundamental properties of living systems, wherein the external and internal body structures are asymmetric and cannot be superimposed on their mirror images. In animals, tissue- and organismal-scale chirality emerge during morphogenesis, termed chiral morphogenesis. In frogs, snails, and Caenorhabditis elegans, previous studies have proposed that asymmetric movement of the cell cortex during early embryonic divisions, termed chiral cortical flow, is regulating chiral morphogenesis. However, the link between chiral cortical flow and chiral morphogenesis has remained correlational. Here, we found that adhesion-cortical flow coupling at the cell-cell interface drives cellular rotation in early C. elegans embryos. Embryonic chirality in C. elegans is specified during cytokinesis of the ABa and ABp cells at the 4-cell stage. The division axes of these cells are orthogonal to the anterior-posterior axis until early anaphase, then undergo a clockwise tilt during cytokinesis. By isolating the individual ABa/p cell, we found that both chiral cortical flow and division axis tilt require neighboring cells. When cultured on artificial adhesive substrates, both phenomena were induced, indicating a crucial role of adhesion during chiral morphogenesis. Consistently, disruption of major cell adhesion proteins cadherin and L1CAM led to defects in embryonic chirality in vivo. Together, we show that adhesion-cortical flow coupling is central to establish embryonic chirality. Interestingly, embryonic chirality is also characterized by the chiral arrangement among the daughter cells of ABa and ABp after cytokinesis, where ABar is in contact with ABpl while ABal is separated from ABpr. However, theoretically, such chiral arrangement cannot be achieved by simple rotation alone. We found that extracellular matrix, such as the embryo's eggshell, is essential for chiral morphogenesis. Indeed, mathematical simulation confirms that steric hinderance imposed by the eggshell, together with cellular rotation and adhesion, can recapitulate the chiral cellular arrangement observed in vivo. By combining biomimetic model, in vitro assays, and mathematical simulations, we demonstrate that cellular rotation and deformation as the primary and secondary mechanisms leading to embryonic chirality. Our study establishes the interplay between cortical

flow, adhesion, and extracellular confinement as the key mechanism in regulating chiral morphogenesis, which is pivotal in shaping the embryonic body plan.

MS207

Contractile dampening of tissue wetting underlies embryo implantation defects in aged females

*Kate Cavanaugh[1], Sneha Rao[4], Elysse Phillips[4], MJ Franco-Oñate[3], Patrick oakes[2], Ricard Alert[3], Diana Laird[4], Orion Weiner[4]; University of California San Francisco[1], Loyola University Chicago[2], Max Planck[3], University of California, San Francisco[4]

Female fertility rapidly declines after the age of 35, with high rates of spontaneous abortions around the time of embryo implantation. With the average age of childbirth globally increasing, age-related infertility represents a significant societal burden. Implantation, mediated by the primitive placental Trophectoderm lineage, is the culmination of a morphogenetic continuum that relies on the early coupling of cytoskeletal mechanics and cell fates. However, we fundamentally lack insight into how maternal age alters this morphogenetic continuum and the embryo's capacity for uterine implantation. Here, we established a mouse model of reproductive aging that recapitulates women's age-related infertility. We leveraged advanced microscopy, classical experimental embryology, synthetic reductionist approaches, and biophysical assays to test how advanced maternal age alters the Trophectoderm tissue output implantation function. Our in vitro implantation assays revealed that maternally aged embryos exhibited reduced trophectoderm spread area than young maternal control embryos. By modeling these data as an active wetting phenomenon, we showed this aged phenotype is due to heightened embryonic contractility and confirmed this by measuring increased Trophectoderm strain energy per area via Traction Force Microscopy. Our theory predicted that, prior to implantation and embryonic wetting, increased blastocyst contractility alone induces less implanting Trophectoderm spread area. Indeed, we can artificially "age" embryos from younger mothers by ectopically increasing contractility via RhoA activity to phenocopy reductions in Trophectoderm spread area in vitro. Aged pre-implantation blastocysts and hypercontractile young embryos further showed increased cell-cell junctional tension and junctional NMIIB, with altered lineage ratios and reduced Trophectoderm cell counts. These data are consistent with hyperactive mechanics and thus defective coupling to cell fates. We further pinpointed the source of this mechanical defect to the 8-cell stage, during which the window of the first Trophectoderm cell fate commitment is accelerated. Here, using high-resolution light-sheet imaging over the entirety of preimplantation development, we found that aged embryonic surface tension rapidly increases, and cell-cell adhesion activation accelerates over the 8-cell stage. This acceleration in developmental tempo is due to altered RhoA-mediated actin dynamics. Together these data reveal a previously undiscovered core mechanical defect emerging in embryos with advanced maternal age. This work highlights the need for integrative approaches in reproductive health, and further shed light on the emerging biophysical defects underlying reproductive aging.

MS205

Epigenetic Remodeling Stores Mechanical Memory and Stabilizes Gene Regulation

*Rohit Joshi[1], Vinayak Vinayak[1], Vivek Shenoy[2]; University of Pennsylvania[1], School of Engineering and Applied Science, University of Pennsylvania[2]

Cells are constantly exposed to physical forces during development, regeneration, and disease, yet how they retain a memory of past mechanical experiences and translate transient inputs into lasting molecular and phenotypic states remains unclear. Here we show that mechanical priming imprints persistent epigenetic and gene-regulatory programs in human mesenchymal stem cells. Cells cultured on physiologically soft or stiff substrates for defined durations retained substrate-dependent morphology and cytoskeletal organization even after removal from the priming environment, establishing a phenotypic signature of mechanical memory. This persistence was accompanied by progressive changes in histone modifications: active marks captured through global acetylation and H3k27ac, while the repressive mark H3K9me3 evolved from transient shifts after short priming to semi-stable patterns with prolonged priming. Super-resolution imaging revealed correlated remodeling of nanoscale chromatin domains and lamina-associated regions, indicating that nuclear architecture encodes the duration of mechanical exposure. Perturbation experiments showed that mechanotransduction pathways are required for memory formation, whereas epigenetic inhibition erases established memory, underscoring complementary roles for cytoskeletal force transmission and chromatin regulation. Transcriptomic profiling further demonstrated reinforcement of gene regulatory networks, with cell–matrix signaling and epigenetic read/write programs becoming progressively stabilized under longer priming. To unify these findings, we developed a mesoscale mechanotransduction–chromatin model that describes a self-reinforcing axis linking mechanical inputs to chromatin remodeling and transcriptional stability. Together, these results establish the epigenome and chromatin architecture as central repositories of mechanical memory and reveal a general principle by which cells record their mechanical history, with broad implications for development, diseases such as cancer and fibrosis, and regenerative medicine.

MS206
Limits of Cell and Nuclear Scaling in a Remodeling Epithelium
*Liam Russell[1], Rashmi Budhathoki[1], Dinah Loerke[1], Todd Blankenship[1]; University of Denver[1]

During epithelial remodeling, cell-level behaviors must be tightly coordinated to ensure successful formation of complex macroscopic topologies without sacrificing the tissue's necessary barrier function. For this reason, epithelia take on solid- or fluid-like characteristics depending on developmental constraints in a regulated and predictable manner. In other soft matter lattice systems, like foams and colloidal glasses, the relative size of substituent components determine the mechanical state of the material at large — to this end, we examined how the relative sizes of cells and nuclei impact tissue mechanics. Here, we assessed cell shape dynamics and convergent extension movements in Drosophila embryos that have double the cell density of wild-type tissues. Packing constraints in these mh mutant epithelia generate cells that, while normal in height, have halved cross-sectional areas, producing a dramatically altered 3D aspect ratio. We next examined how these altered cell dimensions impact the largest organelle in the cell, the nucleus. Interestingly, nuclear volumes scaled non-linearly with cell volumes, and also adopted generally rounder shapes and shorter heights along the apical-basal axis. This suggests a fundamental difference between nucleus and cell scaling, where the mechanically stiff nucleus is more limited in its ability to deform than the much softer cell. These changes in nuclear dimensions create tightly packed nuclei that exhibit characteristics of a jammed epithelium. Indeed, tissue fluidity is reduced, and the cell shape changes necessary for intercalation are inhibited. Thus, unlike in colloidal models, where smaller-sized grains generate lower viscosity materials, we have found that tissues with smaller cells and nuclei exhibit more solid-like qualities than control embryos. Finally, our data indicates that the actomyosin networks that mediate force-generation are insensitive to these smaller cell dimensions, and the biochemical pathways that direct contractility are still capable of generating oscillatory amplitudes and

frequencies similar to those found in control embryos. This suggests a fundamental unlinking of these molecular pathways from cell and cytoplasmic volume. Thus, despite the similar molecular recruitment of contractile proteins, tissue fluidity is deeply impacted in mh embryos with reduced cell and nuclear sizes, potentially suggesting that development has fine-tuned epithelial dimensions to work at time-scales compatible with embryonic requirements.

MS201

Linking metabolic irregularities to neurodegenerative phenotypes in AOA2: Insights from Senataxin knockout cells

*Judith Fishburn[1], Julian Flores[1], Steven Chen[1], Megan Wong[1], Jacqueline Barlow[1]; University of California, Davis[1]

Ataxia with oculomotor apraxia type 2 (AOA2) is a rare neurodegenerative disease that has no cure and virtually no therapeutic options for patients. This disease is caused by mutations in the gene Senataxin. Senataxin is a helicase responsible for resolving RNA:DNA hybrids that form during transcription and plays roles in DNA repair and other cellular processes. Genetic knockout (KO) of Senataxin has several downstream consequences including decreases in genome stability, increased levels of DNA damage, and elevated innate immune activation. We have recapitulated these findings in Senataxin KO (Setx-/-) mouse embryonic fibroblasts (MEFs), which are derived from the AOA2 mouse model. We also observe an increase in reactive oxygen species (ROS), a novel phenotype in these KO cells. These phenotypes are known drivers of neurodegenerative diseases and markers of inflammation and oxidative damage, however, their role in AOA2 remains unclear. In our study we aimed to fill this gap in knowledge by elucidating the molecular causes of DNA damage, innate immune activation, and ROS production in Setx-/- MEFs. Our initial results indicate that mitochondrial function and morphology are dysregulated and abnormal, which may be leading to increased ROS production. We also have identified an irregular glycolytic rate phenotype in these cells. Combined these findings indicate a previously unidentified phenotype in which Setx-/- cells display metabolic difference compared to wildtype cells, indicating a potential link between metabolic dysregulation and AOA2 disease related phenotypes. Future work will dissect if these phenotypes are recapitulated in our AOA2 mouse model and AOA2 patient-derived cells. By understanding the molecular mechanisms of these phenotypes and validating them in both in vitro and in vivo models we can identify biomarkers of AOA2 which is vital for developing potential therapeutic targets for patients.

MS2010

Nanorobot microrheology in vivo: spatiotemporal mapping of the mesenchymal tissue viscoelasticity in development

*Fengtong Ji[1], Weixuan Wang[1], Weida Liao[1], Yi Xing[1], Ana Hernandez-Rodriguez[1], Yisha Lan[1], Lakshmi Balasubramaniam[1], Russell Cowburn[1], Alexandre Kabla[1], Fengzhu Xiong[1]; University of Cambridge[1]

The morphology of tissues and organs, whether normal or diseased, is shaped by cell phenotype changes (e.g., epithelial or mesenchymal) that drive tissue mechanics. One central phenotypic change is the epithelial-to-mesenchymal transition (EMT) that not only controls cell behaviours and fate, but also alters tissue mechanical properties. In particular, highly motile mesenchymal cells create fluid-like mesenchymal tissues whose

mechanical properties are poorly understood due to their high deformability, limited accessibility of deep regions, and heterogeneity. Here, we propose a nanorobotic approach to measure tissue viscoelasticity in situ and in vivo. Magnetic nanorobots, engineered nanoparticles with precise motion control in programmable fields, are actuated to perform out-of-equilibrium rotational dynamics. Using an in vivo self-assembly strategy, we deployed nanorobots in the presomitic mesoderm (PSM) of the chicken embryo, a region of active mesenchymal cell migration. We combined energy-based theoretical modelling with finite element simulations, providing a comprehensive framework for analysing nanorobot dynamics in diverse tissue environments. We generated a spatiotemporal viscoelasticity map of the tail including the PSM and neuromesodermal progenitor (NMP) region during body axis formation. Gradients of the viscosity and shear modulus along the anterior-posterior and medial-lateral axes were established. The temporal changes in the paraxial mesoderm were tracked as the PSM cells migrate. We are currently investigating the tissue structural basis of the mechanical properties, such as extracellular matrix (ECM) expression patterns (e.g., fibrillin, fibronectin, and hyaluronic acid). We anticipate the nanorobotic approach to be a paradigm for probing spatiotemporal tissue mechanics in vivo and to address broader questions in mechanobiological research.

MS222

Single-molecule analysis of morphogen diffusion revealed tightly regulated, dynamic morphogen-matrix interactions that shape the evolution of animal morphology

*Gavin Schlissel[1], Pulin Li[1]; Whitehead Institute[1]

Animals use a small number of evolutionarily conserved morphogens to pattern tissues of varying sizes, but it is not clear how evolution can modulate the effective signaling range of a morphogen. This problem is particularly acute in Hedgehog signaling, where there is deep disagreement about whether the poorly soluble Hedgehog morphogen is capable of extracellular diffusion. Using single-molecule microscopy in live cells, we found that Hedgehog morphogens diffusedextracellularly by sliding along cell membranes and dynamically interacting with membrane proteins or insoluble components of the extracellular matrix. Individual Hedgehog molecules rapidly transitioned among binding partners, and the vertebrate-specific protein SCUBE accelerated Hedgehog diffusion by catalytically accelerating transition between each binding partner, resulting in longer Hedgehog gradients in cells or tissues where SCUBE is expressed. Furthermore, we found that mice and humans with mutations in SCUBE have abnormal craniofacial and skeletal development that reflects tissue-specific defects in Hedgehog diffusion, suggesting that tissue-specific modulation of morphogen diffusion is a novel “knob” that evolution can use to fine-tune animal anatomy. To test our mechanistic understanding of Hedgehog diffusion, we developed synthetic nanobody-derived morphogens that reconstitute core features of the Hedgehog diffusion mechanism, and found that dynamic topological confinement of signaling proteins to the cell surface dramatically enhanced their ability to form long-range signaling gradients. Our work spans a wide range of spatial and temporal scales, connecting single-molecule biophysical measurements to long-range embryonic pattern formation. I will discuss our multiscale understanding of Hedgehog diffusion, and our efforts to extend this framework to understand unrelated signaling proteins, including developmental morphogens and fully synthetic signaling agonists.

MS208

Tissue fluidity: A double-edged sword for multicellular patterning

*Rikki Garner[1], Sean Megason[1]; Harvard Medical School[1]

Tissue fluidity, or the ability of cells to freely move, flow, and rearrange within a tissue, is a universal physical property of multicellular life that plays central roles in development, regeneration, and cancer. While it is clear that tissue fluidity is tightly regulated and varies drastically across biological contexts, we are only beginning to understand (1) how tissue fluidity is biologically controlled; and (2) how fluidity impacts critical tissue patterning mechanisms such as morphogen signaling and cell sorting. In recent work, we showed that tissue fluidity can easily be tuned to freeze, catalyze, or erase multicellular patterns, depending on the biological context (Garner et al., bioRxiv 2025) – suggesting that tissue fluidity is a key regulator of multicellular organization and must be tightly controlled to achieve robust patterning. Interrogating these questions in the context of zebrafish development, we have now established an in toto imaging pipeline to simultaneously track cell movements and morphogen signaling embryo-wide at single cell resolution over many hours. Using this approach, we have identified a fluidity transition in the early embryo in which initially stationary cells suddenly undergo rapid, random migration and scatter globally across the embryo. Similar early-embryonic movements have been reported in chick, mouse, and human. Performing high-resolution imaging of cell morphology and movement, as well as quantitative in toto cell track analysis, we establish that cell scattering is in fact driven by large-scale random migration. The cellular movement is rapid, with cells migrating an average of 1.8 µm/min (IDR 0.5-3.8 µm/min) – approaching the speed of immune cells. Mean squared displacement analysis revealed that cells move diffusively with an average effective diffusivity of 5.6 µm2/min (IDR 0.3-14.1 µm2/min), meaning they exhibit RMS displacements of 63 µm (IDR 14.2-100.8 µm) in just 2 hours, comparable to the size of the embryo (radius ~300 µm). Strikingly, the levels of random motility exhibit a gradient across the embryo, suggesting that the motility is developmentally patterned. Indeed, using an FGF/Erk reporter, we show that the onset of cell motility closely follows the initiation of a transient FGF activity gradient across the dorsal-ventral axis. Overall, our results demonstrate that a fluidity transition induces dramatic cellular mixing during early embryonic patterning. We are now actively investigating the consequences of the observed fluidity transition for early embryonic development. Using quantitative imaging, machine learning, and perturbations to control both motility and signaling, we are determining how major axis formation and fate specification can be robust in the face of such extreme random cell movements.

MS202

Tuning the Emergent, Time and Length Scales Dependent Viscoelasticity of Biomolecular Condensates

*June Ho Hwang[1], Nirnay Samanta[2], Huabin Zhou[1], Michael Cotten[1], Melissa Stuchell-Bereton[2], Andrea Soranno[2], Michael Rosen[1]; Department of Biophysics and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center[1], Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis[2]

Biomolecular condensates are “membraneless organelles” that can specifically organize together functionally related biomolecules and confer spatial and temporal control over cellular processes. The unique physicochemical and material properties of biomolecular condensates, distinct from their surroundings, are increasingly recognized as key determinants of biological functions in both native and disease contexts. Yet, how these material properties and their regulation are related to the underlying molecular and mesoscale interactions and structures remains unclear. Thus, in this study, we aim to establish mechanistic principles that link the molecular determinants of condensate formation to their emergent viscoelastic properties. Guided by the “stickers-and-spacers” model, we engineered polySH3 and polyPRM mutants to systematically and

independently tune sticker and spacer interactions. Our optimized microrheology pipeline enabled precise measurement of condensate viscoelasticity across a wide range of interaction strengths. We show that stronger sticker and spacer interactions increase both the magnitude of condensate viscoelasticity (quantified by zero-shear viscosity) and its characteristic timescale (quantified by terminal relaxation time). Importantly, we observe coupling between sticker and spacer interactions, such that changes in one interaction type show diminished effects when the other is already strong. Extending this framework to biologically relevant systems, we examined condensates formed by the SARS-CoV-2 nucleocapsid (N) protein and the splicing factor PTBP1, both of which recapitulated our previous findings. Our results further reveal that condensates exhibit complex rheological behaviors, with nonlinear responses across timescales that indicate departures from simple Maxwellian mechanics. These behaviors also display length scale dependence: when spacer interactions are sufficiently strong, condensate viscoelasticity is dependent on length scales > 100 nm, indicating heterogenous network organization. To gain structural insight, we performed cryogenic electron tomography (cryoET), which revealed that enhanced spacer interactions promote higher-order structures and heterogeneity at the mesoscale. Altogether, this work provides a generalizable framework in which molecular level interactions define not only the presence of phase separated condensates, but also their complex, time and length scale-dependent rheological behaviors through mesoscale organization. These mechanistic principles advance understanding of how the emergent material properties of biomolecular condensates are tuned and regulated for specific functions, and how their dysregulation may contribute to disease.

Physiological Cell Biology: Adapting Cellular Machines to Cellular Context

SG102
Catching transcription factors for proteasomal degradation
*Xin Gu[1], Christopher Nardone[3], Jingjing Gao[3], Hyuk-Soo Seo[1], Julian Mintseris[3], Lucy Ort[1], Matthew Yip[3], Milen Negasi[1], Anna Besschetnova[1], Nolan Kamitaki[3], Steven Gygi[3], Sirano Dhe-Paganon[1], Nikhil Munshi[1], Mariateresa Fulciniti[1], Michael Greenberg[3], Si; Dana-Farber Cancer Institute[1], Brigham and Women's Hospital[2], Harvard Medical School[3]

The midnolin-proteasome pathway degrades many nuclear proteins without ubiquitination, but how it operates mechanistically remains unclear. Here, we present structures of the midnolin-proteasome complex, revealing how established proteasomal components are repurposed to enable a unique form of proteolysis. While the proteasomal subunit PSMD2/Rpn1 binds to ubiquitinated or ubiquitin-like proteins, we discover that it also interacts with the midnolin nuclear localization sequence, elucidating how midnolin's activity is confined to the nucleus. Likewise, PSMD14/Rpn11, an enzyme that normally cleaves ubiquitin chains, surprisingly functions non-enzymatically as a receptor for the midnolin ubiquitin-like (Ubl) domain, positioning the substrate-binding Catch domain directly above the proteasomal entry site to guide substrates into the proteasome. Moreover, we demonstrate that midnolin downregulation is critical for the survival of myeloma cells by stabilizing the transcription factor substrate IRF4. Our findings uncover the mechanisms underlying the midnolin-proteasome pathway and midnolin downregulation as a driver of multiple myeloma.

SG104
How endo-lysosomal super-organelles maintain proteostasis in mammalian oocytes

*Gabriele Zaffagnini[1]; University of Cologne[1]

Oocytes, the female germ cells, need to preserve their cytoplasm damage-free for up to 50 years in humans, before being employed for reproduction. Protein homeostasis (proteostasis) is essential in all cell types to prevent the accumulation of intracellular damage, such as misfolded protein aggregates. Long-lived cells are particularly sensitive to proteostasis decline during aging. How mammalian oocytes regulate proteostasis during their extended lifespan is unknown. Here I show that, in mice, oocytes sequester and degrade aggregated proteins in novel "super-organelles" that I have named EndoLysosomal Vesicular Assemblies (ELVAs). ELVAs harbour both the major intracellular proteolytic compartments - the ubiquitin-proteasome system and the autophagy-lysosomal pathway - in a liquid-like matrix. Largely inactive in immature oocytes, ELVAs activate protein degradation shortly before fertilization, thereby clearing the sequestered aggregates and allowing correct embryogenesis. These results define a new paradigm for proteostasis maintenance in long-lived oocytes.

SG101

Probing the influences of cell state and sequence variation on the biogenesis and stability of the nuclear lamina

*Abigail Buchwalter[1]; University of California, San Francisco[1]

Understanding the interplay between molecular function and physiological context is a critical challenge facing the future of cell biology. Exploring how protein machines shape intracellular organization and dynamics has revealed the fundamental workings of the cell. However, much as nothing in biology makes sense except in light of evolution, the core logic of cellular function does not make sense without the physiological contexts in which cells exist and operate. The goal of this session is to highlight ongoing work that is bridging this gap. For example, it is critical to determine how the essential functions of core cellular machines are adapted across cell types and what unique demands – and unique vulnerabilities – specific cell types place on essential protein structures. Bridging the gap between mechanism-driven cell biology and physiological context is not achieved without difficulty. "Reductionist" systems and "workhorse" cell lines are such for a reason: they have made intervention, manipulation, and hypothesis testing tractable. However, as we look to the future, emerging technologies may make it possible to bring previously challenging interventions into more physiological contexts. This work promises to reveal new molecular and cellular processes that only arise in the context of a differentiated cell type, mature tissue, or living organism. The importance of examining cell biology in physiological contexts is critical for our work on the nuclear lamina, a core cellular structure that scaffolds almost all mammalian nuclei. It is a mystery why specific tissues are especially vulnerable to "laminopathy" mutations while the lamin proteins themselves are broadly expressed. We discovered that lamin protein and transcript levels are very poorly correlated across tissues, implying that the lamina's functions are shaped by extensive and variable post-transcriptional regulation. By applying a novel in vivo proteomic approach, we found that the lamin proteins are especially long-lived in laminopathy-vulnerable tissues, and that the lifetime of a laminopathy mutant is further prolonged in the diseased cardiovascular system, driving its accumulation over time. This observation raises the possibility that disrupted lamin proteostasis may be a shared feature of laminopathy mutations. Hundreds of pathogenic mutations and over a thousand variants of unknown significance are linked to the LMNA gene. To survey the effects of LMNA sequence variation on protein stability, we are using deep mutational scanning to query the effects of substitutions, insertions, and deletions

at each position along the lamin A protein sequence. We are applying this approach both in pluripotent and differentiated cells to determine how cell state influences lamin assembly and stability.

SG106
Ribosomes in Gene Regulation: Controlling the diversity of proteins made in specific cells, tissues, & organisms
*Maria Barna[1]; Stanford University[1]

Ribosomes, the universal molecular machinery for protein synthesis, have been viewed as structurally and functionally uniform entities since their initial characterization over six decades ago. However, accumulating evidence suggests that ribosomes can contain distinct combinations of ribosomal proteins (RPs) and are regulated by ribosome-associated proteins (RAPs). These heterogeneous ribosomal subpopulations are hypothesized to play specialized roles in translational control, contributing to cellular protein homeostasis. However, the field has lacked tools to directly visualize individual ribosomes, characterize their molecular composition, and map their spatial distribution in cells. Understanding how ribosomal diversity impacts translation could unveil new layers of gene expression regulation. To bridge this technical gap, we developed two synergistic approaches: ribosome expansion microscopy (RiboExM) and ALIBi (an optogenetic proximity labeling method). RiboExM couples physical expansion of cells with standard confocal microscopy to achieve subribosomal resolution, allowing direct visualization of distinct ribosomal populations and their spatial organization within mammalian cells. ALIBi enables biochemical isolation of ribosomes and associated RAPs and mRNAs from specific subcellular compartments, facilitating quantitative proteomics and transcriptomics analyses. Together, these methods offer complementary insights into ribosomal composition and its functional implications, enabling a comprehensive investigation of subcellular translational machinery at the single-molecule level.

SG105
Turning the Oxygen and Vitamin Dials
*Isha Jain[1]; Gladstone[1]

Oxygen deprivation (hypoxia) and excess (hyperoxia) are both toxic to humans. Oxygen deprivation contributes to 3 of the 5 leading causes of mortality in developed nations -- heart attack, stroke, and respiratory failure. On the other hand, hyperoxia is toxic to nearly all organisms and contributes to the pathology of ischemia-reperfusion injury, mitochondrial disease and hyperoxic lung injury. However, the molecular mechanisms underlying hypoxia and hyperoxia toxicity remain unknown. By deciphering these mechanisms, we strive to nominate novel therapeutic candidates. Our recent work highlights such mechanisms of hyperoxia toxicity and the cycle of damage caused by destabilization of specific iron-containing protein complexes. While small molecules and biologics are the most common forms of therapy, we believe we have uncovered a new mode of treating metabolic disorders. We now hope to extend our findings to additional inborn errors of metabolism, as well as more common metabolic disorders, including aging and age-associated damage.

SG103
Uncovering the specificity of gene expression programs in cellular quiescence and senescence
*Yi Fei Tao[1], Jimmy Ly[1], Iain Cheeseman[1]; Whitehead Institute and MIT[1]

Most cells in the human body are not actively dividing and instead exist in states of cell cycle arrest, including quiescence and senescence. To understand the cell biological properties of these distinct physiological states, it is essential to explore the molecular basis governing their functional specificity. Quiescence is a state of reversible cell cycle arrest in which cells exit the cell cycle and yet retain the capacity to divide. In contrast, senescence is defined by an irreversible exit from the cell cycle and is coupled with the induction of an inflammatory secretory program. The physiological functions of quiescent and senescent cells are central to human health and aging, but our understanding of these cell states remains incomplete. Quiescent and senescent cells display substantial differences in their properties with respect to proliferative potential, cellular organization, and other behaviours. To uncover the molecular mechanisms underlying these differences, we analyzed key aspects of gene expression to comprehensively define factors that confer specificity to these cell states. At the global level, quiescent and senescent cells display significant global differences in chromatin organization and transcription and translation activity. Using targeted sequencing and proteomics approaches, we identified gene-specific differences in transcription start site usage, mRNA splicing, translation initiation, and protein decay. We will describe specific proteins and variants that make important functional contributions to these distinct states. Our work reveals that the differential decoding of genes at multiple levels of regulation contribute to the specificity of quiescence and senescence as physiological states of cell cycle arrest.

Plasticity and Cross-talk Between Cytoskeletal Components

MS907
A metaphase, septin-associated myosin filament cage ensures lumen integrity in intestinal organoids by promoting metaphase cell rounding and planar cell division
Dillon Schrock[1], Yang-In Yim[1], Xufeng Wu[1], John Hammer[1], *Sulaiman Yousafzai[1], Kirsten Remmert[2], Anjelika Gasilina[1], Lelia Boley[1], Chris Alexander[1], Antonio Pedrosa[1]; Cell and Developmental Biology Center, NHLBI, NIH, Bethesda MD 20892[1], National Cancer Institute, NIH, Bethesda MD 20892[2]

Here we investigated the relationships between myosin contractility, cell division plane and lumen integrity using mouse intestinal organoids, which normally maintain a single lumen as they grow. Attenuating myosin function inhibits interkinetic nuclear migration and metaphase cell rounding, causing the division plane to shift from planar to orthogonal and resulting in organoids possessing multiple lumens. Following the apical domain marker ZO-1 showed that new lumens originate at the interface between stacked daughter cells created by orthogonal divisions, and that subsequent lumen maturation involves passage through a transitional epithelial state. Super-resolution imaging of rounded metaphase cells revealed a never-before-seen myosin filament cage that we show resists surrounding tissue pressure and contributes to contractile ring formation. Finally, staining showed that septins 7 and 9 co-localize with the myosin filament cage, suggesting that this metaphase contractile structure may be templated by septin filaments. We conclude that a septin-associated myosin filament cage maintains lumen integrity in intestinal organoids by supporting metaphase cell rounding, a prerequisite for planar cell division.

MS902
Cortical microtubule reinforcement enables mechanical resistance of actomyosin-driven contractions in C. elegans and mouse oocytes
*Alyssa Quiogue[1], Takaya Totsuka[2], Takashi Akera[2], Bruce Bowerman[1], Alexander Cartagena-Rivera[2]; University of Oregon[1], National Institutes of Health[2]

The cell cortex is a network of actin filaments, non-muscle myosin II, and membrane-crosslinking proteins that underlies the plasma membrane. Cortical tension drives changes in cell shape and depends on myosin-generated forces and microfilament architecture. Such mechanical properties of the cortex are emerging as key determinants of oocyte developmental potential. While microtubules are considered separate from the cortex, they may play a greater role during oocyte meiotic cytokinesis. We have shown that loss of the C. elegans microtubule stabilizer CLASP/CLS-2 in oocytes results in excessive membrane ingression throughout the cortex, accompanied by defects in contractile ring assembly or stability and failed polar body extrusion. Because cortical actomyosin dynamics appear normal in cls-2 mutants, we proposed that CLS-2, which localizes both to meiotic spindle microtubules and to patches throughout the cortex, stabilizes cortical microtubules to promote a cortical stiffness that resists actomyosin-driven membrane ingressions throughout the oocyte cortex away from the spindle-associated contractile ring. To directly test this, we used Atomic Force Microscopy (AFM) to measure cortical tension in C. elegans oocytes. Tension was significantly reduced in both cls-2 mutants and nocodazole-treated oocytes, which destabilizes microtubules. Conversely, tension increased when microtubules were stabilized with taxol or by depletion of XMAP215/ZYG-9 or Kinesin-13/KLP-7, consistent with microtubules stiffening the cortex. We extended our work to mouse oocytes, where regulation of actomyosin activity plays a functional role in meiosis. However, microtubule reinforcement of the mouse oocyte cortex remains unexplored. In contrast to C. elegans, nocodazole increased cortical tension, whereas taxol had a conserved effect. We are testing whether nocodazole-induced tension increase reflects non-muscle myosin activation. These findings reveal species-specific roles for microtubules in modulating oocyte cortical mechanics and suggest that microtubules may act as mechanical regulators during these highly asymmetric cell divisions.

MS901
Curved microtubule regions mark sites of lattice compaction in cells and neurons
*Nisha Mohd Rafiq[1], Jaya Mishra[1], Polina Volos[1], Ke Wang[1]; University of Tübingen, Germany[1]

Tau is an intrinsically disordered microtubule-associated protein (MAP) best known for its aggregation in Alzheimer's disease. However, similar tau pathology is also present in a substantial subset of Parkinson's disease (PD) patients, especially those with LRRK2 mutations, a connection that remains relatively uncharacterized. On microtubules, tau has been observed to form cohesive "envelope"-like assemblies that preferentially associate with compacted lattices (~41 Å inter-dimer spacing). Here, we demonstrate in cells that lattice expansion - induced by MAP7 or by microtubule-stabilizing agents such as taxol and epothilone D - causes tau to relocalize to regions of high microtubule curvature. At substoichiometric concentrations, tau acts as both a microtubule compactor and curvature sensor, reminiscent of doublecortin. Strikingly, pathogenic LRRK2 mutants (G2019S and R1441C), common in early-onset PD, exhibit microtubule binding patterns similar

to tau and compete with it for binding sites. In human iPSC-derived neurons carrying these LRRK2 mutations, we observe elevated levels of pathogenic, hyperphosphorylated tau, consistent with postmortem findings in PD patients, suggesting that specific microtubule lattice conformations influence tau localization and disease-associated modifications. Finally, we show that neurons display micron-sized tau patches on microtubules, whose localization is sensitive to lattice-expanding perturbations. These findings reveal that mechanical and structural changes to the microtubule lattice can modulate tau distribution in neurons and may contribute to cytoskeletal dysregulation in neurodegenerative disease.

MS904

Mechanism of APC-mediated branched actin assembly at the microtubule tip

*XINGYUAN FANG[1], Tatyana Svitkina[1]; University of Pennsylvania[1]

The branched actin filament network at the cell leading edge generates driving force for membrane protrusion during cell migration and extension of neuronal processes. On the other hand, microtubules largely control cell navigation, but the underlying mechanism remains to be fully understood. Previous study in the lab showed that branched actin can assemble at the microtubule tips in growth cones of cultured hippocampal neurons and that Adenomatous Polyposis Coli (APC) is essential for this process. These observations suggest that microtubules control cell navigation through local formation of branched actin network. Here, we explore the molecular mechanism of this APC-triggered assembly of branched actin networks. We found that knocking-down APC in Ref52 fibroblasts suppressed formation of cell processes and rendered cell morphology. Overexpression of APC constructs containing the N-terminal region of APC promoted process formation, suggesting a key role of the N-terminus of APC in process extension. On the other hand, APC-depleted Ref52 fibroblasts lost the fusiform shape and rounded up. Interestingly, N-APC can rescue APC-depleted Ref52 cells, whereas C-APC failed to rescue the phenotype, suggesting that a functional domain in N-APC is responsible for inducing processes, presumably through local actin polymerization. Given that the N-terminal Armadillo repeat domain of APC can activate the Cdc42 GEF Asef1, we hypothesize that the Asef1 - Cdc42 - N-WASP - Arp2/3 pathway downstream of APC nucleates branched actin filaments at the microtubule tip for growth cone navigation. In support of this hypothesis, we found that depletion of Asef1, Cdc42 or N-WASP, or pharmacological inhibition of Cdc42 or N-WASP, in rat hippocampal neurons closely mimicked the phenotype of the APC depletion characterized by nearly complete elimination of branched but not linear actin filaments in growth cones, as revealed by platinum replica electron microscopy. In conclusion, current results revealed that APC is responsible to induce branched actin formation via its armadillo repeats at the N-terminus of APC. This domain activates Asef1 and downstream effectors Cdc42 and N-WASP. In hippocampal neurons, APC and its downstream effectors are responsible for the formation of branched actin network at the tip of microtubule tips. This crosstalk between microtubule and actin in growth cones is critical for neurite outgrowth in early neuronal development.

MS903

Readers of a tubulin conformational code

*Takashi Hotta[1], Jonathan Singh[2], Robert Keyzers[2], Ryoma Ohi[1]; University of New Mexico Comprehensive Cancer Center[1], Victoria University of Wellington[2]

Microtubules, dynamic polymers of αβ-tubulin heterodimers, are essential for myriad cellular functions. Recent advances in structural biology revealed that tubulin undergoes conformational changes upon GTP hydrolysis, transitioning from an "expanded" GTP-bound state to a "compacted" GDP-bound state. While tubulin isotypes and post-translational modifications (PTMs) are well-established components of the "tubulin code" that regulates microtubule-associated protein (MAP) recruitment, the role of tubulin conformation within the cell is not understood. We hypothesized that the conformational plasticity of tubulin within the microtubule lattice is a third, unrecognized layer of the tubulin code. As a first step to test this possibility, we sought to identify MAPs that selectively bind microtubules with compacted versus expanded lattice conformations. To this end, we assembled microtubules using two distinct microtubule-stabilizing agents, Taxol and Peloruside A, to generate microtubules with different lattice states. Taxol expands the microtubule lattice, whereas Peloruside A stabilizes the microtubule lattice in a compacted state. We assembled microtubules in HeLa cell lysate with each drug and performed quantitative mass spectrometry analysis on the microtubule pellets. This approach revealed MAPs that bind to microtubules in a manner that is sensitive to microtubule lattice conformation. Pursuit of these proteins confirm that a subset of MAPs do indeed selectively bind microtubules in a lattice conformation-dependent manner in cells. Our findings offer novel insights into how the tubulin conformational code can selectively recruit specific MAPs, thereby diversifying microtubule function and properties within the cell.

MS906

Septin crosstalk with microtubules and actin filaments is regulated by phosphorylation of septin 9 by the glycogen synthase kinase 3 beta.

*Md Noor A Alam[1], Sarah Butts[1], Priyanka Bhakt[3], Iliana Kesisova[2], Elias Spiliotis[1]; University of Virginia[1], Massachusetts Institute of Technology[2], University of Pennsylvania[3]

Septins are a family of hetero-oligomeric GTP-binding proteins that contribute to diverse cellular processes including morphogenesis, cell division, migration, and intracellular trafficking. Septins assemble into higher-order polymers and filamentous structures that associate, and crosstalk functionally with both actin filaments, and microtubules (MTs). How septin crosstalk with actin filaments and microtubules is regulated is poorly understood. Here, we show that phosphorylation of septin 9 (Sept9) by GSK3b shifts septins from microtubules to the actin cytoskeleton, a regulation that is critical for neuronal axon generation and the establishment of neuronal polarity. Phosphoproteomic studies had previously identified two serine residues in the N-terminal microtubule- and actin-binding domain of Sept9 as phospho-targets of GSK3β. Using in vitro phosphorylation assays, we found that recombinant Sept9 is directly phophorylated by GSK3β. To assess the functional consequences, we generated phosphomimetic (A-to-E) and phosphonull (S-to-A) mutants of Sept9, and expressed them in MDCK cells while knocking down endogenous Sept9. Microtubule regrowth experiments after nocodazole washout showed that phosphomimetic mutants reduce localization to centrosomal MTs and impair MT growth, whereas phosphonull mutants promote MT regrowth relative to wild type. Interestingly, phosphonull variants displayed decreased co-localization with actin filaments in both MDCK and U2OS cells, while phosphomimetic mutants exhibited enhanced actin association. In vitro binding assays also showed that recombinant phosphomimetic Sept9, and Sept2/6/7/9 complexes, which contained phosphomimetic Sept9, had diminished MT binding and bundling activity. In MDCK cells, inhibition of GSK3β with SB216763 reduced endogenous Sept9–actin association, and shifted Sept9 localization toward centrosomal MTs in nocodazole wash-out assays. In primary rat hippocampal neurons, Sept9 localization was elevated on microtubules with inactivated pSer9-GSK3β. Notably, expression of the phophomimetic Sept9 mutants decreased asymmetric

neurite growth, impairing the development of neuronal polarity. Collectively, these results indicate that septin crosstalk with microtubules and actin is regulated by Sept9 phosphorylation by GSK3beta, which may integrate various signaling pathway outputs into the function of septins in microtubule or actin driven processes.

MS908

Structural Basis of Tubulin Post Translational Modification Recognition by EML Proteins

*Aryan Taheri[1], Jon Fernandes[1], Burak Cetin[1], Ahmet Yildiz[1], Eva Nogales[1]; University of California, Berkeley[1]

Post-translational modifications (PTMs) of microtubule (MT) C-terminal tails modulate MT functions. A family of highly conserved MT binding proteins, the Echinoderm Microtubule-associated protein-Like (EML) family, regulates neuronal outgrowth and mitosis and are readers of MT PTMs. However, resolving the structural basis by which PTM reader proteins recognize PTMs has been challenging due to the intrinsic flexibility of the MT C-terminal tails. Using cryo-electron microscopy and single-molecule imaging, we uncovered the mechanism by which EML proteins recognize PTMs on the tubulin tail. Remarkably, our structures reveal the entire alpha-tubulin tail in a fully ordered conformation, enclosed within a beta-propeller domain of the EML protein. High-resolution structural analysis shows that this interaction is mediated by a unique tyrosine recognition motif, coupled with simultaneous binding across multiple tubulin dimers along the MT lattice. Furthermore, our structures of EML proteins from different species demonstrate that this motif is evolutionarily conserved and essential for their robust MT binding. Through single molecule imaging, we found that EML proteins preferentially associate with the GTP-like MT lattice and promote MT catastrophe events, in contrast to the stabilizing role typically attributed to many MT binding proteins. Together, our findings define a novel tubulin tyrosine recognition motif that is conserved within EML proteins, revealing the structural mechanism of how they bind to MTs and affect their dynamics.

MS905

The Interplay Between the Tubulin- and MAP-Codes and Their Effects on Kinesin Motility

*Jonathan Fernandes[1], Viola Zhao[1], Parnika Kant[1], Ahmet Yildiz[1]; UC Berkeley[1]

Microtubules (MTs) play a critical role in many cellular processes, most significantly in cellular trafficking. It has been proposed that signals that regulate motor-driven intracellular transport are deposited onto MT tracks, either by post-translational modifications (PTMs) and isotypes of tubulin (tubulin-code) or MT-associated proteins (MAP-code). In vitro studies showed that MT motors are tightly regulated by MAPs, but not by tubulin PTMs. Therefore, it remains unclear whether tubulin modifications directly regulate MAPs and motors on the MT. Using recombinant tubulin, we investigated the interplay between the tubulin- and MAP-codes and how they impact motor activity in vitro. We found that different tubulin modifications have a minor impact on kinesin motility when MTs are stabilized by taxol, because taxol expands the MT lattice and outweighs the effects of tubulin PTMs. However, these changes can significantly affect MT-MAP decoration, which in turn strongly affects motility. In dynamically growing MT experiments, we observed different tubulin modifications selectively tune the recruitment of MAPs and motors to the MT. We showed that tubulin PTMs affect the MT affinity of tau by altering its affinity to bind free tubulin. Our results are consistent with a two-layered code, in which the tubulin code selectively recruits MAPs onto MTs, and the MAP code dictates which motors can transport cargo along those tracks.

Quantitative Modeling Insights for Cytoskeleton Dynamics and Cell Polarity

MS2003
Context-dependent spatial multicellular network motifs for single-cell spatial biology
*Assaf Zaritsky[1], Amos Zamir[1], Yael Amitay[2], Yuval Tamir[1], Leeat Keren[2]; Ben-Gurion University of the Negev[1], Weizmann Institute of Science[2]

The clinical state of diseased tissue is caused by complex intercellular processes that go beyond pairwise cell-cell interactions and are difficult to infer due to the combinatorial explosion of such high-dimensionality. We present context-dependent identification of spatial motifs (CISM), a two-step method to identify local cell structures associated with a disease state in single cell spatial data. First, for each tissue, CISM enumerates structures of enriched reoccurring multicellular patterns that define modular 'motifs' in the multicellular network. Second, discriminative motifs are selected according to the context - their presence in patients at different clinical disease states. By applying CISM, we show that modular structures composed of as little as 3-5 cells and their relative spatial arrangement can encode differences in clinical disease states in cohorts of triple-negative breast cancer (TNBC) and melanoma patients. Machine learning validation indicated that discriminative motifs outperform state-of-the-art methods for disease state prediction while enabling interpretation of which interactions in what spatial context are associated with these predictions. CISM-derived discriminative motifs may define an intermediate spatial scale of abstraction and modularity in multicellular organization and function with broad applicability in the domain of spatial single cell omics and beyond.

MS2005
Organelle packing interactions and cell anatomy patterns revealed by whole-cell imaging: a two-pronged approach
*Mary Mirvis[1], Jian-hua Chen[2], Axel Ekman[2], Bieke Vanslembrouk[2], Mark Le Gros[2], Carolyn Larabell[2], Christopher Lee[5], Honor Akenuwa[5], Brooke Weingard[1], Steven Goodman[3], Wallace Marshall[4]; University of California, San Francisco[1], Lawrence Berkeley National Laboratory[2], Stanford University[3], University of California San Francisco[4], University of California, San Diego[5]

The past decade has uncovered the complex interconnectivity of organelles through contact sites and functional crosstalk. The next frontier is how the whole organelle network spatially arranges at the meso- and whole-cell scales. What are the structural patterns and physical rules defining the state of the organelle network? To what extent is cell anatomy a reflection of organelle confinement in the crowded cytoplasm? Addressing these questions requires minimal-artifact, high-resolution whole cell multi-organelle reconstructions, with sufficient throughput to analyze morphological variation and perform robust comparisons of cell populations. Such data are challenging to generate, typically limiting single-study sample size. We address this with two complementary approaches: 1) a high-throughput study in budding yeast, and 2) a synthesis of whole-cell imaging literature. We have leveraged auto-segmentation to create the largest-yet dataset of whole-cell images of S. cerevisiae using soft X-ray tomography, featuring nucleus, vacuole, and lipid droplets for 490 cells across 3 strains at 30-35nm isotropic resolution. Statistical 3D morphometrics show that vacuole enlargement progressively disrupts nuclear scaling, position, and shape. Spherical harmonics-based

shape modeling points to key morphological features driving anatomical variation in wild-type and large-vacuole populations. Quantitative whole-organelle shape analysis and biophysical simulations suggest that asymmetric deformations may arise due to vacuole-nucleus packing, with directionality dependent on differential organelle rigidity. Ongoing work extends our statistical and biophysical modeling to incorporate bulk high-resolution shape metrics. We also adapted evidence synthesis methods to comprehensively curate the whole-cell imaging literature between 2004-2024. Using a "scoping review" methodology, we assembled a corpus of 89 studies representing 13 imaging modalities and a large range of cell types, biological contexts, and organelle combinations. This establishes a knowledge base for cell anatomy research and secondary analysis, and motivates a call for standardized study design, reporting, and data sharing. Ongoing work merges organelle metrics to identify common and context-specific anatomical patterns and organelle relationships. Complementing our yeast SXT study, we find additional evidence that physical packing of organelles in the limited cytoplasmic volume plays an underappreciated role in establishing organelle morphology and global cell patterning. Future outlooks include establishing a community resource dedicated to cell anatomy data, and expanding evidence synthesis methods to drive meta-science work and novel discovery in cell biology.

MS2002

Space Negotiation Strategies of Cells Invading Extracellular Matrix: Lessons from a Physics-Based Model

*Andreas Buttenschoen[1]; University of Massachusetts Amherst - Amherst, MA[1]

Cell migration through extracellular matrix (ECM) presents a fundamental biophysical paradox: cells require ECM fibers as adhesive substrates for traction force generation via filopodia, yet these same structures form mechanical barriers that impede movement. To navigate this trade-off, cells employ three distinct space negotiation strategies—shape adaptation through deformability, mechanical remodeling of elastic ECM networks, and proteolytic degradation via matrix metalloproteinases (MMPs). While each mechanism has been studied in isolation, their integrated dynamics and relative contributions to invasion success remain poorly understood. We developed a computational physics-based model that captures the interplay between these space negotiation strategies and force-driven migration. Our agent-based framework represents cells as deformable entities with explicit filopodia that mechanically interact with an elastic ECM network through a two-component contractile model, capturing force-velocity relationships observed in actomyosin systems. Cells can both mechanically displace ECM nodes through traction forces and enzymatically degrade network components. By systematically varying ECM density, fiber stiffness, and cellular parameters, we identify emergent migration regimes where different strategies dominate. Our simulations reveal that optimal migration occurs in intermediate-density environments where mechanical remodeling through filopodial traction alone suffices, while high-density matrices necessitate coordinated deployment of MMP activity and enhanced deformability. The detailed filopodia model demonstrates how the spatial distribution and dynamics of cell-ECM adhesions critically influence migration efficiency across different microenvironmental contexts. This work provides a computational sandbox for investigating the complex interplay between cellular mechanics, ECM properties, and enzymatic degradation in 3D invasion. By explicitly modeling filopodial force generation alongside cell deformability and ECM remodeling, our framework enables systematic exploration of how perturbations to specific space negotiation mechanisms—such as MMP inhibition or cytoskeletal disruption—alter invasion dynamics, offering testable predictions for experimental validation.

MS2001
STAGED: A Multi-Agent Neural Network for Learning Cellular Interaction Dynamics
*Dhananjay Bhaskar[1], Joao Rocha[3], Ke Xu[3], Xingzhi Sun[3], Ananya Krishna[3], Blanche Mongeon[2], Morgan Craig[2], Mark Gerstein[3], Smita Krishnaswamy[3]; University of Wisconsin-Madison[1], Université de Montréal[2], Yale University[3]

Single-cell technologies have enabled major advances in identifying cellular states and subpopulations, but conventional methods often treat cells as independent points, overlooking the spatial organization and dynamic cell–cell interactions that drive biological function. Spatial transcriptomics now offers a window into these interactions, but new computational frameworks are required to model the complex interplay of intra- and intercellular dynamics. Agent-based modeling provides a natural framework for representing multicellular systems, but conventional implementations depend on handcrafted rules rooted in expert knowledge rather than learned directly from data. To address this gap, we introduce Spatio-Temporal Agent-Based Graph Evolution Dynamics (STAGED), a framework that integrates ABM with deep learning to model how intercellular communication shapes intracellular gene regulatory dynamics. In STAGED, each cell is modeled as an "agent" whose internal dynamics evolve through history-aware, attention-based regulatory graphs, while signals are exchanged between neighboring cells via ligand–receptor interactions. We demonstrate STAGED's capabilities across simulations and real-world data. In tumor microenvironment simulations, STAGED accurately recapitulates heterogeneous population dynamics in response to an oncolytic virus. In oscillatory systems, it recovers temporally varying regulatory interactions and out-of-phase gene expression dynamics across spatially coupled cells. Finally, when applied to spatial transcriptomics data from early Alzheimer's disease, STAGED uncovers microglial subpopulations with distinct regulatory programs, suggesting that diverse gene-gene interaction networks underlie cell-state heterogeneity in disease progression. Together, these results highlight STAGED as a general framework for uncovering biologically grounded mechanisms of cellular communication and decision-making. By bridging mechanistic modeling with deep learning, STAGED provides a powerful tool for generating testable hypotheses about how regulatory networks and intercellular signaling shape complex cellular behaviors.

RNA Biology in Living Cells and Tissues

SG202
Cristae shaped by stochastic mRNA localization and ATP synthase oligomerization
*Tatsuhisa Tsuboi[1], Chenxi Liu[1], Abdul Haseeb Khan[1], Ercan Kuruoglu[1]; Tsinghua University[1]

Mitochondrial cristae form and remodel in concert with the lateral mobility and oligomeric state of ATP synthase, yet the spatial logic underlying this process remains unresolved. Here, we combine quantitative live-cell imaging with graph-theoretic network modeling to map the distribution of nuclear-encoded ATP synthase subunits within intact mitochondrial networks in live Saccharomyces cerevisiae. Components of the peripheral stalk, Atp3p (γ), Atp4p (b), and Atp5p (OSCP) preferentially accumulate at tube termini and branch points, producing periodic banding ("stripe-like" patterns) along tubules. Three-way junctions further enrich these proteins relative to edge centers, indicating position-dependent assembly cues rather than global accumulation in pre-existing cristae domains. Single-molecule tracking of the corresponding mRNAs using

MS2–MCP labeling reveals a stochastic cytoplasmic distribution, resulting in protein delivery biased toward the tube termini, where diffusion-limited multimerization occurs. A stochastic assembly model incorporating network topology recapitulates the observed stripe-like patterns without positing dedicated nucleation sites. Together, the data support a self-organization mechanism in which freely diffusing ATP synthase protomers assemble into higher-order oligomers at geometrically privileged nodes to seed and sculpt cristae. This framework links mobility, local stoichiometry, and membrane geometry to the emergence of functional bioenergetic landscapes within mitochondria. Keywords: Mitochondria; Cristae; RNA localization; Network topology; Modeling.

SG207

Direct interaction of Drosophila Vasa with the core granule protein Oskar regulates its condensation and mRNA localization

Hyosik Kim[1], Harrison Curnutte[1], Tatjana Trcek[1], *Siran Tian[1]; Johns Hopkins University[1]

Drosophila germ granules are ribonucleoprotein condensates that form at the posterior of oocytes and embryos, where they enrich specific proteins and mRNAs essential for development. The short isoform of Oskar (Short Osk) is their nucleator that also recruits the conserved RNA helicase Vasa through direct interactions. Although Vasa is an established core germ granule protein, the mechanisms underlying its essential role within germ granules remain unclear. Here, using quantitative microscopy, genetic manipulation and a human cell line system, we found that while Short Osk-eGFP forms granules independently of Vasa in flies and mammalian cells, Vasa remodels their properties. Specifically, fluorescence recovery after photobleaching revealed that endogenous Vasa in late oocytes and ectopically expressed IRFP-tagged Vasa in human cells enhanced the exchange of Short Osk–eGFP between granules and the surrounding environment. In the absence of Vasa, granules showed minimal recovery, suggesting that Vasa solubilizes Short Osk-eGFP to prevent its condensates from locking into a rigid state. This effect was mediated by the direct interaction between Vasa and Short Osk, as Vasa mutants previously described as RNA-binding defective still interacted with Short Osk-eGFP and promoted granule recovery at wild-type levels in cells. Furthermore, co-expression of Vasa-IRFP and Short Osk-eGFP was necessary and sufficient to localize the germ granule mRNAs and establish their spatial organization within granules in cells, a process critically dependent on the Vasa–Short Osk interaction. Finally, recruitment of germ granule mRNAs into granules reduced Short Osk-eGFP exchange in cells, providing negative feedback on Vasa-mediated solubilization. Together, these findings support a model in which Vasa regulates germ granule formation by promoting Short Osk exchange and mRNA recruitment, which subsequently dampens Vasa's solubilizing activity to fine-tune Short Osk condensation. This research was supported by the NIGMS R35GM142737 grant awarded to TT.

SG206

Investigating the Quality Control Mechanisms that Cope with Defective Ribosomal RNA

*Zachary Stolp[1], Kamena K. Kostova[1]; Stowers Institute for Medical Research[1]

Protein synthesis is one of the most energy-intensive processes in the cell. Disruptions in protein production can compromise cell viability and lead to cellular stress and complex diseases, such as cancer and neurodegeneration. To maintain continuous protein synthesis, cells must monitor the integrity of key

translation components: mRNA, tRNAs, and ribosomes. While quality control mechanisms for defective mRNAs and tRNAs are relatively well understood, less is known about how cells manage faulty ribosomes. The eukaryotic ribosome is a complex molecular machine consisting of 4 ribosomal RNAs (rRNA) and 80 proteins, and all these components are susceptible to mutations, oxidative stress, and UV radiation. Notably, the rRNA carries out the core enzymatic functions of the ribosome during translation. Despite the importance of rRNA to ribosomal function and human health, the quality control mechanisms that cope with the presence of defective rRNA remain poorly defined. To address this knowledge gap, we developed a system to investigate the quality control pathways that cope with ribosomes harboring defective ribosomal RNA in human cells. We engineered a reporter ribosomal RNA carrying tags that allow for fluorescent detection and purification. To model defective rRNA, we engineered a mutation in the conserved rRNA decoding center that disrupts protein synthesis and is actively degraded by an unknown quality control pathway(s). To identify novel factors involved in the degradation of defect, we performed a genome-wide CRISPRi screen. Follow up biochemical characterization of top hits confirmed their crucial role in faulty ribosome detection and degradation. This work advances our understanding of quality control mechanisms that eliminate defective ribosomal RNA in human cells. These insights into ribosomal quality control are essential for understanding cellular proteostasis and how accumulation of faulty ribosomes can drive human disease.

SG205

Mapping and engineering RNA-driven architecture of the multiphase nucleolus

*Sofia Quinodoz[1], Lifei Jiang[1], Aya Abu-Alfa[1], Troy Comi[1], Hongbo Zhao[1], Qiwei Yu[1], Lennard Wiesner[1], Jordy Botello[1], Anita Donlic[1], Elizabeth Soehalim[1], Prashant Bhat[2], Christiane Zorbas[3], Ludivine Wacheul[3], Andrej Košmrlj[1], Denis Lafontaine[3], Clifford B; Princeton University[1], California Institute of Technology[2], Université libre de Bruxelles[3]

Biomolecular condensates are key features of intracellular compartmentalization. As the most prominent nuclear condensate in eukaryotes, the nucleolus is a multiphase liquid-like structure where ribosomal RNAs (rRNAs) are transcribed and processed, undergoing multiple maturation steps to form the small and large ribosomal subunits (SSU and LSU). However, how rRNA processing is coupled to the nucleolus' layered organization is poorly understood due to a lack of tools to precisely monitor and perturb nucleolar rRNA processing dynamics. Here, we developed two complementary approaches to spatiotemporally map rRNA processing and engineer de novo nucleoli. Using sequencing in parallel with imaging, we found that rRNA processing steps are spatially segregated, with sequential maturation of rRNA required for its outward movement through nucleolar phases. By generating synthetic nucleoli in cells through an engineered rDNA plasmid system, we show that defects in SSU processing can alter the ordering of nucleolar phases, resulting in inside-out nucleoli and preventing rRNA outflux, while LSU precursors are necessary to build the outermost layer of the nucleolus. These findings demonstrate how rRNA is both a scaffold and substrate for the nucleolus, with rRNA acting as a programmable blueprint for the multiphase architecture that facilitates assembly of an essential molecular machine.

SG203

Mitochondrial Transcription Sustains Phase Co-Existence of Mitochondrial Nucleoids and RNA Granules

*Nidhi Parikh[1], Surya Penna[1], Eliza Neal[1], Marina Feric[1]; Centre of Eukaryotic Gene Regulation, Department of

Biochemistry and Molecular Biology, Pennsylvania State University[1]

Gene expression is organized into biomolecular condensates in many contexts from transcription of rRNA by Pol I in the multiphasic nucleolus to dynamic bursts of mRNA transcription by Pol II within liquid-like transcriptional clusters. How phase separation affects the flow of mitochondrial gene expression remains largely unknown. Indeed, mitochondria package their own genome (mt-genome, mtDNA) into nucleoprotein complexes called mt-nucleoids, which serve as the transcription sites of long polycistronic mitochondrial RNA (mtRNA). mtRNA transcripts quickly leave mt-nucleoids and undergo further processing and maturation within neighboring ribonucleoprotein complexes known as mtRNA granules (MRGs). Both structures have been shown to behave as biomolecular condensates. Here, we investigated the phase behavior of mt-nucleoids and their neighboring MRGs in relation to mitochondrial gene expression. With super-resolution imaging, we examined the endogenous spatial organization of these two mt-condensates relative to steady-state mtRNA in fixed human cells. Using nearest neighbor analysis, we identified wide distributions of localization, ranging from the mt-nucleoids and MRGs being a few microns apart to being fully mixed. To test if the mixing events are associated with transcription, we performed a series of experiments. Labelled nascent transcripts with EU and overexpression of fluorescently tagged mitochondrial RNA polymerase (POLRMT), showed enrichment of these markers within well-mixed mt-nucleoids and MRGs, confirming that mixing of mt-condensates is associated with active transcription. Moreover, with super-resolution live imaging, we observed frequent mixing and de-mixing events between mt-nucleoids and MRGs, resembling kissing behavior characteristic of other transcriptional systems. In support, small molecule inhibition of POLRMT led to reduced mtRNA levels, and correspondingly, to the dissolution of MRGs, indicating that RNA is a driver of MRG stability. Together, our results suggest that RNA produced upon active transcription sustains the phase coexistence between mt-nucleoids and mtRNA granules, and in this way, organizes the flow of mitochondrial gene expression.

SG201

mRNAbow: A Versatile IVT mRNA Toolkit for Multiplexed Organelle Labeling

*Heejun Choi[1], William Kasberg[1], Sarah Rubin-Elgressy[1], Jennifer Lippincott-Schwartz[1]; HHMI/Janelia Research Campus[1]

In vitro transcribed messenger RNAs (IVT mRNAs) provide a powerful platform for transient gene expression in mammalian cells, enabling rapid and tunable protein production. Unlike DNA plasmids, synthetic mRNAs bypass nuclear entry, allowing robust expression even in post-mitotic or otherwise difficult-to-transfect cell types. Because they do not integrate into the genome, IVT mRNAs offer a safe and versatile alternative to viral transduction. To develop a broadly applicable template for IVT mRNA use, we systematically examined how nucleotide modifications influence transfection efficiency and protein expression across a wide range of mammalian systems, including cancer cell lines, iPSC-derived models, primary cells, and organoids. Live-cell imaging revealed protein production within four hours of transfection, with expression scaling in a dose-dependent manner and persisting for at least 48 hours. Importantly, simultaneous delivery of multiple transcripts enabled efficient multiplexed labeling of distinct organelles within single cells, an approach we term mRNAbow. To further expand accessibility, we generated a publicly available palette of organelle-targeted mRNAs and plasmids encoding these mRNAs, the JLS 4DCP Collection. Collectively, these advances establish a generalizable framework for IVT mRNA design and application, offering both a practical toolkit for cell biology and a foundation for expanding the experimental uses of synthetic mRNAs.

SG208
Paternal effect in early embryos
*Mustafa Aydogan[1], Anand Bakshi[1]; Columbia University[1]

Maternally deposited mRNA and proteins during oogenesis provide a rich source of building blocks for the developing embryo. Termed ‘maternal effect’, the mother’s influence on embryos is the central driver of early animal development. Indeed, early embryos are considered largely transcriptionally silent pre-midblastula transition. In contrast with this classic view, an increasing body of evidence supports the presence of nascent mRNAs (<1.5kb) in early embryos, yet this is challenged with skepticism that they may represent abruptly terminated transcripts destined for degradation. Whether any zygotic transcript can contribute to pre-midblastula development in an effective way, and if so, how their influence could be dissected paternally (without influencing the maternal products) prior to gastrulation remains a significant experimental challenge. Here we innovated using Drosophila a genome-wide RNA expression-guided, nascent RNA imaging-based screening strategy to perform the first ‘paternal effect’ screen in animals. Discovering a cohort of paternal effect genes with a variety of roles in early development, our study is set to act as a springboard for future works focusing on father’s contribution to reproductive biology.

SG204
Regulation of RNA condensation by ribosomes
*Stephanie Moon[1]; University of Michigan[1]

The integrated stress response (ISR) suppresses translation initiation during stress, causing ribosomes to run off mRNAs and driving their condensation into stress granules. At the same time, stress-induced genes encoding proteins that aid cell adaptation to stress are translationally upregulated via upstream open reading frames (uORFs). We tested whether ribosome association with stress-induced gene mRNAs inhibits their condensation into stress granules using polysome profiling, smFISH, chemical genetics, molecular cloning, and live cell imaging. We found that inhibiting ribosome association with stress-induced gene mRNAs increases their localisation to stress granules, while stabilizing ribosomes on mRNAs inhibits their localization to stress granules. Surprisingly, both monosomes and polysomes equally prevented mRNA condensation, and stabilizing a single ribosome on all mRNAs inhibited stress granule assembly. Abolishing uORFs in stress-induced gene reporter RNAs demonstrates that uORFs inhibit mRNA condensation to the same extent as polysome or monosome association. We extended these findings to tRNA synthetase inhibitors and amino acid deprivation, which activate the ISR while preventing ribosome runoff of mRNAs. These stressors fail to induce stress granules despite activating the ISR. Disome and polysome profiling of cells treated with tRNA synthetase inhibitors reveals collided ribosomes that activate the ISR and are cleared within several hours. Remarkably, uncollided ribosomes persist on mRNAs within polysomes for at least 16 hours. These persistent, stalled ribosomes activate the ribotoxic stress response and inhibit RNA-protein granule assembly. Our results together suggest that ribosomes are central regulatory nodes that connect the integrated and ribotoxic stress response pathways to mRNA compartmentalization during stress.

Scholarship for Transformative Change Keynote Lecture

AW201
Education and democracy: Making civic behaviors explicit in STEM courses
*Bryan Dewsbury[1]; Florida International University[1]

There is sometimes an assumption that the affective components of a quality education experience will be easily recognized and internalized by students in our courses. As we seek to create STEM courses that position more students to be successful, we are collectively challenged to revisit the real value proposition of higher education as a whole. The direct tethering of science education to the affective and civic engagement was a powerful movement in the early 1900s. Today, challenges abound inside and outside of the classroom that requires a revisiting of this relationship. In this talk, I will focus on STEM education and position the value proposition of our practice as something far beyond the simple expansion of the technocracy, but also for the preparation of students for meaningful, authentic civic engagement.

Spatiotemporal Control of Cell Signaling Across Scales

MS2402
A Dynamical Systems Framework Applied to Live Cell Imaging of hiPSC-Derived Endothelial-Like Cells Under Flow Uncovers a First-Order Phase Transition Between Morphology-Based Cell States
*Serge Parent[1], Erin Angelini[1], Rebecca Zaunbrecher[1], Ellen Adams[1], Tiffany Barszczewski[1], Antoine Borensztejn[1], Julie Dixon[1], Renske Dupar[1], Jacqueline Edmonds[1], Maxwell Hedayati[1], Caroline Hookway[1], Chantelle Leveille[1], Jacob McCarley[1], Benjamin Morris[1]; Allen Institute for Cell Science[1], Stony Brook University[2], University of Washington[3]

The Allen Institute for Cell Science aims to develop a theoretical understanding of cell state transitions during multicellular morphogenesis, including quantitatively defining cell states. Efforts to quantify a Waddington-like landscape from the perspective of dynamical systems, in which the valleys of the landscape correspond to the attractors of the governing cell state dynamics, have largely focused on describing cell state dynamics in terms of molecular mechanisms such as gene regulatory networks. Here we use morphological data to quantify stable cell states. We use the ability of human induced pluripotent stem cell-derived endothelial-like cells (hiPSC-ECs) to align, elongate, and migrate parallel or perpendicular to the direction of flow under low and high fluid shear stress (FSS), respectively, as a model of an inducible cell state change. We developed a segmentation-free unsupervised machine learning (ML) approach to extract features from transmitted light 2D standard deviation projections from 3D spinning disk confocal timelapse videos of hiPSC-ECs expressing GFP-tagged VE-Cadherin under different FSS. We combined these features with a dynamical systems framework to construct vector field representations of the cell state dynamics. From these vector fields we identified the stable fixed points of the dynamics at varying levels of FSS. We found these stable fixed points change with FSS in a way that suggests a first-order phase transition between low and high FSS, where a fixed point bifurcates into two at an intermediate FSS. We confirmed that these stable states represented qualitative characteristics of the observed biological cell states by using the ML model to reconstruct representative images of the VE-Cadherin channel at these fixed points. We also integrated segmentation-free features with segmentation-based metrics such as cell density and alignment, and individual cell trajectories obtained from tracked hiPSC-

ECs. This analysis supported our earlier biological interpretation of the stable states and showed that cells take varied paths towards the fixed points. Our work complements existing approaches that characterize stable cell states using various types of single cell "omics" data to provide a proof-of-concept framework for quantitative identification of stable cell states from microscopy image-based data.

MS2403

ERK signaling dynamics and alveolar epithelial cell fate

*Evelyn Navarro Salazar[1], Eric Fowler[1], Celeste Nelson[1]; Princeton University[1]

The extracellular signal-regulated kinase (ERK) pathway regulates several cellular functions including proliferation, survival, and differentiation. Given its ubiquity, the specific cellular outcome downstream of ERK signaling depends greatly on the dynamics of ERK activity. The alveolar epithelium of the lung is important for gas exchange and consists of two types of alveolar epithelial cells — type 1 or AT1, and type 2 or AT2 — that are both derived from a common bipotent progenitor (BP) cell. Activation of ERK has been shown to promote differentiation of BP cells into AT2 cells and to prevent differentiation into AT1 cells. Paradoxically, however, ERK signaling is still required for survival of the latter. These findings suggest that the differences between differentiation into AT1 and AT2 cells may lie in the dynamics rather than the magnitude of ERK signaling. Here, we use a fluorescent ERK kinase translocation reporter (ERK-KTR) to track the dynamics of ERK signaling in real time as both mouse embryonic stem cell (mESC)-derived and primary BP cells differentiate into AT1 or AT2 cells. KTR localizes to the cytoplasm when ERK is active and to the nucleus when ERK is inactive. We isolated primary BP cells from ERK-KTR-expressing transgenic mouse embryos at embryonic day 16.5 and treated them with growth factors to induce differentiation. We performed time-lapse imaging to track the dynamics of KTR localization in BP cells with and without growth factors, and calculated the KTR cytoplasmic-to-nuclear (C/N) ratio as a measure of ERK activity, from which we quantified its amplitude, frequency, and duration. We used immunofluorescence to confirm expression of MUC1 (AT2 marker) and PDPN (AT1 marker) at the end of each experiment in order to correlate ERK dynamics to final differentiated fate. We found that the baseline ERK activity in BP cells is pulsatile, but once BP cells are stimulated with ligands that induce differentiation, ERK activity loses its pulsatility and becomes static. We are currently implementing optoSOS, an optogenetic tool, to mimic the temporal patterns of ERK signaling in BP cells and to test whether these dynamics are sufficient to control alveolar epithelial cell fate. We anticipate that the principles uncovered by our work will be broadly applicable to other tissues and organoid models.

MS2404

Integrating molecular dynamics and high-speed single-molecule tracking to link protein structure and function

*Safal Shrestha[1], Christopher Obara[1]; University of California, San Diego[1]

Multi-color, high-speed single-molecule tracking enables quantitative analysis of protein–protein interactions and dynamics in living cells. However, while trajectories reveal how proteins move, they rarely capture how underlying structural features govern these behaviors. To bridge this gap, we developed a general pipeline that integrates computational structural bioinformatics, molecular dynamics (MD) simulations, and correlative single-molecule tracking to directly link protein structure to motion in cells. We apply this approach to two model systems central to metabolic regulation. First, we examine VAMP-associated protein B (VAPB), an ER-

resident tether that mediates contacts with mitochondria, endosomes, and the Golgi. Our analysis identifies specific structural elements in VAPB and its binding partners that regulate the kinetics and stability of inter-organelle contact sites. Second, we investigate Protein Kinase A (PKA), a major signaling hub, and demonstrate how structural features influence its dynamic behavior in live cells. Together, this work establishes a broadly applicable framework for integrating structural and dynamic measurements of proteins, providing new insight into the molecular regulation of organelle communication and signaling.

MS2408

Metabolic-STAMP reveals spatiotemporal kinase signaling and phosphorylation node logic during GPCR-regulated insulin secretion in pancreatic ß-cells

*Mohammad Ovais Azizzanjani[1], Rachel E Turn[1], Peter K Jackson[1]; Stanford University[1]

Pancreatic β-cells rely upon a diverse range of cues to coordinate glucose-stimulated insulin secretion (GSIS), including the sensing of paracrine and metabolic factors by extracellular-facing G protein–coupled receptors (GPCRs). To determine how stimulation of different receptors leads to context-specific mechanisms that modulate GSIS, we developed Metabolic-STAMP (Synchronized Temporal-Spatial Analysis via Microscopy and Phosphoproteomics), which enables time-resolved mapping of receptor-specific, compartmentalized signaling networks on the minutes time scale (GSIS timescale). Using mouse MIN6 cells and human islets as our model system, we have mapped key receptor-specific pathways of kinase signaling cascades that we predicted coordinate differential cellular responses that all impinge on insulin secretion. As proof of principle of this pathway-specific regulation of GSIS, we focused on the differential and convergent signaling mediated by ciliary receptor FFAR4 versus GLP1R. Stimulation of each of these receptors in combination with high glucose led to increased insulin secretion, and combinatorial stimulation further amplified insulin secretion (suggesting pathway specificity). Using a combination of phosphoproteomics and imaging, we have identified key themes of convergence and divergence between the two receptor stimulations. First, we note differences in kinase signaling cascades. GLP1R stimulation, for example, exclusively activates the ERK pathway. Though both stimulate PKA, stimulation of each leads to differential phosphorylation of discrete PKA pathway machinery. While GLP1R stimulation promotes phosphorylation of PDE1C/8A, FFAR4 stimulation enhances AKAP1 phosphorylation and ciliary signaling. Together, these data highlight compartmentalized PKA signaling as a central logic gate—regulated by AKAP and PDE phosphorylation—that shapes local cAMP dynamics and tunes GSIS amplitude. We observe that FFAR4 versus GLP1R stimulation during GSIS leads to differential effects on key cellular compartments. For example, live/fixed imaging shows that FFAR4 lengthens cilia, while GLP1R has no effect. Stimulation of either GPCR reduces glucose-induced microtubule disassembly, though seemingly through different microtubule organizing centers (GLP1R through Golgi and FFAR4 through centrosomes). We observed increased microtubule acetylation in both conditions compared to high glucose, along with differential phosphorylation of HDAC6 and ATAT1 as key drivers. HDAC6 inhibition sharply decreased GPCR-driven GSIS, directly tying context-specific acetylation of microtubules to insulin release. Altogether, these data point to critical aspects of receptor pathway specificity that lie at the center of GSIS regulation.

MS2405

PIP5K-Ras bistability initiates plasma membrane symmetry breaking to regulate cell polarity and migration

*Yu Deng[1], Tatsat Banerjee[1], Peter Devreotes[1]; Johns Hopkins University[1]

Symmetry breaking is a fundamental process that underlies key cellular behaviors such as cell polarity and migration. Yet, how a uniformly structured plasma membrane and cell cortex transition into an asymmetric state, particularly in the absence of cytoskeletal assemblies or receptor-mediated inputs, remains unclear. In this study, we integrate optogenetic manipulations, targeted genetic screening, single-molecule analyses, and computational modeling to show that a core biochemical circuit involving mutually inhibitory interactions between PIP5K and RasGTP is necessary and sufficient for symmetry breaking. The process is initiated by stochastic, localized Ras activation coupled with a decrease the association of PIP5K with the membrane. A resulting localized reduction in PI(4,5)P2 facilitates the recruitment of a RasGEF to that domain, amplifying RasGTP production through a positive feedback loop. Dissociated PIP5K relocates to other membrane regions, where it suppresses Ras activation. These events dynamically segregate the membrane into distinct active and inactive zones. Beyond symmetry breaking, this biochemical circuit guides the spatial organization of downstream signaling and cytoskeletal activities, and thereby, generates protrusions involved in motility and defines the overall axis of a migrating polarized cell.

MS2407

Single-Molecule Tracking Reveals Amphiregulin Mediated Modulation of HER2

*Eric Burns[1], Mark Lemmon[2], Diane Lidke[1]; University of New Mexico School of Medicine[1], Yale School of Medicine[2]

ErbB2/HER2 is a receptor tyrosine kinase frequently amplified in cancer, especially breast cancer, where it preferentially heterodimerizes with other ErbB receptors to amplify MAPK and PI3K/AKT signaling. Unlike the epidermal growth factor receptor (EGFR), HER2 lacks a confirmed specific ligand. Amphiregulin (AREG), a known EGFR ligand, is synthesized as a transmembrane precursor and released as a soluble ectodomain containing an EGF-like domain and a heparin-binding domain (HBD) of unclear function. We asked whether soluble AREG can directly influence HER2 behavior, and whether the HBD and exogenous heparin modulate HER2 dynamics. Using single-molecule imaging and single-particle tracking of recombinant HER2 ectodomain (HER2-ECD) on supported lipid bilayers (SLBs), we find that soluble AREG markedly slows the lateral diffusion of HER2-ECD and subsequent addition of high-molecular-weight heparin further decreases mobility. Moreover, in live cells lacking endogenous EGFR expression, including CRISPR–Cas9 EGFR-knockout lines, treatment with 50 nM AREG reduces HER2 mobility at the plasma membrane. An AREG isoform lacking the HBD also decreases HER2 diffusion, but less so than wild-type AREG. These results support a model in which AREG may act as a putative HER2 ligand, with heparin/heparan-sulfate interactions mediated by the AREG HBD stabilizing HER2 complexes and extending their membrane residence. This putative AREG–HER2 engagement provides a potential mechanistic link between ligand availability, glycocalyx and extracellular matrix composition and HER2-driven signaling and may also suggest new strategies to modulate HER2 activity by targeting the AREG HBD or its heparan-sulfate interactions.

MS2406

Synchronous Erk Oscillations Control the Pace of Zebrafish Notochord Segmentation

*Priyom Adhyapok[1], James Norman[1], Michel Bagnat[1], Stefano Di Talia[1]; Duke University[1]

Building the vertebrate body plan involves segmentation of the anterior-posterior axis, a process that results in duplicated tissue units. This process relies on temporal control through signaling pathways, as segmentation must proceed in parallel with lengthening of the body axis. To achieve this control, oscillatory genetic circuits can act as biological timekeepers, regulating the pace of segmentation and development. In this talk, I will present evidence of an oscillatory system in the zebrafish notochord's outermost epithelial sheath layer. This oscillator, reported by an Erk sensor, demonstrates a periodicity of 10 hours, with cells attaining synchronous states across several hundred microns along the notochord. Using molecular and quantitative approaches, we have shown that this oscillator modulates periodic differentiation of cells to form segments of the vertebral column of the spine. Tracing the oscillator through development shows the emergence of synchronization and periodicity in the tissue leading to the onset of the segmentation process. We report that the Egf pathway, along with Erk inhibitors spry and dusp show dynamical behavior that could serve as the building blocks of the oscillator. Overall, this work provides a new in vivo system to study biological oscillations and identify paradigms in which molecular clocks can coordinate segmentation.

MS2401

Temporal-spatial regulation of protein synthesis and degradation serves as a key checkpoint of G0 transition and fate change

*Rachel Turn[1], Mohammad Ovais Aziz-Zanjani[1], Peter Jackson[1]; Stanford University[1]

Every cell division requires daughter cells to either re-enter the cycle (G1) or exit the cell cycle to G0 and undergo fate change, the predominant state for cells in the body. G0 can manifest as terminally differentiated cells with tissue-specific functions or as stem-like cells poised to re-enter division upon stimulation. However, the sequence of events enabling G0 entry and fate specification remains poorly defined. Multiple processes must coordinate in time and space with internal and external cues, or the cell fails to exit cycle and differentiate. Understanding these transitions is crucial both for basic cell biology and for translational research into how disrupted G0 programs drives oncogenesis or other pathologies. Progress has been limited by the absence of homogeneous, non-cancerous G0 model systems, particularly since many transition steps are highly localized and transient. To dissect checkpoints controlling G0 entry, we developed Spatio-Temporal Analysis via Microscopy and Proteomics (STAMP), in which we synchronized retinal pigment epithelial (RPE) cells at metaphase, allowing them synchronously transition into G0 upon serum starvation. Over the course of 36 hours, we sampled at 1–2 hour intervals for live/fixed imaging and phosphoproteomics, focusing on the known stages of the formation of the primary cilium (a hallmark of non-cycling cells that is a critical, membrane-bound signaling hub) as temporal landmark. These studies, coupled with genetic and pharmacological perturbations, allowed us to track key patterns in the stages of G0 transition both molecularly and in the context of dynamic changes in different cell compartments. One key theme that emerged from our studies was the pivotal role of targeted protein synthesis and degradation (both autophagy and proteosome-mediated) that each occurred in two waves: one immediately after the cells have completed cytokinesis, and one immediately after a fully-formed, GPCR-laden primary cilium has formed. We observed that blocking the first wave of either autophagy or protein synthesis led to failed ciliogenesis and defective establishment of cell fate. Using time-lapse SILAC proteomics and phosphoproteomics to observe what proteins are being newly synthesized and degraded over time, we observe key patterns of specific classes of proteins having time-dependent protein abundance and modification over time (e.g, a number of ciliary, vesicular traffic, and known

tumor suppressors in the first wave, and cell fate specific factors in the second wave). Altogether, these findings set the stage for mapping key signaling cues and cellular functions in time and space, with future work to deconvolve how disrupting these processes in pathology ultimately hinders establishment of a healthy, fated cell program.

Stress Signaling Networks and the Aging Cell

SG805
Beyond unfolded proteins and lipid stress: ER protein influx as a regulator of IRE1 activity
*Diego Acosta-Alvear[1]; Altos Labs[1]

Francesca Zappa, Advait Subramanian, Julia E. Conrad, Tsan-Wen Lu, Benjamin Yang, Stefka Tyanova, Peter Walter & Diego Acosta-Alvear Altos Labs Bay Area Institute of Science, Redwood City, CA The endoplasmic reticulum (ER) is essential for numerous cell functions, including synthesis, folding, and maturation of about one-third of the proteome, lipid biogenesis, and calcium homeostasis. To ensure the ER operates with fidelity, cells rely on the Unfolded Protein Response (UPR), an evolutionarily conserved stress-sensing signaling network that maintains ER homeostasis. In metazoans, the UPR is orchestrated by the ER stress sensors IRE1, PERK, and ATF6, which detect and respond to deviations in ER homeostasis. Current models posit that IRE1 activation occurs in response to two distinct molecular conditions within the ER: (1) an accumulation of unfolded proteins in the ER lumen and (2) perturbations to ER membrane lipid composition leading to changes in membrane thickness and fluidity, or lipid bilayer stress. Here, we identify a novel, third mechanism for IRE1 activation: a reduction in protein influx into the ER. This activation mode operates independently of IRE1's ER-lumenal unfolded protein sensor domain and its ability to detect lipid bilayer stress. Inhibition of translation initiation or ER protein import, using specific small molecule inhibitors and genetic tools, selectively activated the IRE1 arm of the UPR without discernible activation of PERK and ATF6. Mechanistically, a decrease in the rate of new protein entry into the ER leads to the dissociation of a specific pool of IRE1 molecules from the co-translational translocation machinery, resulting in their subsequent activation. Furthermore, activation of the Integrated Stress Response (ISR)—a fundamental homeostatic signaling network that regulates translation initiation and reduces protein synthesis—led to IRE1 activation. Pharmacological inhibition of the ISR significantly attenuated IRE1 activity. These results fundamentally expand our understanding of ER homeostasis and UPR regulation, illuminating a previously unknown role for IRE1 in surveilling ER protein import. Our findings imply that the continuous and adequate loading of the ER with new proteins is as critical for maintaining its functional fidelity as rebalancing the ER's protein-folding capacity to match cellular demands.

SG803
Coordination of Mitochondrial Stress Responses in Human Cells
*Xiaoyan Guo[1]; University of Connecticut Health Center[1]

The DELE1-HRI axis of the integrated stress response (ISRmt) is activated by most mitochondrial stress conditions, yet how the ISRmt regulates mitochondrial homeostasis remains unclear. Here, we investigated its role in regulating mitophagy, a key mitochondrial quality control mechanism. Our findings show that the ISR

suppresses PINK1-dependent mitophagy under many mitochondrial stress conditions by maintaining mitochondrial presequence protein import, independently of ATF4 activation. Mitochondrial presequence protein import efficiency is tightly linked to the rate of protein synthesis. Without ISR, increased protein synthesis overwhelms the mitochondrial import machineries, reducing import efficiency. This impairment can be mitigated by pharmacological attenuation of protein synthesis, such as with mTOR or general translation inhibitors. Under severe depolarizing stress, mitochondrial import is heavily impaired even with active ISR, leading to significant PINK1 accumulation. In contrast, mild mitochondrial stress allows more efficient protein import in the presence of ISR, resulting in lower mitophagy. Without ISR, mitochondrial protein import becomes significantly compromised, causing PINK1 accumulation to reach the threshold level necessary to trigger mitophagy. These findings reveal a novel link between ISR-regulated protein synthesis, mitochondrial protein import, and mitophagy, offering potential therapeutic targets for diseases associated with mitochondrial dysfunction.

SG804

Intercellular Communication is Required for Stress Tolerance in Metabolic Tissues

*Deepika Vasudevan[1], Shyama Nandakumar[1]; University of Pittsburgh School of Medicine[1]

Cells in metabolically active tissues with high biosynthetic and secretory demands often use robust stress-responsive mechanisms to maintain endoplasmic reticulum (ER) homeostasis. Coordinating such robust stress response mechanisms requires intercellular communication and coordination. Such modalities of intercellular communication have been relatively understudied in the context of stress tolerance. We use the Drosophila melanogaster third instar fat body to demonstrate that adipocytes communicate with each other through intercellular bridges called ring canals to buffer endoplasmic reticulum stress. The fat body supports the exponential growth from embryo to late larval stage over a short period of time through its energy storage and secretory functions, enduring a high basal level of ER stress in the process. We discovered that individual cells in the fat body are paired to one neighboring cell through ring canals. We further demonstrate that ring canals mediate rapid and highly specific intercellular cargo and organellar trafficking, and allow the transport of cytoplasmic, ER-bound and Golgi vesicular proteins. Disrupting fat body ring canals resulted in higher levels of ER stress response markers, aberrant cell size, as well as increased cell lethality in response to exogenous stress. We also find that animals with disrupted fat body ring canals display an overall delay in larval development, likely due to reduced secretion of larval serum proteins from the fat body. In sum, our work reveals a novel feature of intercellular communication in adipose tissue that serves to buffer ER stress across cells which is required for both homeostatic secretory function and maintaining tissue viability under exogenous stress.

SG801

Interrogating the function of the nuclear lamina using deep mutational scanning in human iPSCs

*Jessica Mella[1], Willow Coyote-Maestas[1], Abigail Buchwalter[1]; University of California, San Francisco[1]

The nuclear lamina is a meshwork of intermediate filaments that scaffolds and encases the genome. Mutations to the lamin A protein cause a phenotypically diverse group of diseases known as laminopathies, which primarily affect musculoskeletal, adipose, skin, or vascular tissue, even though lamin A is broadly expressed.

Disease-causing mutations can affect the turnover, abundance, and localization of lamin A in affected tissue. However, we do not understand how lamin mutations drive cell type-specific dysfunction. We have implemented deep mutational scanning (DMS) in human induced pluripotent stem cells (hiPSCs). With this approach, we can systematically profile the effects of lamin A sequence variation across pluripotent and differentiated cell types. Using serine integrase cassette exchange, we reconstituted lamin A knockout hiPSCs with a pooled library of ~18,000 untagged missense, insertion, deletion, and nonsense lamin A variants precisely integrated as a single variant per cell. By screening the effects of sequence variation on hiPSC fitness, we found that mutations to lamin A regions important for head-to-tail filament assembly are toxic to stem cells, even though lamin A is not required for hiPSC survival. We then analyzed the expression of the variant pool by immunostaining and flow sorting, which revealed that variants in same the head-to-tail interface are destabilized at the protein level. These toxic variants are rare in the human population and include known laminopathy mutations, highlighting the importance of head-to-tail assembly for the stability and function of the lamin meshwork. We are now screening lamin A abundance during differentiation into cardiac lineages, which are particularly affected in laminopathies. This DMS platform will allow us to explore cell type-specific mutational effects and to understand lamin A genotype-phenotype relationships in disease.

SG802

Using mathematical modeling to understand mechanisms of microtubule dynamics and polarity in neurons

*Anna Nelson[1], Melissa Rolls[3], Maria-Veronica Ciocanel[2], Scott McKinley[4]; University of New Mexico[1], Duke University[2], Pennsylvania State University[3], Tulane University[4]

In neurons, sustained intracellular transport of cargo depends on a stable and correctly oriented microtubule cytoskeleton. At the same time, microtubules also must also be dynamic and reorganize in response to neuronal injury. How this balance is achieved remains an open question. Using information from experimental measurements in Drosophila sensory neurons, we developed a stochastic mathematical model to explore mechanisms that regulate microtubule length. The model incorporates limited tubulin availability and the dependence of shrinking events on microtubule length (length-dependent catastrophe). We also developed a reduced deterministic model that validates and guides our choices of parameters for the more complex stochastic framework. The model recapitulated experimental measurements like microtubule growth velocities and growth durations. With different combinations of length-regulating mechanisms, we achieved a variety of microtubule length distributions. Without length-dependent catastrophe, the distribution of microtubule lengths was exponential-like, with few very long microtubules and many short ones. When length-dependent catastrophe was implemented, the resulting distribution of microtubule lengths was bell-shaped which agrees with biological expectation. This suggests that there could exist some mechanism that increases catastrophe frequency as microtubules become longer. To determine how different tubulin levels may impact microtubule growth dynamics, we manipulated tubulin in the model and found that an increase in tubulin leads to faster growth speeds and longer average microtubule lengths. Finally, the mathematical framework proposed can also be used to validate growth dynamics using tubulin turnover experiments, which can measure microtubule stability and turnover in vivo. We find the timescale of fluorescent tubulin turnover increases with increasing tubulin levels and that dependence of shrinking events on microtubule lengths results in tightly regulated fluorescence decay over all tubulin levels. These findings further support the existence of a length-dependent catastrophe mechanism in neurons. Overall, this modeling framework generates testable predictions for experiments that manipulate tubulin in neurons which could be used to validate hypothesized model

mechanisms. Insights from these models can then be used to understand how microtubules can organize into polarized structures in neurons, and how microtubule dynamics may impact cargo localization post-injury.

Structure, Function and Mechanics of the Nuclear Envelope

MS1002
An ESCRT grommet cooperates with a diffusion barrier to maintain nuclear integrity
*Nicholas Ader[1], Linda Chen[2], Ivan Surovstev[2], William Chadwick[2], Elisa Rodriguez[2], Megan King[2], C. Lusk[2]; University of North Carolina Greensboro[1], Yale School of Medicine[2]

The molecular mechanisms by which the endosomal sorting complexes required for transport (ESCRT) proteins contribute to the integrity of the nuclear envelope (NE) barrier are not fully defined. We leveraged the single NE hole generated by mitotic extrusion of the Schizosaccharomyces pombe spindle pole body to reveal two modes of ESCRT function executed by distinct complements of ESCRT-III proteins, both dependent on CHMP7/Cmp7. A grommet-like function is required to restrict the NE hole in anaphase B, whereas replacement of Cmp7 by a sealing module ultimately closes the NE in interphase. Without Cmp7, nucleocytoplasmic compartmentalization remains intact despite NE discontinuities of up to 540 nm, suggesting mechanisms to limit diffusion through these holes. We implicate spindle pole body proteins as key components of a diffusion barrier acting with Cmp7 in anaphase B. Thus, NE remodelling mechanisms cooperate with proteinaceous diffusion barriers beyond nuclear pore complexes to maintain the nuclear compartment.

MS1003
Lamin-B1 maintains nuclear peripheral protein localization to promote proper genome organization and efficient cardiomyocyte differentiation during early development.
*Katherine Bossone[1], Xiaobin Zheng[2], Youngjo Kim[3], Yixian Zheng[2]; Johns Hopkins University[1], Carnegie Institution for Science[2], Soonchunhyang Institute of Medi-Bio Science[3]

The nuclear lamina consists of a meshwork of type V intermediate filaments called lamins. These filaments have roles in maintaining nuclear integrity and genome organization. In mammalian cells, there are four lamin isoforms that are encoded by three genes: Lmnb1 and Lmnb2 encode for lamin-B1 (LB1) and lamin-B2 (LB2), respectively, and Lmna encodes for lamin-A and the alternatively-spliced lamin-C (LA/C). Most lamin-associated cardiomyopathies involve mutations in LA/C, which have made them a focus of heart-related research. At a cellular level, loss of LA/C in cardiomyocytes results in reduced nuclear integrity, disrupted cytoskeleton, defects in genomic organization, and delays in differentiation. It is debated how much of the disease pathology is due to DNA and nuclear damage and how much is by impaired genome organization and aberrant transcription. In contrast to LA/C, the role of LB1 in cardiomyocytes is not well studied. However, since the heart starts developing and beating at E7.5, well before LA/C expression, LB1 may have an unrecognized role in early heart development. Using a cardiomyocyte-specific knockout of LB1, we had found the mice are viable and fertile but show mild cardiac defects compared to their heterozygous and wildtype counterparts. There is no significant increase in DNA damage or cell death in mutant animals from embryonic to postnatal development. We found that LB1 KO caused significant disruption of H3K9me3 foci and polarization of H3K9me2, that persists from embryonic timepoints into adulthood. Interestingly, although the

LINC complexes and nuclear pore complexes (NPCs) also show asymmetric distribution to one side of the embryonic cardiomyocyte nuclei, these defects are rescued by 1 week of age. Whole chromosome painting shows large-scale and irreversible chromosome decompaction and stretching, which we attribute to chromatin interacting with polarized nuclear proteins being pulled away from the regions tethered to LA/C on the opposite side. These effects on genome organization are transcriptionally relevant, as RNA-seq of embryonic and postnatal hearts indicated dysregulation of genes associated with ion handling and sarcomere formation, suggesting impaired differentiation, signaling, and contractility. Collectively, these results show that LB1 is essential for genome organization, in part by maintaining nuclear periphery distribution, which supports timely cardiomyocyte differentiation and maturation. We further show that cardiomyocytes appear robust to alternative genome organizational states as these cells eventually differentiate and form a viable heart. These results may provide interesting insights to laminopathies or other pathologies where genome organization is disrupted.

MS1008

Membrane dynamics driven by the Arp2/3 complex regulate myofibers growth and myonuclear accretion during skeletal muscle postnatal development

*Catarina Sequeira[1], Silvia Di Francescantonio[1], Gabriele Motta[1], Edgar Gomes[1]; GIMM Foundation - Gulbenkian Institute for Molecular Medicine[1]

Skeletal muscle is a highly organized tissue, composed of large multinucleated cells called myofibers. During postnatal development, muscle growth is mainly driven by the addition of new myonuclei to the myofibers (myonuclear accretion) through the fusion of mononucleated progenitor cells (myoblasts). Cell-cell fusion in skeletal muscle has been extensively studied in myoblasts, but the mechanisms involved in myofibers fusion are largely unknown. The Arp2/3 complex is a seven-subunit complex that polymerizes branched actin networks, having a wide range of cellular functions. In skeletal muscle, the Arp2/3 complex was shown to be involved in the fusion of myoblasts during embryonic development, in the migration of myonuclei to the periphery of the myofibers, and in the transverse organization of T-tubules in triads. Our study aims elucidate how Arp2/3-mediated actin dynamics contribute to myofibers postnatal development in vivo. Using an inducible skeletal muscle-specific knockout mice model, we depleted Arpc4 (a core subunit of the Arp2/3 complex) in myofibers during the first postnatal week (Arpc4-/-). When compared to littermate controls, Arpc4-/- mice presented smaller myofibers with less myonuclei. We found that myoblast fusion into Arpc4-depleted myofibers is severely inhibited both in vitro and in vivo, leading to an accumulation of proliferating myoblasts and a reduction in myonuclear accretion in Arpc4-/- mice. This impairment in myofibers postnatal growth directly affected muscle function in vivo, since P7 Arpc4-/- neonates presented gait abnormalities (asymmetric movement and increased foot angles) and signs of muscle weakness when performing the hindlimb suspension test. Moreover, we developed a co-culture system to study myoblast-myofiber interaction and fusion in vitro. Our results demonstrated that the Arp2/3 complex accumulates at the plasma membrane of differentiated myofibers, specifically in regions of contact with myoblasts. Additionally, myofibers generate membrane extensions enriched in the Arp2/3 complex when interacting with neighboring myoblasts before fusion. The formation of these membrane extensions depends on the activity of the Arp2/3 complex, as they were significantly reduced following Arpc4 depletion in myofibers. Overall, our findings unveil a new fundamental role of the Arp2/3 complex in mediating myofibers growth during skeletal muscle postnatal development.

MS1005

Muscular dystrophy-associated lamin mutations disrupt cytoplasmic macromolecular crowding via a nucleolar-ribosome axis.

*Xiangyi Ding[1], Hongyan Hao[1], Daniel Elnatan[1], Sweta Kumari[1], Patrick Alinaya[1], Shilpi Kalra[1], Abby Kaur[1], Ellen Gregory[1], Liam Holt[2], G. W. Gant Luxton[1], Daniel Starr[1]; University of California, Davis[1], NYU Langone Health[2]

Understanding how cells control their biophysical properties during development remains a fundamental challenge and its regulation in living animals remains poorly understood. Laminopathies, including Emery-Dreifuss muscular dystrophy (EDMD), arise from mutations in nuclear lamins. Most models of how laminopathies lead to pathologies focus on disruptions of nuclear mechanics and/or gene expression. How lamins regulate the biophysical properties of the cytoplasm is largely unexplored. Here, we reveal a novel pathway linking EDMD-associated lamin mutations to broad cellular disorganization through nucleolar and ribosomal dysfunction. We perform in vivo rheology in C. elegans using our recently developed approach with genetically encoded multimeric nanoparticles (GEMs). We previously used GEMs to discover that C. elegans tissues maintain mesoscale properties that differ from those observed across diverse systems, including bacteria, yeast species, and cultured mammalian cells. We identified two conserved mechanisms controlling particle mobility: ribosome concentration, a known regulator of cytoplasmic crowding, works in concert with a previously unknown function for the giant KASH protein ANC-1 in providing structural constraints through associating with the endoplasmic reticulum. Here, we demonstrated that EDMD-associated mutations significantly disrupt cytoplasmic biophysical properties, reducing mesoscale macromolecular crowding and increasing the diffusivity of cytoplasmic particles in C. elegans models of EDMD. These changes coincide with severe nuclear positioning defects and collapsed endoplasmic reticulum architecture, phenocopying the effects of ribosome depletion. We establish that the C. elegans lamin lmn-1 and ribosomes genetically interact to regulate cellular organization. Crucially, the lmn-1(R64P) EDMD model disrupts nucleolar density and reduces ribosome levels, placing lamin dysfunction upstream of ribosome biogenesis. Together, these findings identify a nucleolar-ribosomal axis through which nuclear lamina defects propagate through biophysical defects of the cytoplasm that in turn disrupt cellular architecture. Thus, we establish a new mechanistic framework for understanding EDMD and other laminopathies, suggesting potential therapeutic approaches targeting ribosome biogenesis or cytoplasmic crowding.

MS1006

Nuclear envelope-endoplasmic reticulum junctions regulate the diffusional exchange of proteins to the nuclear envelope.

*Marc Goudge[1], Sylvi Stoller[1], Jesús Rojas Hernández[1], Joerg Bewersdorf[1], Shirin Bahmanyar[1]; Yale University[1]

The nuclear envelope (NE) is a specialized subdomain of the endoplasmic reticulum (ER), primarily responsible for the compartmentalization of the cytoplasm from the nucleoplasm. Key to its successful function is both the specific subset of enriched NE proteins, in addition to the shared pool of resident ER proteins, which collectively serve to maintain compartmental homeostasis. In theory, the direct continuity of the ER to the NE should provide unbiased access to these protein pools. In practice, topological constraints lead to unequal opportunities for molecular exchange between the two compartments. The ER and NE are connected through

a limited number of poorly characterized NE-ER junctions, and any molecular exchange between the two regions will by definition be dependent on their distribution throughout the membrane. Additionally, the ER's complex and convoluted structural topology precludes uniform, omnidirectional molecular movement – protein diffusion can only happen within the trajectories defined by the organelle's membranes. While recent work has highlighted the capacity of such topologically constrained diffusion to asymmetrically alter protein accessibility at the peripheral ER, little is known as to how the ER's topological complexity and connectivity to the NE may influence the availability of proteins broadly at the NE. Borrowing from dynamic trafficking assays within the secretory system, we designed a chemically-inducible dimerization system that allows for the monitoring of protein exchange to the nuclear envelope. Using this system, we demonstrated the capacity of ER organization to influence the effectively available proteome at NE. We show that the NE has diffusionally-buffered regions, which, as a function of their sparse connectivity to the ER, are less readily able to access ER-resident proteins. Finally, we show as a proof of concept that this system allows for the microscopic identification of bonafide NE-ER junctions, where protein flux can be isolated to individual segments of membrane with high-speed live-cell microscopy.

MS1004

Phosphoinositide Phosphatase Myotubularin (MTM1) Regulates Myonuclear Architecture and Regional Chromatin Modifications

*Matthew Haemmerle[1], Mo Zhao[2], Nesrin Sabha[2], Emanuela Pannia[2], James Dowling[1]; University of Pennsylvania[1], The Hospital for Sick Children[2]

X-linked myotubular myopathy (XLMTM) is a severe congenital muscle disease that primarily affects boys and is characterized by ventilator and wheelchair dependence and, in many cases, early death. XLMTM is caused by mutations in the MTM1 gene, which encodes a phosphatase that dephosphorylates second messenger lipids PI3P and PI3,5P2 and has roles in endosomal lipid dynamics and trafficking. Pathologically, the disease is defined by hypotrophic muscle fibers containing disorganized organelles and myonuclei with aberrant appearance and localization. However, despite the fact that XLMTM is partly defined by the appearance of abnormal myonuclei, the contributions of the nuclear alterations to XLMTM pathogenesis are unknown, and the reason(s) for the nuclear aberrations are not well understood. In this study, we provide insight into these longstanding open questions by demonstrating for the first time a role for MTM1 in myonuclear structure and genome architecture, abnormalities in which lead to alterations to the myogenic gene program and muscle development. While previous studies have classified MTM1 as a strictly cytoplasmic protein, we have discovered using high-resolution confocal and immunoelectron microscopy that a subpopulation of MTM1 protein localizes to the nucleus and nuclear envelope. Furthermore, Mtm1 deficiency in vivo gives rise to ultra-structurally abnormal myonuclei ($p<0.0001$), altered genome organization (>30% of the genome), and shifted chromatin accessibility (16% of genomic sequences). Excitingly, introduction of nuclear targeted-MTM1 in the muscle of Mtm1-deficient mice significantly improves the XLMTM phenotype, with increased myofibril size, increased mouse rears (11 vs 6.3, $p<0.05$), and prolonged survival (50 vs 38 days, $p<0.05$), demonstrating an essential role for MTM1 at the nucleus. We are currently investigating the sub-nuclear localization of MTM1 and MTM1 nuclear interactome using high-resolution microscopy and proximity labeling followed by mass-spectrometry. Together, these findings present a paradigm shift in the understanding of MTM1 function during muscle development and provide new insight into the underlying mechanisms of XLMTM pathology.

MS1001
Rainbow Nucleus Charts Dynamic Interactome of Membrane-less Organelles
*Songtao Ye[1], Nadav Goldfajn[1], Choon Leng So[1], Takanari Inoue[2], Danfeng Cai[1]; Johns Hopkins Bloomberg School of Public Health[1], Johns Hopkins School of Medicine[2]

Membrane-less organelles (MLOs) are dynamic cellular structures that carry out essential biological functions without lipid membranes. While individual MLOs have been extensively studied, how MLOs interact and coordinate with each other to carry out cellular functions is unknown. In this work, we developed the "Rainbow Nucleus", a multi-spectral imaging tool to simultaneously visualize the interactions among five nuclear MLOs. First, through multiplexed imaging, we discovered that different MLOs occupy characteristic nuclear spaces and harbor unique cellular biophysical properties. Additionally, we identified two distinctive MLO interaction dynamics, stable and transient interactions, existing among different MLO pairs using time-lapsed spectral imaging. Stable interactions persisted for longer durations with few breakoffs, and occur frequently between functionally coordinated MLOs, such as between Cajal body and nuclear speckle. By contrast, transient interactions formed between functionally unrelated MLOs and exhibited "kiss and run" contact patterns. Furthermore, by plotting the inter-MLO interactome network, we found that the MLO interactions are not random: nuclear speckle serve as the central hub that organizing pairwise interaction networks with Cajal body, histone locus body, and PML nuclear body, while the nucleolus is excluded from the main MLO interaction network. Finally, we demonstrated that active transcription is fundamental for maintaining inter-MLO interactions, as inhibiting transcription completely rewired the MLO interactome network and caused markedly reduced MLO interaction frequency. Collectively, enabled by multi-spectral imaging using "Rainbow Nucleus", our study uncovers different modality of MLO interaction dynamics, and maps the first comprehensive MLO interactome network of five MLOs under different conditions. These findings establish a foundation for future efforts to reprogram the MLO organization as a therapeutic strategy.

The Centrosome in Cell Division, Development, and Disease

MS1607
A KASH-less Msp300 isoform has a novel outer nuclear membrane targeting domain and scaffolds the non-centrosomal microtubule organizing center in fat body cells
*Tania Sultana[1], Chunfeng Zheng[1], Timothy Megraw Ph.D.[1]; Florida State University[1]

In Drosophila fat body cells, nucleus-associated non-centrosomal microtubule-organizing centers (ncMTOCs) coordinate nuclear positioning and intracellular trafficking. The nesprin homolog Msp300 anchors this perinuclear ncMTOC and recruits the microtubule minus-end stabilizer Patronin and other microtubule regulators. Among the 11 Msp300 isoforms, we show that isoform Msp300-PE is necessary and sufficient for ncMTOC formation, despite lacking a canonical KASH domain that anchors conventional nesprins to the outer nuclear membrane (ONM) through association with a SUN domain protein anchored in the inner nuclear membrane (INM). To dissect its unique function in organizing the ncMTOC, we are investigating Msp300-PE localization in fat body cells and examining the phenotypic consequences of targeted CRISPR truncation mutants, focusing on its role in microtubule organization and nuclear positioning. We demonstrate that the unique C-terminus of Msp300-PE, comprising a putative transmembrane helix followed by a 35-aa domain that

resides within the nuclear intermembrane space, is sufficient to target the ONM. This finding identifies a novel ONM-targeting domain at the Msp300-PE C-terminus with no sequence similarity to the canonical KASH domain. Notably, this short tail contains seven Cys residues that we propose form disulfide bridges with an unknown partner residing at the INM, a hypothesis supported by non-reducing SDS-PAGE. We are employing mass spectrometry and genetic screens to identify the putative INM partner required for Msp300-PE anchoring and ncMTOC function. This study aims to define the molecular mechanisms by which Msp300-PE, together with its unique domains and partners, generates a perinuclear ncMTOC in fat body cells.

MS1608
A mechano-chemical model for γTuRC-mediated microtubule nucleation
*Kenneth Tsui[1], Shannon Stewman[1], Ao Ma[1]; University of Illinois at Chicago[1]

Nucleation by the γ-tubulin ring complex (γTuRC) is the primary mechanism for microtubule (MT) multiplication in cells, yet its mechanism remains poorly understood—the prevailing template activation model cannot explain key experimental observations. We present a structure-based mechano-chemical model that integrates MT dynamic instability with a two-step open-to-closed transition of γTuRC. Closure resembles the MT sheet-to-tube transition, but with cone rather than tube geometry. The first step requires straightening of γ-tubulin complex proteins (GCPs), enabling lateral bonding between α,β-tubulin subunits bound to γ-tubulins, which supports sheet growth from γTuRC, suppressing catastrophe and promoting persistent elongation. The second step involves lateral rotation of GCPs and a bent-to-straight transition of γ-tubulins, driving sheet-to-tube conversion and yielding longer, more stable MTs and ensuring efficient nucleation. Simulations reproduce all recent in vitro results on γTuRC-mediated nucleation and reveal the underlying mechanism as an ordered sequence of conformational changes in GCPs and α, β, γ-tubulins.

MS1606
A Novel Mechanism for Centrosome Expulsion Ensures Metabolic Activity in Polyploid Cells
*Renata Basto[1], Riham Salame[1], Simon Gemble[1], Anthony Simon[1], Lin Jawish[1], Ptissam Bergam[1], Graca Raposo[1], Clotilde Cadart[2]; Institut Curie[1], Institut Cochin[2]

Programmed polyploidy is often linked to increase cell size to support enhanced metabolism, barrier function or regeneration. In certain polyploid cells, the cytoskeleton is drastically remodeled- most notably by eliminating centrosomes. However, the purpose and mechanisms underlying centrosome elimination have remained unclear. We investigated this question in Drosophila acentrosomal salivary glands (SGs), a physiological polyploid model where cells reach high chromosome content through endoreplication. Using genetic tools, live imaging approaches, super-resolution microscopy combined with tissue clearing and electron microscopy, our study uncovers a novel centrosome elimination pathway in vivo. This process requires non-muscle myosin II (MyoII) activity and the macroautophagic machinery to drive centrosome release into the lumen of the salivary glands via autolysosomal exocytosis. Failure to eliminate centrosomes disrupts the mitochondria network, impairing respiration and ATP production. Our findings reveal a previously unknown mechanism that removes centrosomes through the secretory autophagy pathway to protect mitochondrial function and support the high metabolic demands of polyploid cells.

MS1604

Dynamic remodeling of centrioles and the microtubule cytoskeleton in the lifecycle of fungi with motile cilia

*Alexandra Long[1], Krishnakumar Vasudevan[4], Andrew Swafford[2], Claire Venard[4], Jason Stajich[5], Lillian Fritz-Laylin[6], Jessica Feldman[4], Tim Stearns[3]; University of Kentucky[1], Middlebury College[2], Rockefeller University[3], Stanford University[4], University of California, Riverside[5], University of Massachusetts, Amherst[6]

Cells reorganize in space and time to move and divide – complex behaviors driven by their internal cytoskeleton. As cells transition between these states, their cytoskeletal structures are remodeled to meet new needs. For example, in many organisms, microtubule-based centrioles transit from serving as the ciliary base to become the center of the centrosome. While the molecular components and general principles of cytoskeletal assembly are well studied, less is known about cytoskeletal remodeling to support different cell states and functions and how remodeling pathways emerge and change in evolution. To study remodeling of the microtubule cytoskeleton in an evolutionary context, we use the chytrid fungus, Rhizoclosmatium globosum, that retains centrioles and cilia lost in most other fungal lineages. Chytrids undergo extensive reorganization of their microtubule cytoskeleton as they grow from a motile zoospore with a cilium to a sessile multinucleated coenocyte in which new zoospores are built. Thus, they provide an ideal system in which to study remodeling of the cytoskeleton as they shift between having motile cilia and centrosomes. We use evolutionary comparison, RNA sequencing, and expansion microscopy to understand this reorganization and further develop this organism as a model for evolutionary cell biology. We find that when motile zoospores transition to sessile sporangia, cilia are retracted into the cytoplasm and degraded, while centrioles detach from the ciliary axoneme yet persist. During the mitotic cycles, short centrioles are associated with a centrosome-like microtubule-organizing center (MTOC) and a dense microtubule array at the spindle pole. After the mitotic cycles, centrioles elongate and form cilia, driven by transcription of genes associated with centriole maturation and ciliogenesis, and microtubule bundles are reorganized. Thus, in chytrids structural remodeling of the centriole is temporally coupled to specific changes in cytoskeletal organization over the coenocytic life cycle.

MS1605

Isotropic ultrastructural imaging reveals novel membrane organization in ciliogenesis initiation

*Quanlong Lu[1], Huijie Zhao[1], Ziam Khan[1], Adam Harned[1], Erina Kamiya[1], Valentin Magidson[1], Abhi Senthilkumar[1], Sumeth Perera[1], Kedar Narayan[1], Christopher Westlake[1]; National Institutes of Health[1]

The primary cilium is essential for development and tissue homeostasis, and its dysregulation is implicated in ciliopathies and cancer. However, the early membrane remodeling events that initiate ciliogenesis remain poorly defined. Using 3D volume electron microscopy and super-resolution imaging, we characterized the ultrastructural and molecular framework governing the initial stages of cilium formation. We show that the enlargement of small distal appendage-docked vesicles marks a critical transition point for ciliogenesis progression. This process is dependent on the distal appendage protein CEP164. These vesicles subsequently fuse to generate distinct tubular intermediates: a C-shaped ciliary vesicle (CCV) structure and a toroidal ciliary vesicle (TCV). These intermediates ultimately assemble into the mature ciliary vesicle that caps the mother centriole distal end. The formation and remodeling of these tubulovesicular structures require the membrane

trafficking regulators EHD1 and RAB8, as well as the intraflagellar transport protein IFT88. Notably, EHD1 promotes ciliogenesis by driving membrane tubulation and facilitating removal of the CP110/CEP97 cap from the mother centriole, an essential step for axoneme initiation. We also find that these tubular intermediates coincide with the recruitment of transition zone proteins, suggesting their role in establishing the ciliary gate. Our findings redefine early ciliogenesis by revealing a novel sequence of membrane remodeling events and identifying key molecular players driving this process. This study underscores the power of isotropic 3D ultrastructural imaging and quantitative analysis for unraveling membrane trafficking and organelle biogenesis mechanisms.

MS1603

Liver regeneration CRISPR screen identifies E3 ligase NEURL1B regulates hepatocyte mitosis by destabilizing microtubule organizing centers

*Dingzi Yin[1], kirk wangensteen[1]; Mayo Clinic[1]

Background and aims: Centrosomes serve as the microtubule organizing centers (MTOCs) of most cells. Supernumerary centrosomes can lead to multipolar spindles, errors in chromosome alignment and prolonged mitosis. Hepatocytes naturally harbor supernumerary centrosomes but they can proliferate extensively with bipolar spindles, accurately separating chromosomes. How hepatocytes overcome these challenges to efficiently divide is unclear. Methods: To understand how hepatocytes cope with mitotic challenges, we established a high-quality in vivo CRISPRi screen in regenerating mouse liver. By cross-referencing with gene essentiality data in cell lines, we identified genes specifically required for hepatocyte proliferation. Knockout mice, RNA sequencing and proteomic approaches were used to understand the function of the candidate gene and the mechanism in regulating hepatocytes proliferation. Result: We found E3 ligase NEURL1B is essential for hepatocyte division during liver regeneration and tumorigenesis, yet it is dispensable in other cell types and liver development. This dependence on NEURL1B arises from a hepatocyte-specific pathway of spindle pole organization. NEURL1B localizes to microtubule-organizing centers (MTOCs) and destabilizes them. NUMA1 is a substrate of NEURL1B. Prophase hepatocytes exhibit multiple MTOCs organized by NUMA1. These MTOCs undergo dynamic remodeling to form two spindle poles through clustering, and a rarely described mechanism of centrosome inactivation. The MTOCs destabilization activity of NEURL1B promotes inactivation of extra centrosomes and facilitates mitosis progression. Human single nucleus RNA-seq and cell culture data support a similar role for NEURL1B in human liver regeneration. Conclusion: Our approach of studying mitosis in vivo reveals a unique mechanism used by hepatocytes to manage supernumerary centrosomes and identifies NEURL1B as a critical regulator in this pathway, paving the way to new treatments for liver regeneration and cancer.

MS1601

Orb2 is required for neural stem cell centrosome and cell size asymmetries

*Joey Buehler[1], Dorothy Lerit[1]; Emory University[1]

Neural stem cells (NSCs) orchestrate repeated rounds of asymmetric cell division to pattern the developing brain with neurons and glia. Each cell cycle, the NSCs differentially segregate cell fate determinants to regenerate one larger, self-renewing stem cell and a smaller daughter cell fated for differentiation. Prior work

by our group and others indicates the asymmetric cell division of NSCs is regulated, in part, by the asymmetric activities of the NSC centrosomes. We determined that Drosophila Orb2, a cytoplasmic polyadenylation element binding (CPEB) protein involved in post-transcriptional regulation, supports NSC centrosome asymmetry and brain growth, but the mechanisms behind these phenotypes remain incompletely understood. We found that Orb2 is required for the transient inactivation of the NSC basal centrosome in the third larval instar. Moreover, we discovered steady state protein levels of the master regulator of centrosome activation, Polo kinase, are significantly decreased in orb2 mutant brains. Consistently, live imaging reveals a significant reduction of Polo localization to orb2 NSC centrosomes. We are currently testing whether downstream centrosome maturation effectors compensate for reduced Polo, leading to basal hyperactivation. In addition to impaired centrosome asymmetry, orb2 NSCs are significantly smaller than controls, highlighting a defect in daughter cell size asymmetry. Live imaging shows orb2 specifically regulates daughter cell size asymmetry near the onset of cytokinesis. Our current efforts are examining whether altered distributions of cortical myosin underlie a more symmetric positioning of the cytokinetic furrow. Additionally, we are using MARCM clones to determine if the proliferative capacity of orb2 NSCs is compromised. Taken together, this work supports a model whereby Orb2 contributes to NSC asymmetry required for proper neurodevelopment.

MS1602
The Unkempt RNA-binding protein reveals a local translation program in centriole overduplication
*Abraham Martinez[1], Alex Stemm-Wolf[1], Ryan Sheridan[1], Matthew Taliaferro[1], Chad Pearson[1]; University of Colorado School of Medicine[1]

Excess centrosomes cause defects in mitosis, cell-signaling, and cell migration, and therefore their assembly is tightly regulated. The divergent Polo kinase, PLK4, controls centriole duplication at the heart of centrosome assembly, and elevated PLK4 levels promote centrosome amplification (CA), a founding event of tumorigenesis. Here, we investigate the transcriptional consequences of elevated PLK4 and find Unkempt (UNK), a gene encoding an RNA-binding protein with roles in mRNA translational regulation, to be one of only two upregulated mRNAs. UNK protein localizes around centrosomes and with CEP131-positive centriolar satellites, promoting CEP131 localization to and around centrosomes. UNK's RNA-binding activity is required for PLK4-induced centriole overduplication. Consistent with the loss in PLK4-induced centriole overduplication, UNK depletion disrupts PLK4 and centriole assembly protein localization. Finally, translation is enriched at centrosomes and centriolar satellites, with UNK and CEP131 promoting this localized translation. In summary, UNK and CEP131 promote PLK4 localization and local translation at centrosomes during centriole overduplication. <div widget-instance-contentmetadata_articlefulltext_article""=""> Subjects:

The Endoplasmic Reticulum: Master Regulator of Cellular Homeostasis

MS2306
Derlins in control: shaping sphingolipid metabolism and mitochondrial dynamics
*Nicola Scott[1], Dhanendra Tomar[5], Zer Vue[4], Antentor Hinton[4], Melanie McReynolds[2], Sonya Neal[3]; University of California, San Diego[1], Pennsylvania State University[2], University of California San Diego[3], Vanderbilt University[4], Wake Forest University[5]

The ER and mitochondria coordinate key processes including protein quality control, calcium signaling, and energy metabolism. While Derlin proteins are classically linked to ER-associated degradation (ERAD), their individual roles in organelle homeostasis remain unclear. Using CRISPR-engineered HEK293T cell lines, we find that Derlin-2 knockout (KO) causes pronounced cellular defects, including increased ER stress, reduced proliferation, and increased apoptosis, unlike Derlin-1 or Derlin-3 KO. Derlin-2 loss also disrupts mitochondrial integrity, resulting in fragmented morphology, decreased calcium uptake, impaired respiration, and alters mitochondria-ER contact sites (MERCs), highlighting a unique role in inter-organelle communication. Furthermore, lipidomic profiling revealed distinct membrane lipid alterations specific to Derlin-2 KO cells. When we conducted a proteomic screen, we identified a negative regulator of sphingolipid metabolism, ORMDL3, as a likely Derlin-2 substrate, which was then confirmed through degradative assays. These findings uncover a non-redundant function for Derlin-2 in maintaining ER-mitochondria homeostasis and challenge the prevailing view of Derlin paralog redundancy.

MS2304
Direct visualization of ER-associated degradation by correlative, real-time single protein tracking.
*Christopher Obara[1], Tim Abel[2], Lilly Castorena[2], Sonya Neal[3]; University of California, San Diego[1], UC San Diego[2], University of California San Diego[3]

Targeted degradation of proteins is an essential aspect of life, as old, damaged, or misfolded proteins must be degraded to avoid the buildup of nonfunctional or aggregating forms. In eukaryotes, approximately 25-35% of proteins are translated into or through the membrane of the endoplasmic reticulum (ER), and these proteins must be extracted back into the cytoplasm for effective degradation by the proteosome if they are damaged. This process is collectively known as ER-associated degradation (ERAD), and it serves as a major quality control system in eukaryotic cells. Despite decades of research, the mechanism of ERAD remains a fundamental mystery, since the process is sufficiently precise to distinguish functional and nonfunctional forms of the same protein, yet it works for a very large range of proteins with no apparent relationship in sequence or structure. The core of ERAD is the process of retrotranslocation, where substrates are trafficked back across the ER membrane by a set of large protein complexes centered on a conserved set of membrane-associated E3 ubiquitin ligases. In this work, we utilize our new multicolor, high-speed single protein tracking system to directly visualize the dynamic assembly and disassembly of the Hrd1 retrotranslocon on the ER memebrane in living cells. Using quantitative single molecule bleaching analysis, we show that Hrd1 complexes are frequently in higher oligomeric states than the existing monomer/dimer models predict, and these higher order structures are entirely dependent on the HAF-H domain. Additionally, we show for the first time the real-time translocation of single misfolded proteins, and association with other ERAD factors including the p97/VCP ATPase, several Derlin pseudoproteases, and the Hrd3 substrate adaptor.

MS2307
ER-Shaping Proteins and Contact Site Organization in the Differentiated Sarcoplasmic Reticulum
*Ana Pereira[1], Assia Amazit[2], Catarina Bento[1], Alexis Filachet[2], Edgar Gomes[1]; GIMM - Gulbenkian Institute for Molecular Medicine, Avenida Prof. Egas Moniz, 1649-028 Lisboa, Portugal[1], UFR Science de la vie, Université Paris Cité[2]

During development, the skeletal muscle cell endoplasmic reticulum transforms into the sarcoplasmic reticulum (SR), forming a highly organized structure with two main morphologies: longitudinal SR and junctional SR. The longitudinal SR consists of elongated tubules running parallel to muscle fibers and is primarily responsible for reabsorbing calcium following muscle contraction. In contrast, the junctional SR forms enlarged, sac-like terminal cisternae positioned at regular intervals, creating membrane contact sites with plasma membrane invaginations and specializing in the rapid storage and release of calcium during contraction. While the role of ER-shaping proteins (Climp63, Kinectin, p180, Atlastin3, Lunapark, Rtn2, Rtn3 and Rtn4) in establishing ER structure is well characterized in non-differentiated cells, their involvement in shaping the SR during development has not yet been explored. Furthermore, the relationship between SR morphology and its functional properties remains unclear. Here, using an in vitro skeletal muscle system, we performed a loss-of-function screen to identify ER-shaping proteins involved in the development of longitudinal and junctional SR subdomains. We discovered that ER shaping proteins are localized near the junctional SR. Additionally, loss of function of ER shaping proteins impacted the normal distribution of junctional SR proteins, which localized as elongated filaments instead of puncta. Notably, plasma membrane proteins that form membrane contact sites with the junctional SR were also mislocalized as filaments. Furthermore, we investigated the impact of ER shaping proteins on calcium dynamics and muscle contractility, linking SR structural integrity to its physiological function.

MS2305
Organization of specialized ER subdomains for lipid droplet biogenesis.
*Amit Joshi[1], Morgan House[1], Karan Khadayat[1]; University of Tennessee[1]

Lipid droplets (LDs) are highly dynamic and ubiquitous storage organelles that form in the ER membrane subdomains. Several proteins such as Nem1, Spo7, Yft2, Sei1, and Pex30 are enriched at the sites of LD formation in Saccharomyces cerevisiae. However, the assembly of these ER subdomains and the mechanism by which proteins are recruited to them is poorly understood. Pex30, an ER membrane protein, has the reticulon homology domain (RHD) that generates ER tubules and a dysferlin (DysF) and DUF domain of unknown function. Pex30 is localized at ER subdomains where new LDs form. How is Pex30 recruited to these ER subdomains? Deletion of SEI1, that codes for seipin, a highly conserved protein required for LD biogenesis, results in accumulation of Pex30 at ER-LD contact sites. This suggests that the Pex30 distribution in the ER membrane can be regulated. In addition to Pex30, phosphatidic acid (PA) also accumulates at these ER-LD contact sites. We show that PA recruits Pex30 at ER subdomains by binding to the DysF domain. Using, MD simulation, we identified the amino acid residues that preferentially interact with PA. Mutating these residues affects binding of Pex30 to PA. Taken together, we propose that PA regulates the distribution of Pex30 at ER subdomains that plays a critical role in driving the formation of LDs in the ER membrane. We are currently investigating the spatiotemporal distribution of seipin and Pex30 at LD biogenesis sites and role of Pex30 domains in the formation of LDs.

MS2303
Roles of ubiquitin-fold modifier 1 (UFM1) in protein quality control at the endoplasmic reticulum
*Yihong Ye[1], Mandy Ennis[1], Lihui Wang[1]; NIH[1]

UFM1 (Ubiquitin-fold modifier 1) is the most recently identified ubiquitin-like protein, but its biological roles remain poorly defined. Like ubiquitin, UFM1 modifies lysine residues through an E1–E2–E3 cascade, with the only known E3 ligase being a UFBP1–UFL1–CDKRAP53 complex. UFBP1 anchors this complex to the endoplasmic reticulum (ER), and UFMylation is essential for ER homeostasis, though the underlying mechanisms were unclear. Using biochemical purification and mass spectrometry, we identified RPL26 of the large ribosomal subunit as a key UFMylation substrate. This modification is strongly induced when ER-bound ribosomes undergo persistent stalling, producing truncated nascent chains that clog the ER translocon. UFMylated ribosomes recruit downstream factors, including the UFM1-binding protein SAYSD1, to route stalled nascent chains for degradation via lysosomes or the proteasome. Genome-wide CRISPR screens further revealed two distinct branches of translocation-associated quality control (TAQC). mRNAs with ribosome-stalling poly(A) sequences direct their products to lysosomes via Golgi trafficking, whereas substrates from non-stop mRNAs are degraded by an unconventional ERAD pathway involving ER-to-Golgi trafficking and KDEL-dependent retrieval. The choice between these routes appears to be regulated by NEMF-mediated CATylation. Importantly, blocking RPL26 UFMylation impairs red blood cell differentiation in vitro and causes secretion of truncated collagens in fly larvae. In summary, UFMylation of RPL26 enables stalled ribosomes to offload defective nascent chains that would otherwise obstruct the Sec61 translocon, targeting them to lysosomes or the proteasome. This mechanism safeguards ER protein biogenesis and links ribosome quality control to organismal physiology.

MS2301
SLC33A1 exports oxidized glutathione to maintain endoplasmic reticulum redox homeostasis
*Shanshan Liu[1]; Rockefeller University[1]

The endoplasmic reticulum (ER) requires an oxidative environment to support the efficient maturation of secretory and membrane proteins. This is in part established by glutathione, a redox-active metabolite present in reduced (GSH) and oxidized (GSSG) forms. The ER maintains a higher GSSG:GSH ratio than the cytosol; however, the mechanisms controlling ER redox balance remain poorly understood. To address this, we developed a method for the rapid immunopurification of the ER, enabling comprehensive profiling of its proteome and metabolome. Combining this approach with CRISPR screening, we identified SLC33A1 as the major ER GSSG exporter in mammalian cells. Loss of SLC33A1 leads to GSSG accumulation in the ER and a liposome-based assay demonstrates that SLC33A1 directly transports GSSG. Cryo-EM structures and molecular dynamics simulations reveal how SLC33A1 binds GSSG and identify residues critical for its transport. Finally, an imbalance in GSSG:GSH ratio induces ER stress and dependency on the ER-associated degradation (ERAD) pathway, driven by a shift in protein disulfide isomerases (PDIs) toward their oxidized forms. Altogether, our work establishes SLC33A1-mediated GSSG export as a key mechanism for ER redox homeostasis and protein maturation.

MS2309
The kinesin-14 Kar3-Vik1 is required for Endoplasmic Reticulum-Associated Degradation
*Eric Rubenstein[1], Emmanuel Akoto[1], Ellen Doss[1], Kieran Claypool[1], Sophia Owutey[1], Kyle Richards[1], Katie Lehman[1], Mahmoud Daraghmi[1], Samantha Turk[1], Christopher Indovina[1], James Avaala[1], Olivia Crooks[1], Melissa

Evans[1], Abigail Scott[1], Hayden Schneider[1], E; Ball State University[1]

Eukaryotes maintain quality control in the endoplasmic reticulum (ER) by destroying aberrant and overabundant proteins via ER-associated degradation (ERAD). While significant advances have been made in defining ER protein quality control pathways, the full complement of genetic requirements for ERAD remains unknown. We conducted a genetic screen in Saccharomyces cerevisiae to identify novel contributors to ERAD. Our screen revealed that KAR3, which encodes a kinesin-14 motor protein that regulates microtubule behavior during mitosis, is necessary for efficient destruction of ER proteins. KAR3 deletion profoundly and specifically impaired turnover mediated by multiple ERAD ubiquitin ligases and sensitized cells to proteotoxic stress. Loss of cytosolic Kar3 cofactor Vik1 (but not nuclear cofactor Cik1) similarly impeded ERAD. This work uncovers a novel role for microtubule-associated motor proteins in ER quality control, with implications for neurodegenerative diseases marked by disrupted cytoskeletal dynamics. Over 60 undergraduate biology students participating in course-based undergraduate research experiences (CUREs) generated preliminary data for this project. A team of 15 high school, undergraduate, and master's-level students conducted the screen, validated CURE findings, and further characterized the link between Kar3-Vik1 function and ERAD. These results demonstrate the power of CUREs and very-early-career scientists to contribute meaningfully to the ERAD and broader research communities.

MS2302

The Role of the GRP170 Molecular Chaperone in Endoplasmic Reticulum, Cellular, and Organismal Proteostasis

*Jeffrey Brodsky[1]; University of Pittsburgh[1]

The Unfold Protein Response (UPR) and Endoplasmic Reticulum Associated Degradation (ERAD) play complementary roles to support ER proteostasis. Although the contributions of the BiP Hsp70 chaperone to both pathways is defined, how a BiP nucleotide exchange factor, Lhs1 in yeast or GRP170 in mammals, regulates the UPR and ERAD is more mysterious. Nevertheless, Lhs1/GRP170 possesses "holdase" activity, suggesting a more direct effect on the UPR and ERAD. To test this hypothesis, we used first used the yeast model to demonstrate that Lhs1 delivers an orphaned subunit that normally resides in a membrane protein complex—the epithelial sodium channel (ENaC)—to the ERAD pathway. The subunit, alpha-ENaC, is also targeted for ERAD if transmembrane contacts within the ENaC complex are weakened. In yeast and human cells, we then discovered that Lhs1/GRP170 interacts with core glycosylated and ubiquitinated alpha-ENaC, thus suggesting Lhs1/GRP170 acts at a late step during ERAD by preventing substrate aggregation. Indeed, when GRP170 was depleted in mouse embryo fibroblasts, an aggregation-prone ERAD substrate accumulates. Perhaps not surprisingly, the UPR was also induced, which was followed by PERK/CHOP-dependent apoptosis. Next, because ENaC resides in the nephron, a nephron-specific inducible GRP170 knockout mouse was constructed. Similar to outcomes when ENaC function was disabled, GRP170-/- mice were unable to maintain salt and water homeostasis, which was followed by UPR induction and PERK/CHOP-dependent renal cell apoptosis. These outcomes mirror effects seen in patients suffering from acute kidney injury (AKI), although a cause-and-effect relationship was previously undetermined. Therefore, to establish if the UPR is directly triggered by loss of salt and water homeostasis, GRP170-/- mice were maintained on a high salt diet. While this diet ameliorated some physiological outcomes associated with AKI, UPR induction in the nephron—followed kidney failure—remained. Together, our data support the use of UPR-targeted therapies for AKI, a disease afflicting up to 50% of all patients in intensive care units.

MS2310

The Small Molecule, BRD4780, Prevents ER-export to the Cell Surface and Promotes ER-degradation of Misfolded GPI-anchored Proteins

*Prasanna Satpute-Krishnan[1], Alexandra Graninger[1]; Uniformed Services University of the Health Sciences[1]

Cells express more than 160 glycosylphosphatidylinositol-anchored proteins (GPI-APs) that play diverse and essential roles in cellular physiology. When these proteins misfold, they not only fail to perform their function, but in the case of misfolded variants of prion protein, form toxic extracellular aggregates that lead to fatal neurodegenerative prion diseases. During steady-state conditions, misfolded GPI-APs, including misfolded variants of prion protein, gain access to the plasma membrane via an ER-to-Golgi export pathway called RESET. After accessing the plasma membrane, they are rapidly endocytosed and degraded in lysosomes. ER-export of misfolded GPI-APs through the RESET pathway involves dissociation from the ER-resident chaperone, calnexin, and association with the p24-family members, TMED10, TMED2 and TMED9. Conditions that induce the dissociation of misfolded GPI-APs from calnexin, such as ER-stress, accelerate traffic through the RESET pathway. Conversely, conditions that stabilize binding to calnexin or reduce binding to p24-family members by misfolded GPI-APs slow down traffic through the RESET pathway. For example, siRNA-mediated depletion of TMED10, TMED2 and TMED9 each inhibit RESET. Using a yellow fluorescent protein (YFP)-tagged misfolding mutant of prion protein (YFP-PrP*) as a model misfolded GPI-AP substrate, we present data that the established TMED9 inhibitor, BRD4780, rapidly decreases binding of YFP-PrP* to TMED10, TMED2, TMED9, within 30 min, and blocks ER-export of YFP-PrP* during steady-state and ER-stress conditions. Using a new semi-quantitative western blot-based approach to measure antibody-uptake, we show that BRD4780 inhibits approximately ≥90% of the antibody-uptake during steady-state or ER-stress induced conditions. This indicates that BRD4780-treatment prevents nearly all of the YFP-PrP* molecules from accessing the plasma membrane. Notably, we present evidence that upon inhibiting RESET with BRD4780, YFP-PrP* was rerouted to and cleared by an alternate degradation pathway that bypassed exposure on the plasma membrane and lysosomal degradation. We will discuss potential physiological implications of this discovery with regard to secretion, protein quality control and clearance of disease-associated mutants of GPI-APs including prion protein, whose access to the extracellular environment has been shown to be associated with disease.

MS2308

Unraveling the architecture of an ER stress sensing complex

*Malaiyalam Mariappan[1], Sikha Thoduvayil[1]; Texas Tech University Health Sciences Center[1]

Misfolded proteins in the endoplasmic reticulum (ER) activate the endoribonuclease IRE1α, which mediates the splicing of XBP1 mRNA to produce an active transcription factor. This promotes the expression of specific genes to alleviate ER stress, thereby attenuating IRE1α activity. Our previous studies discovered that IRE1α exists in a complex with the Sec61/Sec63 translocon complex, and that this interaction is critical for mediating chaperone BiP binding to IRE1α and inactivating it. In the absence of Sec61/Sec63, IRE1α remains persistently activated even after stress is resolved, ultimately leading to cell death. Despite its essential role in regulating ER stress signaling, the architecture of this complex remains poorly understood. We address this by establishing a novel recombinant system to express all five transmembrane proteins in mammalian cells and

purify them as a complex. Our biochemical and reconstitution studies suggest that IRE1α exists in inactive monomeric and active oligomeric forms within the Sec61/Sec63 complex, and that BiP regulates these states. Thus, our studies establish a recombinant system that faithfully recapitulates the endogenous IRE1α signaling.

The Peroxisome: From Molecular Mechanisms to Cellular functions

SG406
How Peroxisomes keep Mitochondria Respiring
*Peter Kim[1]; Hosp for Sick Children[1]

Peroxisomes contain one of the most potent antioxidants, catalase, which has long been believed to play a role in regulating cellular redox homeostasis by scavenging reactive oxygen species (ROS). Interestingly, genetic mutations in peroxisomal genes, collectively referred to as Zellweger Spectrum Disorders (ZSD), lead to the loss of peroxisomal structures while sparing catalase. However, in tissues from patients and animal models with ZSD, an accumulation of oxidatively damaged mitochondria is observed in nearly all cell types. This presentation will discuss how peroxisomes regulate mitochondrial redox homeostasis in mammalian cells. Our findings suggest that peroxisomes directly interact with respiring mitochondria to maintain mitochondrial redox balance. Finally, we will propose a model illustrating how peroxisomes support mitochondrial health during cellular stress conditions.

SG402
Import mechanism of peroxisomal proteins with an N-terminal signal sequence
*Michael Skowyra[1], Tom Rapoport[2]; UT Southwestern Medical Center[1], Harvard Medical School[2]

Peroxisomes are conserved membrane-bounded organelles essential for human health, with crucial functions in lipid metabolism and redox homeostasis. Proteins are imported into peroxisomes from the cytosol by soluble receptors, which remarkably can transport even folded proteins and protein complexes across the peroxisomal membrane. Most peroxisomal proteins contain a C-terminal import signal that is recognized by the receptor PEX5, which then shuttles the cargo through a nuclear pore-like conduit formed by the tyrosine/glycine-rich YG domain of the peroxisomal membrane protein PEX13. After cargo release inside the organelle, PEX5 returns to the cytosol by being pulled out through a separate retrotranslocon. Some peroxisomal proteins instead contain an N-terminal signal that is recognized by the receptor PEX7, but how PEX7 transports its cargo into peroxisomes and then returns to the cytosol has been unknown. Here, we present a comprehensive model of the import cycle of PEX7. We show that PEX7 hijacks PEX5 to move cargo through the YG conduit into peroxisomes. After cargo release inside the lumen, PEX7 returns to the cytosol by moving back through the same conduit, a mechanism distinct from that used by PEX5. We further identify a chaperone called PEX39 that extracts PEX7 out of the YG conduit on the cytosolic side, and then reloads PEX7 with new cargo and a receptor to start another round of translocation. Our work unveils the unique pathway by which PEX7 imports proteins into peroxisomes, paving the way toward understanding how defects in this process cause the devastating human disorder rhizomelic chondrodysplasia punctata and Refsum disease.

SG403
Molecular Mechanisms of Peroxisomal Fatty-Acid Uptake
*Meghna Gupta[1]; Oregon Health & Science University[1]

Peroxisomes are highly dynamic subcellular organelles critical for lipid metabolism and cellular detoxification, including roles in fatty acid oxidation and bile acid synthesis. Fatty acyl-CoA substrate translocation across the peroxisomal membrane is mediated through ATP-binding cassette transporters of subfamily D (ABCD1-3). ABCD3 displays broad substrate specificity, facilitating the transport of branched-chain fatty acids, very long-chain fatty acids, bile acid precursors, and dicarboxylic acids into the peroxisomal matrix. Mutations in ABCD3 are linked to metabolic disorders such as congenital bile acid synthesis defects. Using cryo-electron microscopy, we determined high-resolution structures of human full-length ABCD3 in apo and substrate-bound conformations. Our biochemical and biophysical characterization reveals that substrate binding stimulates ATP hydrolysis, demonstrating allosteric communication between substrate-binding and nucleotide-processing sites. Molecular dynamics simulations further provide a detailed insight into substrate recognition and alterations in the binding pocket of the transporter. Comparative structural analysis identifies substrate-dependent reorganization of nucleotide-binding domains, elucidating key mechanistic features of the transport cycle. These insights advance our understanding role of ABCD3 in peroxisomal metabolite trafficking and associated diseases.

SG404
Peroxisomal Regulation of Host Recovery After Infection
*Jie Sun[1]; University of Virginia[1]

Peroxisomes are vital yet often overlooked metabolic organelles. We demonstrate that exuberant interferon signaling remodeled the macrophage peroxisome compartment after severe COVID-19. Lack of macrophage peroxisomes resulted in impaired inflammation resolution and defective lung repair, causing heightened acute morbidity and mortality during SARS-CoV-2 infection. Peroxisomes exhibited cell-type specific modulation of lipid metabolism, enhancing mitochondrial health critical for lung macrophage repair program and alveolar cell self-renewal. Peroxisomes also prevented excessive inflammasome activation and IL-1β release, thereby limiting the accumulation of KRT8high dysplastic epithelial progenitors and facilitating their proper differentiation into functional alveolar cells post viral injury. Dysfunction in macrophage peroxisomes contributed to chronic lung pathology and fibrosis post SARS-CoV-2 infection. Furthermore, pharmacologically enhancing peroxisome biogenesis mitigated acute symptoms and limited chronic sequelae post SARS-CoV-2 infection (PASC). Our data reveal a peroxisome-centric pro-recovery therapeutic strategy for managing both acute and chronic conditions after respiratory viral infections.

SG401
Peroxisome Dynamics in Arabidopsis

*Bonnie Bartel[1]; Rice University[1]

Peroxisomes, essential for life in metazoans and plants, sequester various oxidative reactions to improve metabolic efficiency while protecting cytosolic constituents from oxidative damage. We are studying the dynamics of this vital organelle in Arabidopsis thaliana; the peroxisomal functions, small size, and facile forward and reverse genetics of this plant allow straightforward impairment and enhancement of peroxisomal processes in an intact multicellular organism. Moreover, the large size of seedling peroxisomes (compared to yeast and mammalian peroxisomes) offers unique opportunities to view peroxisome dynamics via live-cell imaging. Arabidopsis peroxisomes have extensive internal membranes that derive from the outer peroxisomal membrane and accumulate over time. Moreover, these intralumenal vesicles do not form normally in certain peroxin mutants or when fatty acid β-oxidation, a core function of peroxisomes, is impaired. Our findings have implications for multiple aspects of peroxisome biogenesis and function. (This research is supported by the National Institutes of Health.)

SG405
Peroxisomes in immunity and inflammation
*Francesca Di Cara[1]; Dalhousie University Libraries[1]

Research over the past decade has established metabolism as a fundamental requirement underlying immune system function in health and disease. Our team has been exploring the mechanisms by which peroxisomal metabolism controls cellular and systemic innate immune responses. These versatile organelles orchestrate lipid synthesis and modulate reactive oxygen species for the production of immunoregulatory molecules in immune and inflammatory cascades. Our studies in Drosophila, mouse, and human samples showed that peroxisomes are involved in a variety of immune processes, including phagocytosis, cytokine and antimicrobial peptide production, hematopoiesis, and antigen presentation. Additionally, we found that peroxisome dysfunction is linked to autoinflammatory diseases and significant modulation of inflammatory signaling, such as the TNF pathway. Finally, we established that Peroxisome Biogenesis Disorders can exhibit hematopoietic defects, including a reduction in memory/effector T and B cells. Our studies established peroxisomes as a hub in immunity and defects in immunity as overlooked features of PBDs.

Visionary Awards Session: E.B. Wilson, E.E. Just, EMBO Gold Medal, Masur Senior Leadership Awards

AW302
Can we cure HIV? Lessons from the elite.
*Joel Blankson[1]; Johns Hopkins University Sch Med[1]

HIV can be treated effectively with potent antiretroviral drugs, but latently infected resting CD4+ T cells present a barrier to viral eradication. A rare subset of people living with HIV can control viral replication without antiretroviral drugs. These individuals are commonly referred to as elite suppressors or elite controllers. While early studies suggested that infection with defective virus was the cause of elite control, we demonstrated that some of these patients are infected with fully pathogenic viral isolates. Genome wide association studies have

shown an overrepresentation of certain Class I HLA alleles suggesting that HIV-specific CD8+ T cells are responsible for elite control. Thus, therapeutic vaccination to enhance the HIV-specific CD8+ T cell response may be one way of achieving a functional cure. We have studied the viral reservoir of an elite suppressor before, during, and after chemoradiation for metastatic lung cancer. We observed a transient but marked decline in the frequency of clonally expanded latently infected resting CD4+ T cells. We tested the hypothesis that this was due to the fact that these cells were actively proliferating and as such were more susceptible to the drugs. Our results have implications for developing HIV cure strategies to eliminate clonally expanded infected resting CD4+ T cells. We have accomplished this while training students who are underrepresented in science.

AW304
Genetic Perturbation of Gut Bacteria with Engineered Phage Vectors and CRISPR
*David Bikard[1]; Institut Pasteur[1]

The mammalian gut microbiota harbors a complex ecosystem of genetically diverse bacteria that profoundly impact host health and disease. Understanding the precise genetic determinants of bacterial adaptation in vivo and developing tools to engineer them are critical goals for microbiome research. I will first discuss the development and application of an in vivo CRISPR interference (CRISPRi) platform to create high-resolution functional maps of E. coli's genetic requirements within the mouse gut. This functional genomics approach reveals how factors like dietary shifts, host inflammation, and strain-specific genetics (commensal vs. pathogenic) profoundly reshape E. coli's metabolic landscape, stress responses, and colonization strategies.Our results provide a high-resolution genetic atlas of E. coli's functional adaptation and demonstrate the utility of functional genomics to probe the gut environment itself. Second, building on the need to move from probing to precise modification, we engineered novel phage-derived particle to deliver CRISPR base editors to bacteria in situ. We demonstrated the success of this system in the mouse gut, achieving high-efficiency and stable, long-term editing of a target gene. We further demonstrate the platform's versatility by editing therapeutically relevant genes, including those involved in antibiotic resistance and virulence, in pathogenic E. coli and Klebsiella pneumoniae. Together, these approaches provide a powerful toolkit for both understanding and engineering the gut microbiome.

AW303
Intracellular trafficking by microtubules and kinesin motor proteins
*Kristen Verhey[1]; University of Michigan[1]

The research in my lab is focused on understanding microtubule-based intracellular trafficking in mammalian cells. We combine biophysical, biochemical and cell biological approaches to probe the structure, function and regulation of microtubules and associated kinesin motor proteins. Our work has ranged from exploring the motility and force-generating properties of various kinesins at the single-molecule level to understanding how kinesins work in teams (coordinate their motility with other motors attached to the same cargo) and navigate the complex cytoskeleton (directional cues, obstacles on the microtubule track, etc.) within the viscoelastic environment of a mammalian cell. Our work has revealed general mechanisms of kinesin regulation by autoinhibition of motor activity, spatial regulation of kinesin motors by post-translational modifications of

microtubule tracks, and temporal regulation by cargo binding. A major factor of our success has been the interdisciplinary collaborations with scientists from around the world with expertise in physics, chemistry, structural biology, mathematical modeling, and genetics. Another major factor has been the support of ASCB in driving forward our science, promoting scientific knowledge and policy, and developing a diverse, global, and inclusive scientific community.

AW301
Musings on the Future of Cell Biology
*Sandra Schmid[1]; CZ Biohub -SF[1]

Current upheavals in the biomedical research enterprise can certainly lead to pessimism, especially for cell biologists early in their careers. However, the revolution in technologies over the past 2 decades, including CRISPR gene editing, affordable 'omics, new imaging technologies and modalities and most recently AI breakthroughs provide tremendous opportunities for biomedical research. Given that cells are the fundamental unit of life, cell biologists can be optimistic about their essential role in contributing to our understanding of human biology and disease. Yet, despite these technological advances and the opportunities they present, in many ways not much has changed in the way we conduct our research and train our students, at least over the time course of my 40-year research career. AI has the potential to revolutionize the research enterprise, but it's not there yet. I'll discuss my own musings as to how cell biology might adapt to best contribute to and eventually benefit from the power of AI.

What's Next: Cell Biology from ASCB MOSAIC Program Alumni

SG1203
AAV Delivery of MET Channel Subunits (Tmc, Cib2, Tmie) Restores Hearing and Balance in KO Mice
*Evan Ratzan[1], Cristobal Von Muhlenbrock Rodriguez[1], Caroline Li[2], Zubair Ahmed[3], Gwenaelle Geleoc[1], Jeffrey Holt[1]; Boston Childrens Hospital/Harvard Medical School[1], Boston Children's Hospital/Harvard Medical School[2], University of Maryland[3]

Background: Unlike typical ion channels, mechanotransduction (MET) channel expressed in stereovilli of hair cells of the inner ear is composed of protein complex consisting of: TMC1, CIB2, TMIE, PCHD15 and LHFPL5. Together these proteins form a nonspecific ion channel that is mechanosensitive to displacement from sound waves or changes in body position. Tmc1, Cib2, or Tmie mutations can result in loss of hearing loss in humans but vestibular deficits are not well characterized. These components are thought of as necessary for hair cell MET function but are not uniformly expressed. We sought to characterize expression profilies of MET genes, audiovestibular phenotypes in MET KO mice, and restorative potential for AAV mediated gene replacement therapy to treat hearing and balance. Methods: We used vestibular stimulated evoked potentials (VsEP), rotarod, open field, and infrared palm reader analysis single KO and DKO mice to assess vestibular phenotypes. We also used auditory brainstem response (ABR) to examine restoration of auditory function in injected mice. Hairpin chain reaction fluorescent in situ hybridization (HCR-FISH) was used to assess Tmc1, Tmc2, Cib2 and Cib3 regional expression differences across inner ear organs. RT-qPCR was completed to assess the relative distribution of MET genes in utricles, saccules, crista, and cochlea over time. Functional assessment of hair cell

mechanotransduction was completed using styryl dye FM1-43 labeling of acutely dissected tissues. Results: We show that Tmc1 KO, Cib2 KO and Tmie KO mice mice fail to produce VsEP responses to head jerk stimuli, or ABR responses to sound. Interestingly, Tmc2 KO and Cib3 KO mice have normal responses under the same conditions; however, Tmc1/2 DKO and Cib2/3 DKO phenotypes are more severe. HCR-FISH shows Tmc1/2, and Cib2/3 are expressed in regionally non-overlapping patterns. Similarly, Tmc2 KO and Cib2 KO vestibular organs fail to uptake FM1-43 dye in the striola of the utricle and saccule. Unilateral neonatal utricle injection of AAV is sufficient to restore VsEP and ABR responses to near WT thresholds in both ears. Conclusions: Tmc1 expression in the extrastriola, and Cib2 expression in the striola are necessary for VsEP generation. Additionally, despite expression in vestibular organs, endogenous Tmc2 cannot compensate for Tmc1 loss, and Cib3 cannot compensate for loss of Cib2. These results suggest that MET components Tmc1 and Tmc2, as well as Cib2 and Cib3 are not functionally redundant in the vestibular system. Tmie KO severity is comparable to Tmc1/2 DKO and Cib2/3 DKO which suggests that though not redundant, Tmc2 and Cib3 contribute to vestibular function during development.

SG1201

How augmin establishes the angle of the microtubule branch site

*Jodi Kraus[1], Sophie Travis[2], Rui Zhang[3], Sabine Petry[1]; Princeton University[1], University of Nebraska[2], Washington University in St. Louis, School of Medicine, St. Louis[3]

How microtubules (MTs) are generated in the proper orientation is essential to understanding how the cytoskeleton organizes a cell and MT-dependent events such as cell division. In the spindle, most MTs are generated through the branching MT nucleation pathway. In this pathway, new MTs are nucleated from the side of existing MTs and oriented at a shallow angle by the branching factor augmin, ensuring that both MTs have the same polarity. Yet, how augmin binds MTs and sets the branch angle has remained unclear. Here, we report the cryo-electron microscopy structure of an augmin subcomplex on the MT. This structure resembles that of NDC80 bound to the MT, with the conserved CH domain of augmin's Haus6 subunit directly proximal to the MT lattice. We find that the Haus6 CH domain is a bona fide MT binding site that increases augmin's affinity for the MT and helps establish branch angle. A second binding site, located in the disordered N-terminus of Haus8, also establishes branch angle,. Thus, we find that augmin regulates MT branching using two domains, each tuned to modulate MT affinity and MT branch angle. This work expands our mechanistic understanding of branching MT nucleation and thus spindle formation.

SG1202

Loss of indiscriminate GLDR-2 tailing enhances body length and reduces fertility and embryonic viability in C. elegans

*Karl-Frédéric Vieux[1], Brian Riley[1], Danni Li[1], Shuling Lin[1]; Worcester Polytechnic Institute[1]

During the oocyte-to-embryo transition RNA tailing is dynamic. Yet, we don't fully understand how tailing patterns are established in gametes and early embryos, how they coordinate RNA biology, or why they are required for embryonic viability. gldr-2 is one of two C. elegans orthologues of the vertebrate noncanonical poly(A) polymerase Gld2, critical for mouse oocyte maturation. In vitro, CeGLDR-2 displays indiscriminate transferase activity, while in vivo, it is responsible for A-tailing miRNA precursors. Its effect on other RNA

biotypes and its physiological relevance, however, remain elusive. Its paralogue, GLD-2 has a key regulatory function in C. elegans meiosis and oogenesis, which our work, using a Nanopore-based direct cDNA sequencing method monitoring RNA tail length and composition (Nano3P-seq), has recently expanded on. Here, we use traditional genetics and Nano3P-seq to determine the molecular targets, and the cellular and physiological function of GLDR-2-mediated tailing. Imaging of GFP tagged GLDR-2 reveals a discrete but robust expression in both hermaphrodite and male germlines. Yet, gldr-2 null hermaphrodites remain fertile while male mutants are severely subfertile. Interestingly, in a pup-1/2 mutant background, loss of gldr-2 in hermaphrodites results in smaller brood and an exacerbated decrease in embryonic viability of F1 offsprings, suggesting an epistatic relationship between gldr-2 and the pup genes. Nano3P-seq data shows a loss of A-tails in the absence of GLDR-2. We also observed a loss of U- and mixed tails, further supporting some redundancy with PUP-1/2. We identified 433 genes with a decrease in average transcript tail length and a decrease in the proportions of A-, U- or mixed-tailed reads in gldr-2 null mutants. Gene Ontology analysis shows that these putative GLDR-2 targets are enriched in processes important for early embryonic viability and cell proliferation, including energy metabolism and cell division, and correlates well with the observed GLDR-2 expression and mutant phenotypes. Future work will determine the effects of A-, U- and mixed-tail loss on stability and translation, confirm physical interactions between targets and GLDR-2 and validate their function. Without an RNA recognition motif, GLDR-2, like GLD-2, must rely on RNA-binding proteins to interact selectively with targets. Our data also shows an enrichment for RNA Pol III and ETR-1 associated transcripts amongst GLDR-2 targets, providing insight into the mechanisms behind GLDR-2 recruitment. This work expands the list of RNA tailing enzymes and the mechanisms responsible for complex gene expression patterns during the oocyte-to-embryo transition and provides novel candidates for therapies targeting RNA mechanisms to impact embryonic viability.

SG1204

The Biology of Longevity: From Energy Metabolism in the Brain to Reproductive Aging

*Ewa Bomba-Warczak[1], Tristan Wrong[1], Molly Healey[1], Timothy Hark[2], Seby Edassery[2], Karen Velez[3], Luhan Zhou[2], Francesca Duncan[2], Jeffrey Savas[2]; University of Pennsylvania[1], Northwestern University[2], University of Chicago[3]

Cellular longevity requires sustained energy production and the preservation of organelle integrity over extended lifespans. Our objective was to uncover mechanisms by which mitochondria and their protein constituents contribute to the longevity of both post-mitotic brain cells and reproductive systems. Using in vivo stable isotope pulse-chase mass spectrometry, mitochondrial isolation, and functional assays, we interrogated the persistence and turnover of mitochondrial proteins across diverse tissues. We found that select inner membrane and cristae organizing proteins exhibit exceptional stability in the mammalian brain, persisting for months in neurons where long-lived proteins support synaptic activity and metabolic resilience. In parallel, studies of reproductive tissues revealed that oocytes harbor extraordinarily stable mitochondrial structures and proteins that endure throughout the female reproductive lifespan, enabling developmental competence despite chronological aging. Complementary conceptual frameworks from comparative metabolism further highlight that mitochondrial adaptations underlie species-specific differences in reproductive span and brain function. Collectively, these findings establish that longevity in neurons and oocytes is supported by conserved principles of mitochondrial protein persistence and metabolic economy, while also exposing unique vulnerabilities in aging. We conclude that the biology of longevity cannot be understood without integrating mitochondrial proteostasis across cell types that rarely, if ever, regenerate. This work defines mitochondria not

only as engines of energy metabolism but also as guardians of lifespan in cells central to cognition and reproduction.