2025 CELL DYNAMICS SYMPOSIUM

Hosted by Cell and Developmental Biology

May 22-23

Carmichael Conference Center 1406
Vanderbilt University

Sue Biggins, Ph.D.
Fred Hutch Cancer Center

Margaret Gardel, Ph.D.
University of Chicago

Tobias Meyer Ph.D.
Weill Cornell Medical College

Loïc Royer Ph.D.
Chan Zuckerberg Biohub San Francisco

Orion Weiner Ph.D.
University of California San Francisco

The Company of Biologists

VANDERBILT
School of Medicine Basic Sciences
Department of Cell and Developmental Biology

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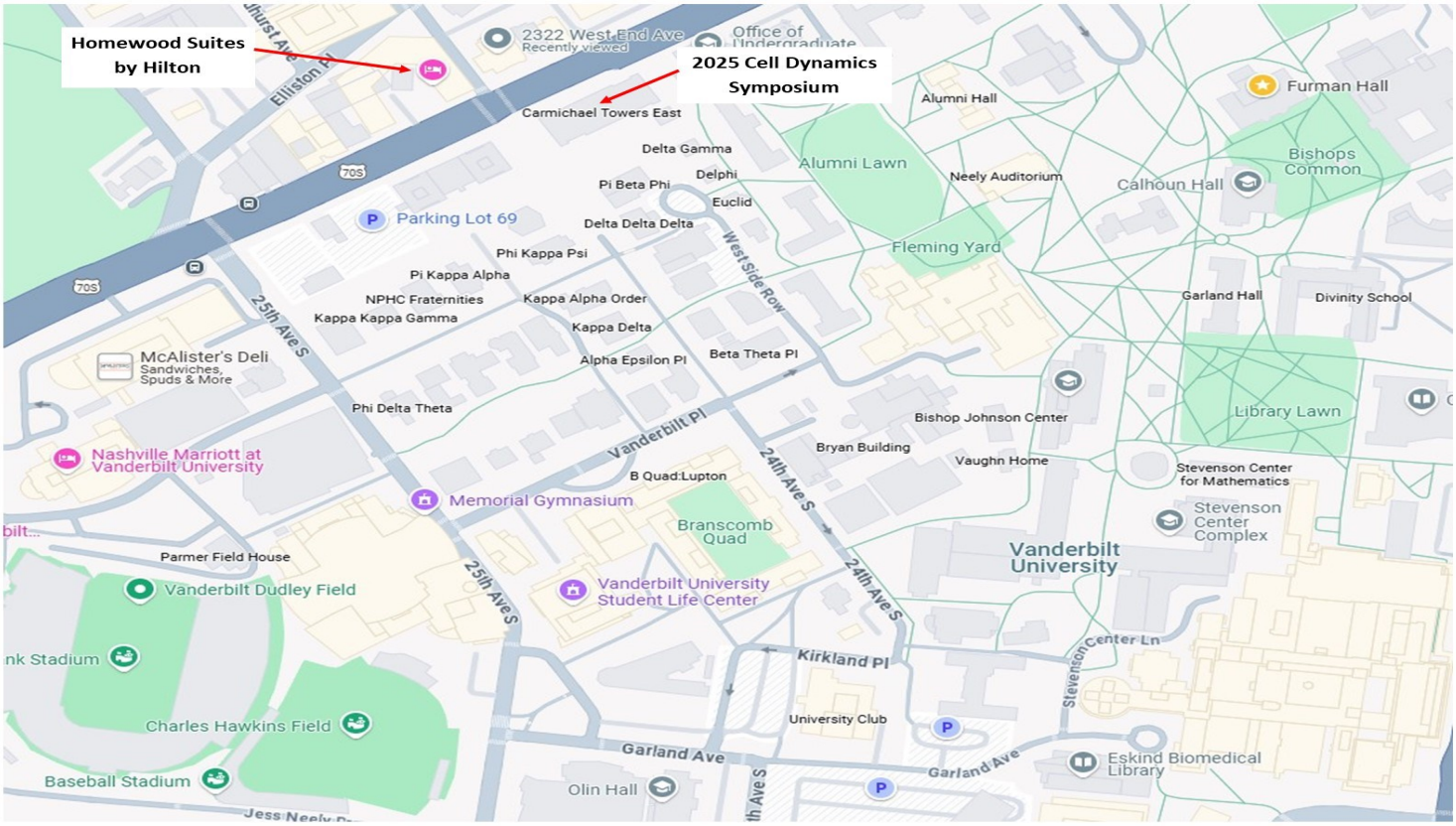
Office of the Vice Provost for Research & Innovation, Program in Developmental Biology

Carmichael Conference Center

Located at the corner of 24th Ave S and West End Ave



2025 Cell Dynamics Symposium

Thursday, May 22

2:30 pm Registration
3:00 pm Welcome Remarks: Dean Kuriyan
3:10 pm Tobias Meyer
4:10 pm Lightning Talks
4:30 pm Reception & Poster Session #1

Friday, May 23

8:30 am Coffee and Conversation
9:00 am Welcome Remarks
9:05 am Margaret Gardel
10:05 am Presentations by Achievement Award Winners
    Eric Donahue (Burkewitz lab)
    Loïc Fort (Macara lab)
10:35 am Coffee Break
11:00 am Orion Weiner
12:00 pm Lightning Talks
12:30 pm Lunch / Round Tables
1:30 pm Poster Session #2
3:00 pm Sue Biggins
4:00 pm Loïc Royer
5:00 pm Conclusion of Symposium

Oral Presentations

“A structural and signaling foundation for directed cell migration”

Tobias Meyer, Ph.D.

Dr. Meyer will discuss how directed cell migration is driven by the front–back polarization of the internal cell signaling machinery and actin cytoskeleton. Various models proposed how cells direct migration, but two observations suggest that a different explanation is needed: previous models assumed (1) that cells sense gradients of receptor stimuli across the cell but receptor tyrosine kinases (RTK) and GPCRs are activating local signals much more strongly at the front; (2) the other contradicting finding has been that cells polarize the internal signaling and actin machinery to the same extent whether a uniform external stimulus or a gradient is applied, resulting in similar migration speeds. My talk will discuss how structural mechanisms can explain these conflicting observations. Specifically, I will focus on two polarized structures in migrating cells, membrane proximal actin (cortical actin) and endoplasmic reticulum–plasma membrane (ER–PM) contact sites. We showed that both structures are polarized in single and collectively migrating cells and are crucial for polarizing the cell’s signaling and protrusion machinery, restricting the sensing of external signals and actin polymerization to the front. I will focus on molecular mechanisms explaining this intriguing structural and signaling polarization. We propose that directed cell migration is the result of an emerging front, which requires that cortical actin and ER-PM contacts are locally low, while they keep receptor signaling and the protrusive actin machinery inhibited in the rest of the cell. The initial polarization can occur in random directions and the ongoing cell migration is stabilizing the structural gradient. We further propose that directed cell migration/chemotaxis are secondary mechanisms that sense external stimuli only at the front, orthogonal to the direction of migration, thereby steering the direction of migration through local asymmetric signaling at the front.

“Mechanical Information Processing in Multicellular Tissue”

Margaret Gardel, Ph.D.

Dr. Gardel will describe our recent efforts to understand the design principles of the active, soft materials that drive morphogenesis of multicellular tissue. The ability of cells to process mechanical cues to control their shape and motion mechanical information processing controls diverse processes including cell proliferation, barrier function and cell fate determination. In this talk, In particular, I will discuss design principles by which the cellular cytoskeleton senses, generates, and adapts to mechanical forces and couples to biochemical and transcriptional pathways.

“Self-organization of cell migration: from single cell polarity to multicellular swarms”

Orion Weiner, Ph.D.

Dr. Weiner will discuss how cell movement requires long-range coordination of the cytoskeletal machinery that organizes cell morphogenesis. We have found that reciprocal interactions between biochemical signals and physical forces enable this long-range signal integration. Through a combination of optogenetic inputs, mechanical measurements, and mathematical modeling, we resolve a recent controversy regarding the role of membrane tension propagation in this process and reveal the requirements for long-range transmission of tension in cells. Most cells don't move in isolation-- they collectively migrate by sharing information similar to the flocking of birds, the schooling of fish, and the swarming of ants. We reveal a novel active signal relay system that rapidly and robustly ensures the proper level of immune cell recruitment to sites of injury and infection.

“Chromosome inheritance during cell division”

Sue Biggins, Ph.D.

Dr. Biggins will discuss how the precise regulation of cell division is critical to processes such as self-renewal, proliferation and development. A key event in the cell cycle is the partitioning of every pair of duplicated chromosomes to daughter cells. Chromosomes segregate using their kinetochores, the specialized protein structures that are assembled on centromeric DNA sequences and attach to spindle microtubules. I will discuss our recent development of a single molecule assay to monitor kinetochore assembly in real time and what it has revealed about centromeric nucleosome assembly. In addition, I will discuss our recent finding using optical trapping techniques that kinetochores intrinsically make stronger attachments to the plus ends of microtubules, a property that likely contributes to their ability to make proper attachments to highly dynamic microtubules.

“Building a Virtual Embryo, one cell at a time”

Loïc Royer, Ph.D.

Imagine having an interactive digital twin of a developing embryo—one you could pause, rewind, or zoom into, exploring how every cell divides, moves, and differentiates. To make this vision real, we created **Zebrahub**, a living atlas of zebrafish embryogenesis that combines cutting-edge microscopy, powerful computational lineage tracking (**Ultrack**), and precise molecular mapping into a unified, interactive resource. Our journey begins with advanced multiview light-sheet microscopy, capturing millions of cells in living zebrafish embryos over days of continuous development (**DaXi**). Next, our uncertainty-aware cell tracker, **Ultrack**, transforms these enormous terabyte-scale datasets into coherent cellular histories, reconstructing precise lineages even in challenging imaging conditions. Combining these detailed lineages to single-cell transcriptomes, **Zebrahub** enables users not only to visualize developmental events in unprecedented detail but also to explore the molecular decisions underlying them. In this talk, I will highlight how we use this "virtual embryo" to conduct in-silico fate-mapping experiments, uncover hidden properties of neuro-mesodermal progenitor cells, and test developmental hypotheses. Join me in exploring how our integrated pipeline—spanning microscopy, AI-driven lineage reconstruction, and molecular profiling—opens new avenues to unravel the fundamental rules by which genomes build complex living organisms, one cell at a time.

Achievement Award Winner

"ER-phagy Drives Conserved, Age-Related Remodeling of the Endoplasmic Reticulum and Promotes Longevity"

Eric Donahue, Ph.D.

As we age, a progressive accumulation of molecular damage drives homeostatic failure, thus increasing disease burden in our rapidly aging society. Targeting the mechanisms of aging can therefore mitigate disease progression, reduce late-life morbidity, and promote healthier lives. At the center of cellular function and homeostatic decline is the endoplasmic reticulum (ER), which comprises structurally distinct sub-domains that serve as specific hubs for specialized cellular functions. Although altered ER functions are increasingly linked to age-related pathogenesis, whether these functional changes are driven by underlying structural shifts is unclear. We therefore hypothesize that ER structure-function remodeling is a pivotal upstream event in the aging process.

Our findings demonstrate that ER remodeling is a conserved feature of aging across yeast, *C. elegans*, and mammals. Focusing on *C. elegans* as exemplar of metazoan aging, we reveal striking reductions in ER mass across diverse tissues and a morphological shift from rough ER sheets to tubular ER. This morphological transition corresponds with large-scale shifts in ER proteome composition from protein synthesis to lipid metabolism.

We identify selective ER degradation, or ER-phagy, as a driver of this remodeling. Tissue-specific factors, including a novel ER-phagy receptor, TMEM-131, and the IRE-1/XBP-1 branch of the unfolded protein response, regulate ER-phagy to mediate the loss of predominantly rough ER. Notably, ER remodeling is not merely a consequence of aging but a proactive, protective response. Diverse lifespan-extending interventions promote profound ER remodeling, and ER-phagy is required for lifespan extension via TOR suppression in both yeast and *C. elegans*. These findings establish ER dynamics and ER-phagy as critical, underappreciated mechanisms in both normal aging and enhanced longevity, suggesting that modulating ER morphology via ER-phagy could offer novel therapeutic strategies for promoting healthy aging.

Achievement Award Winner

"Actomyosin contractility is a potent suppressor of mesoderm induction by human pluripotent stem cells"

Loïc Fort, Ph.D.

During morphogenesis, tissue organization is mediated by complex cell-cell and cell-extracellular matrix interactions, dependent on intracellular cell tension and tissue-scale force sensing. However, it remains unclear how, mechanistically, cells integrate the state of their environment to coordinate developmental responses. We hypothesize that mechanical forces, such as cell tension, regulate cell identity by de-sensitizing mesodermal genes to WNT activity. During mesoderm specification, we noticed that induced pluripotent stem cells (hiPSCs) experience high contractility, leading to stem cell colony retraction and increased myosin light chain phosphorylation. Surprisingly however, pharmacological or genetic inhibition of cell contractility increased mesoderm gene expression and sped up epithelial-to-mesenchymal transition, a critical step for differentiation. Conversely, expression of a constitutively active Rho kinase (ROCK) to increase tension caused a total blockage of cardiac mesoderm conversion. Together, these data suggest that actomyosin contractility inhibits mesoderm identity in hiPSCs. Abolishing cell-cell force transmission using EGTA-mediated disruption of adherens junctions (AJ) or by creating *CTNNA1* knock-out to uncouple AJ to actomyosin network, promoted mesoderm differentiation and mimicked ROCK inhibition, suggesting that force transmission across neighboring cells is important. Mechanistically, reduced contractility increased levels of active β- atenin in the cytoplasm and nucleus, and promoted WNT target gene expression, suggesting that lowering cell contractility licenses WNT pathway signaling. In addition, low contractility promoted β-catenin-TCF4 interaction and CUT&RUN exper ment confirmed increased occupancy at *TBXT* promoter (encoding Brachyury).

Together, these data support a mechanism through which cell-cell-mediated actomyosin contractility modulates AJ stability to regulate free β-catenin, WNT signaling and mesoderm induction in hiPSCs.

Poster Presentations
Thursday, May 22

1. Syed Barmaver
2. Al Borhan Bayazid
3. Caraline Bekas
4. Caroline Bodnya
5. Monica Brown
6. Owen Burroughs
7. Leah Caplan
8. Aiyana Cyr
9. Mikiyas Daniel
10. Andrew Dixson
11. Vaishna Vamadevan
12. Xinyu Dong
13. Adam Ebert
14. Loïc Fort
15. David Gonzalez
16. Sander Haigh
17. Marina Hanna
18. Caleb Hayes
19. Eric Donahue
20. Junmin Hua
21. Halee Scott
22. Deronisha Arceneaux

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Role of KIF21A in Islet β-cell Peripheral MT Organization and Heterogenous Insulin Secretion

Syed Nooruzuha Barmaver[1], Gu Guoqiang[1] and Irina Kaverina[1]

[1] Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN

Diabetes mellitus is a chronic metabolic disorder characterized by dysregulation of glucose homeostasis. Understanding the underlying mechanisms is crucial for developing targeted therapies. This research aims to investigate the role of the motor protein KIF21A in regulating the configuration of microtubules (MTs) at the peripheral regions of pancreatic β-cells and its impact on insulin secretion hot-spots. We have observed that KIF21A is localized at the periphery of β cells and is associated with the end-on microtubules (MTs). Additionally, we have observed that increased expression of KIF21A in β cells is positively correlated with an increase in tubulin levels.

We hypothesize that KIF21A modulates the organization of MTs at secretion hot-spots, resulting in heterogeneous availability of insulin-containing secretory granules (IGs) for exocytosis. We also hypothesize that KIF21A plays a role in IG transport, and its distribution in the pancreatic islets may be associated with the pathogenesis of diabetes.

Rac1 controls cilia-dependent mechanosensing to enhance kidney tubular differentiation after birth.

Al Borhan Bayazid, Richard Zhang, Matthew Tantengco, Olga Viquez, Ambra Pozzi, Roy Zent, Fabian Bock

Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Abstract:

The nephron is the functional unit of the kidney that consists of a filter, called the glomerulus, and ciliated tubular epithelial cells that reabsorb the primary filtrate. During early development nephrons undergo a complex folding and proximal-distal patterning process. After birth and with rapidly accelerating tubular flow, tubular cells undergo additional differentiation to become mature reabsorbing proximal tubular cells. The signals that specifically initiate postnatal differentiation are largely unknown. Rac1 is a small Rho GTPase and actin cytoskeleton regulator with critical functions in epithelial development. We deleted Rac1 in nephron progenitor cells (Six2+) and unexpectedly found no major kidney developmental phenotype with intact folding, patterning and glomerulus formation. However, postnatally Rac1-null proximal tubular cells rapidly lost their differentiated phenotype, underwent cystic dilatation and the mice died within 4 weeks of birth. We found that despite intact initial differentiation the major defect in newborn Rac1-null tubules was abnormal primary cilia. *In vitro,* Rac1-null tubular cells showed impaired ciliogenesis after serum starvation and failed to further differentiate upon application of fluid shear stress, an effect that could be recapitulated with cilia-deficient (Ift88-Knock Down) cells. Rac1-null cells had excessive actomyosin activation resulting in impaired ciliogenesis. Direct myosin inhibition in Rac1-null cells reversed the cilia defect and rescued fluid shear stress-induced tubular differentiation. Collectively, our findings demonstrate that Rac1 is dispensable for early nephron specification but is essential for postnatal tubular differentiation via cilia-dependent sensing of apical mechanical stress.

CRISPR-Cas9 KO of Genes Involved in the Formation of RNA-Containing Extracellular Vesicles

Caraline Bekas[1,2*], Youn Jae Jung [1,2,3*], Bahnisikha Barman [1,2], Lizandra Jimenez[1,2], T. Renee Dawson[1, 2], Alissa M. Weaver [1,2]

[1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee. [2]Center for Extracellular Vesicle Research, Vanderbilt University School of Medicine, Nashville, Tennessee. [3]Department of Chemical and Biomedical Engineering, Vanderbilt University School of Engineering, Nashville, Tennessee.

*co-authors

Extracellular RNAs (exRNAs) carried by extracellular vesicles (EVs) directly influence gene expression in recipient cells and promote aggressiveness and metastasis in various cancers. Our lab previously identified a crucial phenomenon where we established that the key endoplasmic reticulum membrane contact sites (ER MCS) protein, VAP-A, and its binding partner CERT on the late endosome membrane play crucial roles in the biogenesis of RNA-containing EVs. These VAP-A-CERT complexes govern the transfer of ceramide from the ER to multivesicular bodies, leading to the formation of intraluminal vesicles (ILVs). Ceramide synthesis is regulated through L-serine availability, which can be limited by the enzyme phosphoserine aminotransferase 1 (PSAT1). Many cancers, including colorectal cancer (CRC), show overexpression of PSAT1. Currently, our lab uses stable CRISPR-Cas9 knockout (KO) cell lines to investigate how both upstream and downstream regulation of ceramide synthesis impact the biogenesis of RNA-containing EVs. We will use these cell lines *in vitro* to study how disruptions in ceramide synthesis affect EV abundance and RNA content. Additionally, we will conduct *in vivo* studies to evaluate the effects on the tumor microenvironment and growth.

Examining the Impact of Peroxisomal Metabolism on Cellular Transitions During Human Neurogenesis

Caroline Bodnya[1] and Vivian Gama[1,2].
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[2]Vanderbilt Center for Stem Cell Biology, Vanderbilt University, Nashville TN.

Peroxisomes are single membrane-bound organelles that contain over 50 different enzymes involved in a range of metabolic processes. Peroxisomal biogenesis disorders (PDBs) are characterized by dysfunction in any one of 13 peroxisomal biogenesis proteins (peroxins) and result in severe neurological phenotypes, including seizures and developmental delay. Life expectancy for patients rarely surpasses a few years. The peroxisomal biogenesis factor 3 (PEX3), which is essential for the insertion of proteins into the peroxisomal membrane, is among the peroxins mutated in PDBs. Mutations in PEX3 lead to a loss of functional peroxisomes; however, the exact mechanisms by which peroxisomal metabolic dysfunction may contribute to disease pathophysiology remain unclear. To manipulate peroxisomal function during neurogenesis, I generated PEX3 knockout human induced pluripotent stem cells (hiPSCs), differentiated them into neural progenitors (NPCs), and characterized how knocking out PEX3 affects the expression of key neural identity markers and metabolic function. PEX3 KO hiPSCs maintain stem cell identity and can differentiate into NPCs. Consistent with previous findings in other models, we found that PEX3 KO iPSCs and NPCs lack detectable peroxisomes, with no changes in mitochondrial morphology. Furthermore, PEX14, an essential component of the peroxisomal import machinery, localizes to the mitochondria in the absence of PEX3. Notably, PEX3 KO NPCs exhibit changes in the neural identity markers *PAX6, TBR2,* and *TUBB3* at day 8 of differentiation. These changes in neural identity are coupled with changes in mitochondrial respiratory function. Furthermore, PEX3 neural organoids at days 30 and 90 of differentiation display depletion of the neural progenitor pool, cortical disorganization, and loss of deep-layer neuronal markers. These findings may help elucidate how peroxisomal metabolic functions are regulated during neurodevelopment and perturbed in disease

Magnifying the Structure of Tuft Cell Cytospinules

Monica E. Brown, Taewoo Kim, Ken S. Lau

Tuft cells are found in both the small intestine and colon and are a chemosensory cell that communicates between the lumen and other gut epithelial and non-epithelial cells. Tuft cells have many unique architectural characteristics including lateral and basal protrusions, termed cytospinules. Methods to identify these cellular protrusions outside of electron microscopy have made their study challenging, leaving little known about their basic structure and any underlying function. Using automated near super-resolution microscopy in thick sections, cytospinules have been identified in immunofluorescence. Previously, actin filaments alone were thought to be localized to these protrusions, but utilizing the microtubule-associated protein DCLK1, cytospinules are also found to contain microtubules. Additionally, tuft cell specific membrane marker, pEGFR, can be found to wrap around these microtubules and ‘colocalize’ with components of adjacent nuclear envelope lamins. Furthermore, expansion microscopy in formaldehyde fixed paraffin embedded tissue has been optimized to produce physically expanded tuft cells to better identify cytospinules and their proteins within. Together these techniques have allowed for the identification of multiple new proteins within tuft cell cytospinules and helped to separate these cytospinules into multiple variants allowing for future steps to focus on more functional features of these protrusions.

Defining macrophage-induced stressors of *Bacillus anthracis* at early stages of anthrax disease

Owen Burroughs, Bradley Akin, Eric Skaar

Bacillus anthracis, the causative agent of anthrax, is a Gram-positive, spore-forming bacterial pathogen known for its bioterror potential. During inhalation anthrax, the most lethal form of the disease, inhaled spores are phagocytosed by macrophages in which they germinate, persist, and disseminate throughout the body. The stressors encountered within these macrophages, and the mechanisms by which the bacterium resists these stressors, remain incompletely understood. A better understanding of these early host-pathogen interactions may reveal targets for novel therapeutics to treat anthrax. Therefore, the objective of this study is to comprehensively identify host genes that contribute to the cell envelope stress and iron starvation experienced by *B. anthracis* within macrophages. This study will also identify host genes that impact the bacterium's ability to germinate and persist within macrophages. To accomplish this, fluorescent *B. anthracis* strains were engineered to express mNeonGreen constitutively, and express mScarlet-I driven by promoters that are responsive to cell envelope stress or iron starvation. Using a robotic liquid handler, a comprehensive arrayed library of CRISPR guides will be used to inactivate murine macrophage genes in microwell plates. These mutant macrophages will be infected with the fluorescent reporter bacteria, and high-content imaging will be used to assess the impact of the gene knockout on bacterial germination, growth, and stress signaling. A similar comprehensive screen has been successfully employed to identify genes which impact the growth and stress signaling of *Staphylococcus aureus* in macrophages. Preliminary work has validated that the experimental setup can visualize *B. anthracis*-macrophage interactions in a high throughput manner. Additionally, the required fluorescent reporter strains have been generated and are currently being validated. Once completed, this study will yield a comprehensive molecular atlas of host genes that impact the growth of *B. anthracis* in macrophages. Further studies will elucidate the impact of these interactions on anthrax pathophysiology.

Investigating a noncanonical role for cofilin in epithelial microvilli

Leah Caplan, Zach Sanchez, & Matthew Tyska

Transporting epithelial cells, such as intestinal enterocytes, form apically localized, membrane wrapped, actin-based protrusions called microvilli. These structures are integral to the cellular function of absorption and pathogen protection, and therefore proper microvillar development and maintenance is critical. Microvilli are composed of a core bundle of parallel actin filaments (F-actin) where the barbed (polymerizing, ATP-rich) end is oriented at the tips of microvilli and the pointed (depolymerizing, ADP-rich) end is anchored in the terminal web, a meshwork of actin and intermediate filaments held together by various cross-linking proteins located below the apical surface. To sustain their morphology, microvilli must maintain a balance between F-actin stabilization and turnover. The core bundle is stabilized by actin-membrane linking proteins and actin bundling proteins including villin, MISP, and fimbrin. Core bundle turnover is aided by non-muscle myosin 2C (NM2C), an actin motor that forms bipolar filaments and localizes to the terminal web. NM2C was previously shown to increase actin turnover of the rootlet and therefore actively regulate microvillar length. Cofilin, an actin severing protein important for actin turnover, preferentially binds ADP-rich actin like the actin state of the microvillar rootlet. While cofilin is primarily thought of as an F-actin severing protein, previous *in vitro* studies and in neurons demonstrate cofilin's ability to saturate and stabilize F-actin bundles in response to increased concentration or cellular stress. My preliminary data in mouse tissue and intestinal cell culture models localizes cofilin to the microvillar rootlets, specifically below NM2C. Given that previous studies demonstrate both cofilin's severing and stabilizing abilities, it is unclear if cofilin is acting as an actin severing or stabilizing protein in the rootlet. I hypothesize that cofilin serves as a stabilizing protein in the rootlet core bundle. Using a combination of innovative techniques including live cell imaging, CRISPR-mediated endogenous tagging, and native tissue immunolabeling, we collected evidence demonstrate that cofilin competes with NM2C for F-actin binding and effectively stabilizes microvillar rootlets leading to microvillar elongation. Collectively, these data suggest an unconventional role for cofilin in regulating cytoskeletal architecture – likely switching between severing and stabilizing in a filament/bundle dependent manner, respectively, as well as spatially regulating binding of actin-binding proteins.

Genetic screen to identify yeast genes that promote *de novo* telomere addition at a double strand break

Aiyana Cyr[1], Esther Epum[1], Katherine Friedman[1]
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Cells have evolved mechanisms to promote genome stability and survival upon induction of DNA damage. Many factors regulate which DNA damage response pathway is used, including the sequence of the damaged chromosome. The budding yeast genome contains hotspots of *de novo* Telomere Addition (dnTA) where repair of double strand breaks (DSB) occurs at thymine and guanine (TG)-rich sequences that resemble telomeres (termed Sites of Repair-associated Telomere Addition, or SiRTAs). Resection of the 5' strand following a DSB can render a SiRTA single-stranded, facilitating the association of the telomere-binding protein Cdc13. Cdc13 recruits telomerase to add telomeric sequence to the end of the break, truncating the end of the chromosome. *De novo* telomere addition is measured by the inducible generation of a single DSB upstream of a SiRTA. Cells survive persistent cleavage by mutating or eliminating the cleavage site, commonly through telomere addition at the SiRTA.

We have developed a genetic screen to identify trans-acting factors that promote dnTA using a strain in which survival of a persistent DBS upstream of the SiRTA is almost fully dependent on dnTA. Mutations are generated by random transposon insertion. Colonies with insertions are screened for those with reduced survival following DSB induction, similar to a strain lacking the SiRTA. High throughput sequencing is used to identify the sites of insertion.

We have screened several thousand insertion strains and have identified numerous candidates with decreased dnTA levels. Candidate genes are verified by deleting the gene via one step gene replacement and comparing the phenotype to the parent strain and the control strain lacking the SiRTA. As expected, we have identified numerous components of the telomerase complex, including *EST2* and *TLC1* (encoding the reverse transcriptase and RNA, respectively). Genetic and physical interactions with telomere relevant genes (*CDC13*, *EST1-3*, *RIF1* and *RIF2*, and *PIF1*) are greatly increased among candidates identified in the screen compared to a list of random, non-essential genes. Furthermore, gene ontology analysis of positive candidates shows highly significant enrichment of multiple telomere-related pathways. Most prominently, we identify genes involved in localization of DSBs and telomeres to the nuclear periphery, telomere maintenance, the DNA damage response, gene silencing, and chromosome organization and transcription regulation.

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How Do New Cells Invade into Old Monolayers? – Investigating The Mechanism of Apical Intercalation

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Epithelial cell intercalation is a fundamental process that plays important roles in embryonic development, wound healing, and tissue morphogenesis. Apical intercalation involves top-down insertion between cells of a confluent epithelial monolayer. It is a multi- step processes involving adhesion, tight junction (TJ) re-organization, and cell polarity changes (Pfannenstein, Dev Cell 2023). This study investigates the rate-limiting role of tight junctions in apical intercalation into mammary epithelium. I compared the eSiciency of intercalation of WT Eph4 mammary epithelial cells into a WT monolayer versus one lacking the TJ polarity protein Pals1. This protein is required for barrier function and TJ assembly. Dr. De Caestecker and his mentees (Mathew and Savi) used Cas9-mediated gene editing to create an Eph4 cell line deleted for Pals1. In the same cell lines, they also endogenously labeled another TJ protein, ZO1, with the highly stable fluorophore mStayGold (mSG), as a marker of TJ integrity. With this marker, loss of Pals1 resulted in the visible disintegration of the TJs. I then used an in vitro intercalation assay in which fluorescently tagged cells are plated onto the top of confluent monolayers and live imaged over time. Strikingly, intercalation was accelerated at least 10-fold by loss of Pals1, demonstrating that TJ integrity is rate-limiting for apical intercalation. Re-expression of Pals1 restored junctions and suppressed intercalation.

There is evidence from work in Drosophila that intercalation involves a zippering mechanism by homophilic interactions between adhesion proteins on the lateral cell membranes. To test this idea in a mammalian context, I asked if the adherens junction protein E-cadherin is important for intercalation. E-cadherin was deleted from Eph4 cells that were plated onto WT monolayers or Pals1-KO monolayers and imaged to track intercalation eSiciency. A substantial reduction was observed for integration into the Pals1- KO monolayers, consistent with the loss of zippering between Ecadherin molecules on the incoming and monolayer cells. Interestingly, some Ecad- cells did temporarily intercalate but were unstable and would frequently extrude after one to several hours. A corollary to the zipper hypothesis is that active migration, driven by acto-myosin contractility, would not be required for intercalation. To test this requirement, I am using a new genetic tool

developed by Dr. Fort that consists of an active fragment of CA-MYPT1, a regulatory subunit of Myosin Phosphatase, to inhibit actomyosin contractility by dephosphorylating myosin light chain. My goal is to test if inducible expression of CA-MYPT1 blocks apical intercalation when expression is limited to the incoming cells, or the monolayer, or both.

Overall, I hope to elucidate a molecular mechanism for the later stages of apical intercalation, when the incoming cells penetrate between the lateral membranes of the monolayer cells. These studies are likely to be of importance for cancer cell dissemination as well as for development of organs such as the mammary and salivary glands.

Localization of the ceramide transfer protein (CERT) to GW bodies

Authors: Andrew Dixson and Alissa Weaver

Abstract:

Extracellular vesicles (EVs) are membrane-bound carriers of protein, RNA, and lipid cargo and are involved in cell-cell communication. An RNA-rich subpopulation of EVs is regulated by the membrane contact site protein VAP-A and its binding partner CERT, which was previously found to target multivesicular bodies which are sites of EV biogenesis. In DLD-1 cells, I did not find evidence of CERT colocalizing with the multivesicular body / lysosome marker CD63 by immunofluorescence. Instead, I found that CERT localizes to bright cytoplasmic puncta in ~12% of DLD-1 cells. I used colocalization analysis with a panel of markers to identify the organelle corresponding to CERT puncta. I found that CERT puncta colocalize strongly with myc-GFP-GW182, a marker of GW bodies. GW182 is a member of the RNA-induced silencing complex and is involved in translational repression of mRNAs. Based on the finding that CERT localizes to GW bodies, I propose to test whether CERT plays a role in GW182-mediated RNA interference or in GW body formation.

Two-factor signaling unlocks mesoderm commitment in human pluripotent stem cells

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The early stages of human development remain elusive due to the complexity of intrauterine embryogenesis and the limitations of animal models, as significant differences exist between mouse and human embryonic development. Human pluripotent stem cells have been instrumental in elucidating early transcriptional changes, while advanced 3D gastruloid models provide valuable insights into blastocyst remodeling and germ layer formation. Among the critical processes of early embryogenesis is the specification of lateral mesoderm, essential for heart development. While WNT signaling is known to drive mesoderm specification, the Macara lab discovered that nucleotides, released from apoptosing human stem cells, are critical for surviving cells to undergo mesoderm commitment (Fort et.al, 2022). This permissive signaling occurs through nucleotide activation of purinergic receptor P2Y2, an entirely novel and unexpected mechanism. However, the link through which P2Y2 licenses stem cells to respond to WNT signaling remains unknown. Hence, we are exploring how P2Y2 and WNT/β- catenin signaling function together as a novel two-factor mechanism to drive mesoderm commitment in human pluripotent stem cells.

WNT activation stabilizes β-catenin, which then enters the nucleus to bind to the transcription factor TCF4 at WNT target gene promoters. TCF4 is constitutively associated with these promoters. We proposed that P2Y2 signaling would promote β-catenin binding only at genes involved in mesoderm induction. However, using CUT&RUN assays to directly measure β-catenin occupancy at these promoters, we discovered that inhibition of P2Y2 signaling, by multiple strategies, strongly reduces β-catenin binding at all WNT target promoters, not just those involved in mesoderm induction. It indicates that in human stem cells (contrary to somatic cells) P2Y2 signaling is essential to induce WNT target genes.

P2Y2 signaling appears to license WNT responsiveness through mechanisms that are still not defined. Although we initially hypothesized that P2Y2 promotes TCF4 binding at WNT target gene promoters, inhibition of P2Y2 does not reduce TCF4 occupancy. This points to alternative roles for P2Y2, such as enhancing β-catenin activity or nuclear localization, or facilitating β-catenin-driven transcription via chromatin remodeling. These possibilities are currently being explored.

Funding: This work is supported by RO1 GM070902 to IGM.

Integrin adaptor protein α-parvin coordinates cytoskeletal dynamics with DNA integrity during kidney branching morphogenesis

Xinyu Dong[1,2], Colton Miller[3], Hugo D. Arce-Santamaria[3], Fabian Bock[2], Shensen Li[2], Olga Viquez[2], Meiling Melzer[2], Ambra Pozzi[2], Roy Zent[1,2]

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Branching morphogenesis requires coordination between cytoskeletal dynamics and cell proliferation. The integrin-associated adaptor protein α-parvin is essential for actin organization and cell migration; however, its involvement in genome stability and DNA repair is unknown. We previously showed α-parvin regulates ureteric bud (UB) morphogenesis through Rho–cofilin–mediated actin turnover. However, inhibition of Cdc42 only partially rescued branching defects in α-parvin knockout kidneys, suggesting that α-parvin has additional roles beyond cell motility.

Here, we identify a novel role for α-parvin in regulating the cell cycle. α-parvin knockout kidneys showed reduced Ki67 staining, and parvin-null UB cells exhibited diminished BrdU incorporation, indicating impaired proliferation. Flow cytometry following double thymidine block revealed a G1/S transition defect: 81.2% of parvin-null cells remained in G1 with minimal progression into G2/M over 24 hours, in contrast to wild-type cells, which progressed normally within 4 hours. Despite intact ERK and Akt signaling, cyclins D, E, and B were downregulated in parvin-null cells, suggesting a blockage independent of mitogenic signaling.

Interestingly, γ-H2AX was elevated in parvin-null UB cells as well as in embryonic day 18.5 kidney collecting ducts, indicating increased DNA double-strand breaks. Parvin-null cell nuclei were also enlarged and irregular. Since α-parvin contains a nuclear localization signal (NLS) and we confirmed α-parvin protein expression in the nucleus, we tested whether nuclear α-parvin was required for proliferation. Re-expression of wild-type α-parvin rescued the proliferation defect, while an 3xNLS-α-parvin mutant did not, indicating that nuclear localization is not required and that cytoplasmic α-parvin is necessary for proper cell cycle progression.

Taken together, we hypothesize that α-parvin prevents DNA damage accumulation by modulating cytoplasmic actin dynamics, thereby indirectly influencing nuclear architecture and genome integrity through mechanosensitive pathways.

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Sphingolipid metabolism drives mitochondria remodeling during aging and oxidative stress

Adam C. Ebert, Nathaniel L. Hepowit, Thyandra A. Martinez, Henrik Vollmer, Hayley L. Singkhek, Kyrie D. Frazier, Sophia A. Kantejeva, Maulik R. Patel, Jason A. MacGurn

One of the hallmarks of aging is a decline in the function of mitochondria, which is often accompanied by altered morphology and dynamics. In some cases, these changes may reflect macromolecular damage to mitochondria that occurs with aging and stress, while in other cases they may be part of a programmed, adaptive response. In this study, we report that mitochondria undergo dramatic morphological changes in chronologically aged yeast cells. These changes are characterized by a large, rounded morphology, decreased co-localization of outer membrane and matrix markers, and decreased mitochondrial membrane potential. Notably, these transitions are prevented by pharmacological or genetic interventions that perturb sphingolipid biosynthesis, indicating that sphingolipids are required for these mitochondrial transitions in aging cells. Consistent with these findings, we observe that overexpression of inositol phospholipid phospholipase (Isc1) prevents these alterations to mitochondria morphology in aging cells. We also report that mitochondria exhibit similar sphingolipid-dependent morphological transitions following acute exposure to oxidative stress. These findings suggest that sphingolipid metabolism contributes to mitochondrial remodeling in aging cells and during oxidative stress, perhaps as a result of damaged sphingolipids that localize to mitochondrial membranes. These findings underscore the complex relationship between mitochondria function and sphingolipid metabolism, particularly in the context of aging and stress.

Actomyosin contractility is a potent suppressor of mesoderm induction by human pluripotent stem cells

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During morphogenesis, tissue organization is mediated by complex cell-cell and cell-extracellular matrix interactions, dependent on intracellular cell tension and tissue-scale force sensing. However, it remains unclear how, mechanistically, cells integrate the state of their environment to coordinate developmental responses. We hypothesize that mechanical forces, such as cell tension, regulate cell identity by de-sensitizing mesodermal genes to WNT activity.

During mesoderm specification, we noticed that induced pluripotent stem cells (hiPSCs) experience high contractility, leading to stem cell colony retraction and increased myosin light chain phosphorylation. Surprisingly however, pharmacological or genetic inhibition of cell contractility increased mesoderm gene expression and sped up epithelial-to-mesenchymal transition, a critical step for differentiation. Conversely, expression of a constitutively active Rho kinase (ROCK) to increase tension caused a total blockage of cardiac mesoderm conversion. Together, these data suggest that actomyosin contractility inhibits mesoderm identity in hiPSCs. Abolishing cell-cell force transmission using EGTA-mediated disruption of adherens junctions (AJ) or by creating *CTNNA1* knock-out to uncouple AJ to actomyosin network, promoted mesoderm differentiation and mimicked ROCK inhibition, suggesting that force transmission across neighboring cells is important. Mechanistically, reduced contractility increased levels of active β-catenin in the cytoplasm and nucleus, and promoted WNT target gene expression, suggesting that lowering cell contractility licenses WNT pathway signaling. In addition, low contractility promoted β-catenin-TCF4 interaction and CUT&RUN experiment confirmed increased occupancy at *TBXT* promoter (encoding Brachyury).

Together, these data support a mechanism through which cell-cell-mediated actomyosin contractility modulates AJ stability to regulate free β-catenin, WNT signaling and mesoderm induction in hiPSCs.

The ubiquitin protease Ubp10 suppresses the formation of translocations at interstitial Cdc13 binding sites

David I Gonzalez, Allison Westerbeek, Esther Epum, Katherine L Friedman

Genomic maintenance is essential for human well-being since alterations in the genetic code can result in cancer or inherited genetic disorders. Certain DNA sequences are particularly prone to damage or incorrect repair; yet our understanding of how these sequences impact genome stability remains incomplete. Destabilizing sequences include those found at interstitial sites that mimic the telomeric sequences located at the ends of linear chromosomes. If these sequences become exposed after a double-strand break (DSB), telomerase, the reverse transcriptase canonically used to lengthen telomeres, can act on these sequences to create a *de novo* telomere addition (*dn*TA). We have previously characterized telomere-like sequences in *Saccharomyces cerevisiae*, termed Sites of Repair-associated Telomere Addition (SiRTAs) that have a propensity to stimulate *dn*TA. Notably, SiRTAs contain two TG-rich regions: a site at which telomerase acts to synthesize the new telomere (Core) and a site that stimulates *dn*TA through its ability to recruit the single-stranded telomere-binding protein Cdc13 (Stim). An unpublished genetic screen designed to identify genes that promote *dn*TA at SiRTAs yielded the ubiquitin protease *UBP10*. To determine the effects of *UBP10* on DNA repair, a persistent DSB is induced through expression of the HO endonuclease. Cells that survive cleavage are screened for those in which a distal marker has been lost through a gross chromosomal rearrangement (GCR) event. We utilize whole genome sequencing to determine the frequency of GCR events at the SiRTA and to map those events with nucleotide precision. Aligning sequence reads to the reference genome and filtering by mapping quality identifies split reads that are subsequently classified as *dn*TA, translocation, or large internal deletion. Loss of *UBP10* reduces *dn*TA frequency while simultaneously increasing the frequency of nonreciprocal translocations at SiRTAs of various efficacies. These non-reciprocal translocations join DNA at the SiRTA with subtelomeric sequences of donor chromosomes, particularly in or adjacent to the Y' element. In some cases, we observe translocations between SiRTAs and an interstitial telomeric sequence (ITS) at the junction between the X and Y' element. We provide evidence that these effects are specific to SiRTAs and that Cdc13 is both necessary and sufficient to promote translocations in the absence of *UBP10.* Interestingly, a subset of translocations at the SiRTA occur via the *RAD51*-independent break-induced replication (BIR) mechanism and require Sir4 and Sir2 of the silencing information regulator (SIR) complex. This work underscores the diversity and complexity of DNA repair mechanisms at interstitial Cdc13-binding sites and their broader implications for genomic stability.

Characterizing the role of RADX in regulation of RAD51 and fork reversal

Sander Haigh, Madison B. Adolph, Swati Balakrishnan, Walter Chazin, David Cortez

RAD51 is an essential regulator of the DNA damage response and functions in multiple stress tolerance pathways including homologous recombination and replication fork reversal. How RAD51 is regulated during fork reversal is unclear. Recently, RADX has been identified as a regulator of RAD51 and fork reversal. Biochemically, RADX destabilizes RAD51 nucleoprotein filaments. In cells, RADX promotes fork reversal under conditions of replication stress. We hypothesize that RADX regulates fork reversal by destabilizing RAD51 filaments. Here, we aim to determine how RADX regulates RAD51 and how this regulation influence fork reversal. Additionally, we aim to conduct a genome wide CRISPR/Cas9 synthetic lethal screen to identify potential unknown functions of RADX.

Pharmacological inhibition of MCL-1 disrupts mitochondrial cristae and fatty acid oxidation in human neural progenitor cells

Marina R. Hanna, Madison Yarbrough, Melanie Gil, and Vivian Gama

Myeloid Cell Leukemia-1 (MCL-1) is an anti-apoptotic protein that is crucial for early neurodevelopment. Loss of MCL-1 results in embryonic lethal neurodevelopmental defects that cannot be rescued by other anti-apoptotic proteins of the BCL-2 family. However, the noncanonical functions and mechanisms of MCL-1 during neurodevelopment are yet to be uncovered. We hypothesize that MCL-1 modulates fatty acid oxidation (FAO) either directly through interaction with FAO enzymes or indirectly by maintaining mitochondrial cristae morphology. FAO is a metabolic process critical for energy production and cellular homeostasis that occurs within the mitochondrial matrix and produces metabolic precursors, such as acetyl-CoA, that can impact cell identity and fate decisions. Our studies in human neural progenitor cells (hNPCs) show that pharmacological inhibition of MCL-1 disrupts mitochondrial cristae morphology and decreases levels of cristae shaping proteins. Targeting MCL-1 with small molecule inhibitors alters levels of FAO enzymes, accumulates lipid droplets, and downregulates two critical hNPC transcription factors – *PAX6* and *EOMES*. Our data reveal that MCL-1 is crucial for the maintenance of intermediate progenitor cells. These findings shed light on the complex molecular interactions between mitochondrial cristae morphology and metabolic signaling in the context of cell identity during neurogenesis.

Interrogating the mechanisms of mitochondrial cristae remodeling during neurogenesis.

Authors: Caleb S. Hayes, Vincent Ling, Caroline Bodnya, & Vivian Gama

As neural stem cells differentiate into neural progenitor cells and eventually mature neurons, they undergo a metabolic shift from glycolysis towards oxidative phosphorylation (OXPHOS). This new metabolic context, along with myriad biochemical and signaling requirements, relies on dynamic changes to mitochondrial morphology. In addition to changes in overall morphology, the mitochondrial cristae, invaginations of the inner mitochondrial membrane, undergo remodeling that results in increased density to facilitate increased OXPHOS and metabolic signaling. The major regulator of cristae formation and maintenance is the mitochondrial contact site and cristae organizing system (MICOS) complex, comprised of the MIC60 and MIC10 subcomplexes which are bridged by MIC13. My study aims to investigate the role of MICOS and cristae remodeling within neurogenesis using induced pluripotent stem cell (iPSC)-derived neural progenitor cells and neurons. Previous work interrogating MICOS and its role in cristae architecture in a human context has been done using non-neuronal systems, despite many disease-associated mutations resulting in neurological phenotypes.

Due to the MIC60 subcomplex having critical roles outside of cristae architecture, I knocked out MIC13 in iPSCs to disrupt the incorporation of the MIC10 subcomplex into MICOS. This approach allows for examining how cristae architecture and remodeling impact neurogenesis without ablating non-cristae functions. The MIC13 deficient iPSCs retained their pluripotency capability and differentiated into neural progenitor cells (NPCs). Using advanced microscopy techniques, I was able to visualize cristae morphology changes in the MIC13 KO NPCs, and detected possible rewiring of lipid metabolism through lipidomic and lipid droplet analyses. Our results provide insight into how cristae remodeling and metabolic regulation impact neural cell identity throughout neurogenesis. We are currently investigating whether MIC13 KO neural progenitor cells have any defects in their capacity to give rise to functional cortical neurons and forebrain organoids. This work aims to show how critical cristae morphology is for neurogenesis and to identify the MICOS-dependent metabolic signals that coordinate neurogenesis. We also aim to increase our understanding of the pathophysiology of mitochondrial rare diseases caused by mutations in genes encoding MICOS proteins which cause devastating neurodevelopmental diseases.

ER-phagy Drives Conserved, Age-Related Remodeling of the Endoplasmic Reticulum and Promotes Longevity

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As we age, a progressive accumulation of molecular damage drives homeostatic failure, thus increasing disease burden in our rapidly aging society. Targeting the mechanisms of aging can therefore mitigate disease progression, reduce late-life morbidity, and promote healthier lives. At the center of cellular function and homeostatic decline is the endoplasmic reticulum (ER), which comprises structurally distinct subdomains that serve as specific hubs for specialized cellular functions. Although altered ER functions are increasingly linked to age-related pathogenesis, whether these functional changes are driven by underlying structural shifts is unclear. We therefore hypothesize that ER structure-function remodeling is a pivotal upstream event in the aging process.

Our findings demonstrate that ER remodeling is a conserved feature of aging across yeast, *C. elegans*, and mammals. Focusing on *C. elegans* as exemplar of metazoan aging, we reveal striking reductions in ER mass across diverse tissues and a morphological shift from rough ER sheets to tubular ER. This morphological transition corresponds with large-scale shifts in ER proteome composition from protein synthesis to lipid metabolism.

We identify selective ER degradation, or ER-phagy, as a driver of this remodeling. Tissue-specific factors, including a novel ER-phagy receptor, TMEM-131, and the IRE-1/XBP-1 branch of the unfolded protein response, regulate ER-phagy to mediate the loss of predominantly rough ER. Notably, ER remodeling is not merely a consequence of aging but a proactive, protective response. Diverse lifespan-extending interventions promote profound ER remodeling, and ER-phagy is required for lifespan extension via TOR suppression in both yeast and *C. elegans*. These findings establish ER dynamics and ER-phagy as critical, underappreciated mechanisms in both normal aging and enhanced longevity, suggesting that modulating ER morphology via ER-phagy could offer novel therapeutic strategies for promoting healthy aging.

A Novel Mechanism of Wound-Induced Cell Fusion Primed by Plasma Membrane Damage

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Tissue repair is a dynamic and intricately coordinated process. Understanding cellular behaviors that promote wound healing has both basic biological and clinical significance, as non-healing wounds form ulcers and over-healing wounds result in fibrotic scars. After most tissue injuries, some cells surrounding the wound suffer collateral cellular damage. For example, epithelial cells within about 100 μm from pulsed laser ablation have various degrees of plasma membrane damage. These peripheral cells need to manage their own cellular injuries while actively repairing the nearby epithelial breach. How are the two levels of repair integrated? We report a novel crosstalk between the cell-level plasma membrane damage and a tissue repair strategy – cell-cell fusion.

We and others have previously reported that, after tissue injury, cells near the wound fuse frequently to form multinucleated syncytia. Using *Drosophila* pupal notum as a model epithelium, we demonstrated that these syncytia are better able to heal wounds than the neighboring diploid cells. However, one outstanding question remains: what allows these epithelial cells, which are not pre-programmed to fuse during development, to undergo fusion after tissue injury? We found that fusion is restricted to cells with plasma membrane damage. A wounding technique that eliminates plasma membrane damage also eliminates cell fusion. However, plasma membrane damage, albeit necessary, is not sufficient to achieve wound-induced fusion. Cell-cell fusion requires, first, the formation of nanometer-scale fusion pores that overcome repulsive electrostatic forces between adjacent membranes, and second, the expansion of the pore to several microns to fully connect the cells. We demonstrate that, aside from mechanical damage, fusion after injury is biologically regulated, and this process appears to require endocytosis and high cortical tension. Post-wound cortical tension is decreased in the two mutants, dynamin and its binding partner endophilin, identified by us to impair wound-induced fusion. Hence, we propose a novel framework of wound repair in which a threshold of plasma membrane damage potentiates epithelial cell fusion, and the progression of fusion requires endocytosis and high cortical tension.

Inhibited DNA methylation drives metaplastic cell lineage differentiation and progression

Authors: Halee Scott, Eunyoung Choi

Gastric cancer frequently arises after distinct cellular changes in the gastric mucosa, including the transdifferentiation of chief cells into spasmolytic polypeptide-expressing metaplastic (SPEM) cells, the main cell type of pyloric metaplasia (PM). PM often progresses to intestinal metaplasia (IM) and neoplastic stages like dysplasia and cancer. One event that occurs during this progression is the upregulation of DNA methyltransferase 1 (Dnmt1) in the SPEM cell population. Dnmt1 maintains global DNA methylation in dividing cells and its dysregulation is a hallmark of many cancers. In this study, we characterized Dnmt1 expression during SPEM cell proliferation, maturation and differentiation using *in vitro* and *in vivo* models. Following this, we examined the effects of both Dnmt1 knockdown and global inhibition of DNA methylation on SPEM cell lineages using PM, IM, and dysplastic mouse organoid lines.

Immunofluorescent (IF) staining of Dnmt1 in mouse and human metaplastic stomach tissue reveals increased expression of Dnmt1 in SPEM cell lineages. Furthermore, Dnmt1 is strongly expressed in SPEM and IM organoids but is absent in dysplastic organoids, suggesting that Dnmt1 plays a role in maintaining metaplastic cell types. PM and IM organoids exhibit distinct proliferative capacities, with Dnmt1 expression peaking one-week post-passage during the proliferative growth phase and declining by two weeks, when maturation occurs. PM and IM organoids were cultured in minimal media lacking growth factors typically present in standard media. These organoids differentiated into surface cell lineages after one week, with reduced expression of SPEM markers and increased expression of surface cell markers Uea1 and Muc5ac These differentiated surface cells lost Dnmt1 expression, suggesting the role of Dnmt1 in maintaining the stem-like nature of proliferative SPEM cells.

To elucidate the function of Dnmt1 in metaplastic cell types, siRNA-mediated knockdown of Dnmt1 was performed in 2D cultures derived from PM and IM organoid lines. Cells were transfected with Dnmt1 siRNA using lipofectamine-mediated delivery and harvested at 48 hours post-transfection. RNA was isolated and Dnmt1 expression was examined using qPCR. Preliminary results show a significant reduction of Dnmt1 gene expression with one of the siRNA duplexes in both PM and IM cells. However, qPCR analysis of SPEM-associated genes following Dnmt1 KD revealed a decrease in SPEM-related genes in PM cells but not IM cells, suggesting that Dnmt1 may be crucial in early PM rather than IM. Overall, these results indicate that Dnmt1 plays a key role in maintaining the metaplastic cell population.

To determine the impact of inhibiting global DNA methylation during metaplasia progression, we treated SPEM and IM organoids with the DNA methylation inhibitor 5-Aza-2'-deoxycytidine (5-AZA-CdR) for one week. After treatment, organoids were analyzed using histology, IF and qPCR. Treatment with 5-AZA-CdR resulted in a decrease in SPEM cell lineage markers, like Aqp5, in both PM and IM organoids. PM organoids showed increased Villin and Tff3 expression, features of intestinal cells, and retained organization. In contrast, IM organoids lost epithelial character and gained dysplastic cell features, including multilayering and complicated budding structures, along with increased expression of dysplastic cell markers, Trop2, Cldn7 and MMP7. Together, our results suggests that DNA methylation changes contribute to both cellular differentiation and lineage evolution of metaplastic cells during gastric carcinogenesis.

Non-Stem Cell Lineage Gives Rise to Atoh1-independent, Succinate-Activated Tuft Cells

Deronisha Arceneaux[1], Amrita Banerjee, Yilin Yang[1], Lucy Chen[1] , Bob Chen[1], Alan Simmons[1], Yanwen Xu[1], Ken Lau[1]

[1]Cell and Developmental Biology

Tuft cells of the intestinal epithelium are categorized into two distinct subtypes with specialized functions. Type 2 tuft cells are involved in regulating type 2 immunity, whereas type 1 tuft cells exhibit neuronal-like properties. These subtypes arise from different developmental origins and are regulated by distinct mechanisms: the microbiome influences type 2 tuft cells, while the master secretory transcription factor Atoh1 governs type 1 tuft cells. Recent advancements, including single-cell RNA sequencing (scRNA-seq) and clump-seq, have identified and characterized these tuft cell populations. Spatially, type 2 tuft cells are predominantly localized within the villi, while type 1 tuft cells reside in the intestinal crypts. Transcriptomic analyses reveal that type 2 tuft cells share features with enterocytes and express immune-related genes, suggesting a differentiation trajectory linked to enterocyte progenitors. Lineage tracing following type 2 stimulation demonstrates that immune-activated type 2 tuft cells originate from committed precursors rather than crypt-based columnar stem cells. These findings highlight the existence of two molecularly and functionally distinct tuft cell subtypes, each defined by unique origins and mechanisms of specification.

Poster Presentations
Friday, May 23

1. Rajdeb Banerjee
2. Anna Cassidy
3. Kylie Jozwik
4. Andrew Kjar
5. Zachary Lehmann
6. Briana Markham
7. Thyandra Martinez
8. Krishna Mudumbi
9. Alexandra Mulligan
10. Alessandra Norris
11. Olivia Perkins
12. Kianna Robinson
13. Elizabeth Ruark
14. Avishkar Sawant
15. Lauren Schnitkey
16. Rahul Sharma
17. Jennifer Silverman
18. Yang Song
19. Pankajam Thyagarajan
20. Diego Torres-Martinez
21. James Hayes
22. Aishwarya Venkataravi

Integrin α3β1: A Molecular Key Player in Metanephric Mesenchyme Differentiation and Kidney Development

Rajdeb Banerjee, Jongmin Sim, Meiling Melzer, Olga Viquez, Ambra Pozzi, Fabian Bock, Roy Zent

Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Kidney development begins when the ureteric bud invades the metanephric mesenchyme, initiating the formation of the collecting duct system and nephrons, respectively. Integrins (Itg) α3β1, α6β1, and α6β4—key receptors for extracellular matrix components such as Laminin—are essential for renal development, function, and pathology. Among them, mutations in Itgα3 have been associated with neonatal lethality due to severe kidney and lung defects; however, the specific role of Itgα3β1 in metanephric mesenchyme development remains poorly understood. To investigate this, we conditionally deleted the Itgα3 subunit in the metanephric mesenchyme. This resulted in perinatal lethality with profound kidney abnormalities, including reduced organ size, a significantly lower nephron count, and widespread defects in glomerular and tubular architecture. Specifically, we observed fewer glomeruli with abnormal mesangial and capillary loop structures, indicating a disruption in cellular proliferation during nephrogenesis. In vitro analyses confirmed a proliferation defect, characterized by delayed cell cycle progression. Using DNA content profiling, cyclin and CDK expression analysis by synchronized cell cycle studies via a double thymidine block, we observed a clear delay at the G1/S transition. These findings were corroborated using the PIP-FUCCI sensor, which further demonstrated impaired cell cycle dynamics in Itgα3-deficient cells. Mechanistically, loss of Itgα3 disrupted AKT and ERK signaling pathways, both of which are critical for cell cycle regulation. Notably, increased levels of p-γH2AX indicated elevated DNA damage in the absence of Itgα3, suggesting impaired S-phase DNA replication fidelity. Together, our data reveal that integrin α3β1 signaling is vital for maintaining DNA synthesis integrity during the cell cycle, thereby ensuring normal cell proliferation and nephron formation during kidney development.

The GTP-Tubulin Cap is Not the Determinant of Microtubule End Stability in Cells

Anna Cassidy[1,+], Veronica Farmer[1,2,+], Goker Arpağ[1,3] and Marija Zanic[1,4,5]

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Microtubules are dynamic cytoskeletal polymers essential for cell division, cell motility, and intracellular transport. Microtubule dynamics are characterized by dynamic instability—the ability of microtubules to switch between phases of growth and shrinkage. Dynamic instability can be explained by the GTP-cap model, suggesting that a 'cap' of GTP-bound tubulin subunits at the growing microtubule end has a stabilizing effect, protecting against microtubule catastrophe—the switch from microtubule growth to shrinkage. Although the GTP-cap is thought to protect the growing microtubule end, whether the GTP-cap size affects microtubule stability in cells is not known. Notably, a family of microtubule end binding proteins, EBs, recognize the nucleotide state of tubulin, and their comet-like localization at growing microtubule ends can be used as a proxy for the GTP-cap. Previous work *in vitro* demonstrated that EB comet size increases with increasing microtubule growth rates, achieved using microtubule polymerase XMAP215. Surprisingly, the rate of microtubule catastrophe also increased, despite the presence of a larger GTP-cap. To what extent these relationships observed *in vitro* relate to microtubule dynamics in complex cellular environments in the presence of many different microtubule associated proteins is not known. Here, we use high spatiotemporal resolution imaging to directly compare the relationship between EB comet size and microtubule dynamics in interphase LLC-PK1 cells to that measured *in vitro*. Our data reveals that GTP-cap size in cells scales with the microtubule growth rate the same as *in vitro*. However, we find that microtubule ends in cells can withstand transition to catastrophe even after the EB comet is lost. Taken together, our data reveal that, while the GTP-cap size is similarly modulated in response to microtubule growth rate in the two investigated systems, the presence of the GTP-cap is not the determinant of microtubule end stability in cells.

Leveraging natural genetic variation as a tool to study mitochondrial DNA regulation

Kylie Jozwik, Maulik Patel

Mutations in the mitochondrial genome (mtDNA) are associated with severe childhood diseases and age-related degeneration. Unlike the nuclear genome, mtDNA exists in tens to thousands of copies per cell that encode essential components of the electron transport chain. When mtDNA mutations accumulate beyond a critical threshold, energy deficiencies and mitochondrial diseases can arise. Although the presence of multiple mtDNA variants—termed heteroplasmy—is common, predicting inheritance patterns and pathogenicity of mutant mtDNA remains challenging. The nuclear genome encodes all machinery responsible for mtDNA replication, transcription, and maintenance. Therefore, heteroplasmy must be regulated by the nuclear genome. Previous studies have utilized human biobank data to uncover nuclear genome variation that influences heteroplasmy, but human studies lack functional validation and mechanistic insights. I aim to overcome these limitations by utilizing the genetically tractable model organism, *C. elegans*, in which wild isolate genetic variation can be systematically interrogated in an unbiased and controlled setting. Using a recently developed genetic tool, I can enforce uniparental inheritance of the nuclear genome while preserving maternally inherited mtDNA. This approach enables analysis of the same mtDNA heteroplasmy in genetically diverse nuclear backgrounds. After identifying strains that differentially regulate heteroplasmy levels, I will chromosomally map the variants responsible and investigate their mechanisms of action. Ultimately, this work seeks to provide a mechanistic understanding of heteroplasmy dynamics which is essential for improving the ability to predict, prevent, and treat mitochondrial diseases.

Spatial and temporal control of tau pathology in neural organoids

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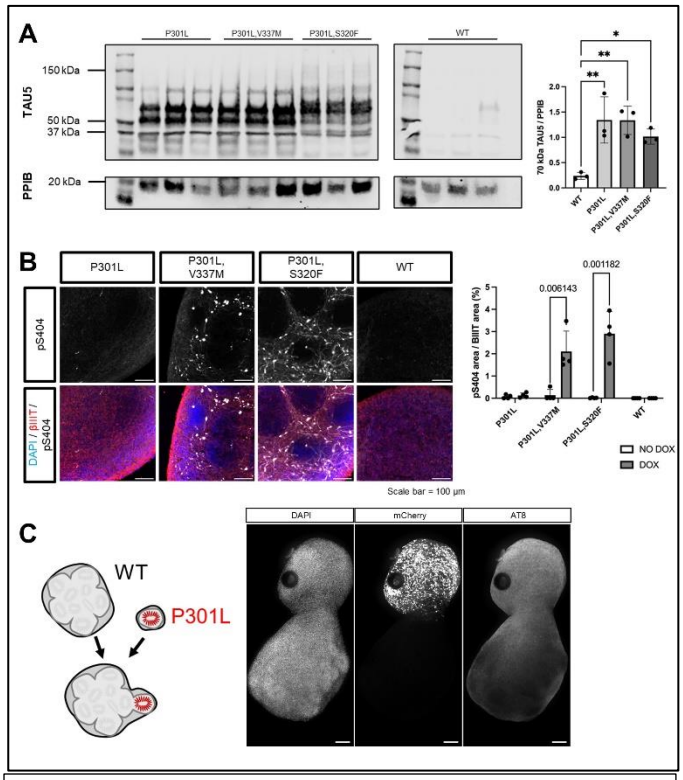
Protein aggregation of the microtubule associated tau (encoded by MAPT) is a key feature of Alzheimer's disease, and other tauopathies. Toxic protein aggregates are known to spread between cells and propagate across brain regions during the course of disease.

In this work we focus on building a next-generation model of tau aggregation and spread. While previous *in vitro* models have succeeded in producing cell populations which harbor tau mutations, they generally produce homogenous cell populations that constitutively express the mutant protein, thus making interrogation of temporal and spatial spreading impossible. To overcome this challenge, we utilize assembloid neural organoids, with inducible pathology to gain spatial and temporal control of pathology.



A) Western blot analysis of HEKs after induction of cassette, displaying robust tau induction. **B)** Analysis of pS404 phospho-tau signatures in day 60 organoids, showing tau buildup after cassette induction.
C) Representative schematic and image of a day 40 engineered assembloid, displaying segregation of reporter signal (mCherry), and putative spread of phosphorylated tau (AT8).

We constructed a synthetic MAPT overexpression cassette, which is induced by doxycycline addition. We constructed a cassettes with an aggregation prone missense mutation in MAPT, P301L, or dual mutations P301L+V337M and P301L+S320F. We then screened these cassettes in HEKs and neural organoids, verifying construct efficacy (**Figure 1A-B**). We now have begun assembling wild-type (WT) and mutant organoids together into long-term assembled cultures which retain spatial control of inducible pathology (**Figure 1C**). Our work demonstrates a novel paradigm by which to understand the cellular dynamics of tau aggregate spread.

The junctional actin belt is a novel site of microvillar growth.

Zachary Lehmann, Jennifer Silverman, Caroline Cencer, Matthew J. Tyska

Transporting epithelial cells across a diverse set of tissues depend on the assembly of apical microvilli: actin-bundle supported membrane protrusions that increase the apical surface area. While the molecular composition of apical microvilli has been well characterized, the mechanisms promoting their assembly are poorly understood. Previous reports characterized *de novo* growth of microvilli on the medial regions of the apical surface, preceded by the accumulation of actin filament elongation-promoting factors. More recent studies have elucidated an intimate connection between nascent apical protrusions and clathrin-coated endocytic pits, where clathrin-mediated endocytic events on the apical surface serve as a local source of actin polymer for the assembly of microvilli-supporting core actin bundles. Drawing from this mechanism, we questioned whether other actin-rich resources at the apical surface can serve as a platform for microvillar growth. During differentiation, microvillar protrusions can be visualized accumulating at the planar boundary of the apical surface in native tissue and cell culture models. Using live-cell imaging, we demonstrated that nascent protrusions also emerge at this location, directly above the actin-filament rich apical junctions. Further, we demonstrated that microvillar core actin bundles extend into the region of the apical junctions, suggesting that actin filaments may be continuous between the two structures. These data suggest apical junctions are a key source of actin polymer for the assembly of core actin bundles that support microvilli.

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Regulation of Golgi phosphoinositides and protein trafficking by the lipid flippase Neo1

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Membrane asymmetry, marked by a difference in phospholipid composition between membrane leaflets, is a fundamental feature of eukaryotic cells. This asymmetry plays a crucial role in processes like apoptosis, signaling, and vesicle trafficking. The *Saccharomyces cerevisiae* P4-ATPase Neo1 is a Golgi-localized phospholipid flippase that maintains lipid asymmetry by flipping phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol 4-phosphate (PI4P) from the lumenal to the cytosolic leaflet of the Golgi membrane. The PI4P flipped by Neo1 is initially synthesized at the cytosolic leaflet of the plasma membrane and is transported to the endoplasmic reticulum (ER) by Osh6, where it can avoid dephosphorylation by the PI4P phosphatase Sac1 by scrambling to the ER lumen. This PI4P is then transported by COPII vesicles to the Golgi, where Neo1 flips the PI4P to the cytosolic leaflet to prevent its cell surface exposure. This action blocks the aminoglycoside antibiotic neomycin from binding to extracellular PI4P and entering the cell. Specific mutations in Neo1's substrate translocation pathway lead to neomycin sensitivity and exposure of PI4P on the extracellular leaflet of the plasma membrane. Here, we show that these Neo1 mutants surprisingly display a loss of PI4P in the cytosolic leaflet of the Golgi even though the PI 4-kinase Pik1 remains localized to the Golgi. Intriguingly, confining Sac1 to the ER restored Golgi PI4P levels in these *neo1* mutants. Our results suggest that PI4P flip by Neo1 induces retrograde transport of Sac1 from the trans-Golgi network (TGN) to the cis-Golgi, thereby preserving PI4P levels in the cytosolic leaflet of the TGN.

Sphingolipid metabolism drives mitochondria remodeling during aging and oxidative stress

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Abstract

One of the hallmarks of aging is a decline in the function of mitochondria, which is often accompanied by altered morphology and dynamics. In some cases, these changes may reflect macromolecular damage to mitochondria that occurs with aging and stress, while in other cases they may be part of a programmed, adaptive response. In this study, we report that mitochondria undergo dramatic morphological changes in chronologically aged yeast cells.

These changes are characterized by a large, rounded morphology, decreased co-localization of outer membrane and matrix markers, and decreased mitochondrial membrane potential. Notably, these transitions are prevented by pharmacological or genetic interventions that perturb sphingolipid biosynthesis, indicating that sphingolipids are required for these mitochondrial transitions in aging cells. Consistent with these findings, we observe that overexpression of inositol phospholipid phospholipase (Isc1) prevents these alterations to mitochondria morphology in aging cells. We also report that mitochondria exhibit similar sphingolipid-dependent morphological transitions following acute exposure to oxidative stress. These findings suggest that sphingolipid metabolism contributes to mitochondrial remodeling in aging cells and during oxidative stress, perhaps as a result of damaged sphingolipids that localize to mitochondrial membranes. These findings underscore the complex relationship between mitochondria function and sphingolipid metabolism, particularly in the contact aging and stress.

The V659E transmembrane mutation in ErbB2 causes misfolding and mislocalization of the receptor

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The human epidermal growth factor receptor 2 (ErbB2/HER2) is a receptor tyrosine kinase (RTK) involved in regulating cell growth, division, and repair. Unlike other ErbB receptors, ErbB2 does not have a ligand and relies on homodimerization and heterodimerization to activate downstream signaling pathways. Missense mutations in the transmembrane domain of ErbB2 have been identified as oncogenic in non-small-cell lung cancer (NSCLC), but treatment with the HER2 antibody trastuzumab has induced limited responses. The transmembrane V659E mutation is thought to promote ErbB2 dimerization by forming hydrogen bonds between the polar glutamic acid residues, strengthening the lateral interactions between transmembrane domains and initiating signaling. However, previous efforts to experimentally validate this hypothesis have shown conflicting results. Here, we use immunofluorescence microscopy and single-particle tracking (SPT) to show that the Val to Glu mutation, while activating, alters receptor trafficking, with a large fraction of the receptor stuck in the endoplasmic reticulum, hindering treatment with the antibody trastuzumab. Our data suggests that tyrosine kinase inhibitors (TKIs) traffic the misfolded receptor to the plasma membrane, improving the efficacy of targeted therapy with antibodies such as trastuzumab or antibody drug conjugates (ADCs).

Investigating the function of EPS8 in actin-based protrusions

Alexandra G Mulligan, Matthew J Tyska

Actin-based protrusions such as filopodia, microvilli, and stereocilia, are evolutionarily conserved structures that support a variety of cellular functions ranging from nutrient absorption to mechanosensation. Although these structures have been well characterized, how their dimensions are regulated remains unknown. Many accessory proteins are associated with actin filaments (F-actin) in protrusions including proteins that form “dense tip material” at the barbed ends, which are well positioned to play roles in regulating protrusion dimensions. One protein that localizes robustly to the distal tips of filopodia, microvilli, and stereocilia is Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8). Previous studies have shown that a constitutive loss of EPS8 in epithelial cell culture and mouse models results in shorter microvilli and stereocilia, suggesting that EPS8 is functioning specifically at the barbed ends of actin filaments to promote elongation. Furthermore, EPS8 has a C-terminal actin-binding domain that has proposed actin capping and bundling motifs from *in vitro* and *in vivo* experiments, respectively. Despite the consistent localization of EPS8 across various models, how it supports the growth and/or function of surface protrusions remains unknown. Preliminary data show that upon addition of a known barbed end binder, Cytochalasin D (CytoD), EPS8 is displaced from the distal ends of protrusions, suggesting that EPS8 is directly binding the barbed ends of F-actin. Previous work from our lab demonstrated that in epithelial cells, EPS8 puncta appear at the apical membrane prior to the genesis of a new microvillus, remain at the distal tip for the lifetime of the structure, and then disappear right before the microvillus collapses. Taking advantage of CRISPR editing technology, we have endogenously tagged EPS8 in HeLa cells. Using this system, we also observed that EPS8 puncta are found at the distal tips of growing filopodia. Together, these data suggest that EPS8 regulates the dimensions of actin-based protrusions by controlling actin incorporation at filament barbed ends in each core bundle, however further investigation is required to understand the direct role of EPS8. Through a combination of gene edited cell lines, and fixed and live cell microscopy, I will examine how perturbations to native EPS8 impact the architecture of the actin filament bundle in protrusions.

Implementing multi-isotope mass spectrometry (MIMS) with scanning electron microscopy (SEM) to observe metabolic shifts under lifespan-extending paradigms in *C. elegans*

Alessandra Norris, Chris Acree, Tyler Butsch, Kelsey Mabry, Rafael Arrojo e Drigo and Kristopher Burkewitz

Dietary restriction (DR) extends lifespan from worms to primates, while also protecting against diverse chronic diseases. DR is thought to confer these benefits in part by reprogramming metabolism, triggering adaptive responses that prioritize energy efficiency, maintenance, and resilience at the expense of growth and reproduction. Together, these responses impact nutrient flux and utilization between tissues as well as between organelles within individual cells. However, we lack any complete model on how these changes occur throughout tissues and within individual cells, which could provide potential therapeutic targets. Here we attempt to generate a framework for DR-mediated metabolic reprogramming across an organism with a new microscopy modality that combines spatial multi-isotope mass spectrometry (MIMS) with scanning electron microscopy (SEM), both at nanoscale resolution. We pioneered MIMS-EM for the *C. elegans* model, as its simple and compact body plan enables us to simultaneously visualize not only all tissues, but also all organelles inside those tissues within a single SEM cross section. Experimentally, we developed a sample processing pipeline similar to conventional SEM protocols and have performed *in vivo* pulse-chase experiments with stable isotope-labeled nutrients in worms fed either standard diets or life-extending DR. Subsequently, the correlated MIMS and SEM images enable us to visualize and quantitatively measure where metabolic incorporation and turnover are occurring across timepoints at both the tissue and organelle level *in situ*. This approach will point to physiological and cell biological metabolic adaptations that may underpin DR's protective effects against aging.

Apical clathrin-coated pits control the location, timing, and scale of microvillar growth

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Epithelial cells from diverse contexts assemble apical specializations to serve tissue-specific functions. In virtually all cases, these features consist of arrays of microvilli: micron-scale, actin bundle-supported protrusions that mediate biochemical and physical interactions with the external environment. Despite their importance for epithelial physiology, how microvilli grow during cellular differentiation remains poorly understood. Using genetic and small molecule perturbations, we found that an epithelial cell's potential for growing microvilli of normal size is limited by an adjacent actin-dependent process: apical clathrin-mediated endocytosis. Unexpectedly, timelapse imaging of individual microvillar growth events revealed tight spatial and temporal coupling to sites of clathrin-mediated endocytosis. Ultrastructural characterization of undifferentiated epithelial monolayers also showed that most nascent microvilli are in contact with an apical endocytic pit. Finally, inhibition of the Arp2/3 branched nucleation complex, which drives actin polymerization on coated pits, significantly reduced the accumulation of new microvilli on the surface of differentiating epithelial cells. Based on these discoveries, we conclude that clathrin-mediated endocytosis and its associated Arp2/3-based actin nucleation activity control the timing and location of microvillar growth, as well as the dimensions of the resulting protrusions.

Determining the mechanism of MYO7B motility.

Kianna L. Robinson[1], Matthew J. Tyska[1*]

The intestinal epithelium is responsible for both nutrient absorption and pathogen protection via the brush border, composed of thousands of actin-supported finger-like protrusions, known as microvilli. The intermicrovillar adhesion complex (IMAC) drives length matching and tight packing of these protrusions to maximize the number of them on the apical surface. The IMAC localizes to the distal tips of microvilli and consists of two modules: extracellular adhesion links and cytoplasmic proteins. The adhesion links are composed of the transmembrane protocadherins CDHR2 and CDHR5 that form an extracellular threadlike ~50 nm link between microvilli. IMAC cytoplasmic proteins include the actin-based motor myosin-7B (MYO7B), which contains a C-terminal tail with an SH3 domain flanked by two MYTH4/FERM domains, and scaffolding proteins ankyrin repeat and sterile alpha motif domain containing 4B (ANKS4B) and Usher syndrome 1C (USH1C). MYO7B was previously shown to interact with other IMAC proteins through its tail domain and promote their enrichment at the distal tips of microvilli. However, MYO7B lacks a clear dimerizing motif like that found in most processive motors (e.g. a coiled coil), and the mechanisms that allow it to move to the distal tips are currently unknown. Interestingly, we find in our current studies that the tail of MYO7B can promote tip localization of Myosin 10 motor domain in the absence of its native coiled coil. To gather preliminary data on if MYO7B tail promotes tip localization of motor domains by self-associating, we next expressed soluble differentially tagged MYO7B tail constructs and quantified their colocalization. We find that these MYO7B tail constructs exhibit significant colocalization and arrange into robust puncta throughout the cytoplasm. This data suggests that MYO7B tails can form a complex with other MYO7B tails within the cell by self-associating into puncta. Further studies will focus on directly testing the hypothesis that MYO7B tails self-associate to support MYO7B tip directed motility.

InsP3R coordinates adaptive responses to promote mitochondrial quality control during chronic mitochondrial stress-induced longevity

Elizabeth M Ruark, Gaomin Feng, Alexandra G Mulligan, Brianne Jacquet-Cribe, and Kristopher Burkewitz

Perturbing mitochondrial function can elicit cellular responses that range from detrimental effects which trigger pathogenesis to beneficial effects which promote extension of lifespan. However, limited understanding of the adaptations that distinguish between these opposing outcomes hinders therapeutic progress. One underexplored target for modulating mitochondrial roles in aging is the endoplasmic reticulum InsP3R Ca^{2+} channel which promotes mitochondrial homeostasis through both direct matrix Ca^{2+} flux and diverse cytosolic pathways. Using *C. elegans*, we find that the InsP3R is uniquely required for lifespan extension induced by impairment of the electron transport chain (ETC) but is dispensable of the mitochondrial calcium uniporter indicating InsP3R signaling promotes adaptation independently of matrix Ca^{2+} uptake. Utilizing long-lived ETC Complex I mutants, we find that the InsP3R prevents maladaptive hyper-expansion of dysfunction mitochondrial networks and coordinates transcriptional reprogramming to promote mitochondrial biogenesis. Further, we show that the InsP3R promotes mitochondrial turnover via control of the actin cytoskeleton for pruning of dysfunctional mitochondria from the network and show that rescuing mitochondrial fission and turnover restores longevity in InsP3R mutants. Overall, these findings reveal a cytosolic inter-organelle Ca^{2+} signaling pathway essential for controlling the balance between mitochondrial biogenesis and degradation while highlighting critical new roles for stress-induced actin damage responses in mitochondrial longevity paradigms.

Cell cycle-regulated tug-of-war between microtubule motors positions major trafficking organelles

Avishkar V. Sawant and Irina Kaverina

Rapidly dividing cell populations must maintain efficient membrane trafficking while constantly remodeling their interior in preparation for cell division. Efficient protein processing and sorting in the mammalian Golgi apparatus relies on the integrity of this organelle. The integral Golgi is assembled around the centrosome by microtubule minus end-directed molecular motor cytoplasmic dynein. However, the Golgi must dissociate from the centrosome to allow for unperturbed centrosome separation in mitosis, which we have previously shown to occur as early as the G1/S transition. In addition, the Golgi exists in a constant membrane exchange with the Endoplasmic Reticulum (ER) through ER exit sites (ERES), which are also transported by microtubule molecular motors. Cell cycle signaling and molecular mechanisms that coordinate Golgi-centrosome and Golgi-ERES association still need to be understood. Here, we apply live cell imaging and loss-of-function approaches to show that cell cycle signaling tunes tug-of-war between the plus-end and minus-end-directed molecular motors, resulting in differential positioning of Golgi and ERES in the interphase sub-stages. Specifically, we find that in G1, the Golgi and ERES are brought to the centrosome by the minus-end-directed action of dynein and KIFC3, respectively. On the onset of the S-phase, kinesin-1-dependent activity at both the Golgi and ERES overpowers minus-end directed motors, driving the Golgi away from the centrosomes and spreading ERES throughout the cytoplasm. Out of known kinesin-1 motors (KIF5s) and kinesin light chains (KLCs), we have identified KIF5B and KLC1 as drivers for Golgi translocation in S/G2. In contrast, our preliminary data suggest that kinesin-dependent ERES transport in S/G2 is driven by KIF5C and KLC3 rather than KIF5B and KLC1. Interestingly, CDK1 inhibition in S-phase reverses the ERES and Golgi transport toward the minus end-directed motor activity, leading to a compact ERES/Golgi configuration around the centrosome, similar to G1. An acute kinesin-1 inhibition at this stage causes similar retrograde repositioning of the Golgi and ERES. This suggests that CDK1 activity in the S phase rises sufficiently to facilitate the switch of molecular motors favoring kinesin-1-dependent transport of these organelles. Our data indicate that CDK1 likely regulates KLCs, enhancing recruitment of respective kinesin-1 variants to the Golgi and ERES in S/G2. Overall, we conclude that CDK1 signaling regulates Golgi and ERES positioning via kinesin-1 recruitment to the membranes and that the differential positioning of these two organelles reflects the association of these organelles with different sets of molecular motors.

Characterization of the STK38 Kinase in Wnt Signaling and Cardiac Development

Lauren Schnitkey, Muhammad Taha, Yashi Ahmed, Ethan Lee

Precise coordination of Wnt signaling dynamics is essential for heart development and its dysregulation underlies many congenital heart defects (CHDs)—the most prevalent class of birth anomalies. We have identified the serine/threonine kinase **STK38** as a novel and evolutionarily conserved regulator of Wnt signaling, with pivotal functions in cardiogenesis across ***drosophila***, **zebrafish**, **mice**, and **mammalian** systems.

Loss-of-function studies in zebrafish reveal that **STK38 is essential for proper cardiac morphogenesis**, positioning it as a critical node in Wnt-dependent developmental processes. In human cells, STK38 depletion leads to a marked reduction in Wnt/β-catenin reporter activity and downstream target gene expression, acting **downstream of the β-catenin destruction complex**. These findings point to a functional role for STK38 at the **transcriptional level**, beyond the cytoplasmic signal cascade.

Mechanistically, **co-immunoprecipitation and mass spectrometry** uncover a direct interaction between STK38 and the transcriptional co-repressor **TLE3**, suggesting that STK38 may modulate **nuclear Wnt signaling output** by influencing repressor–activator dynamics within the Wnt transcriptional complex.

Ongoing work leverages **induced pluripotent stem cells (iPSCs)** to map STK38 expression and function across stages of cardiac differentiation. Through temporally controlled depletion, we aim to define **critical windows of STK38 activity** and its role in directing Wnt-driven transitions from progenitors to differentiated cardiomyocytes.

Together, our findings illuminate a previously unrecognized nuclear function of STK38 in shaping Wnt signaling outcomes during heart development. This work provides a new lens through which to understand cell fate dynamics in cardiogenesis and opens the door to novel therapeutic strategies for CHDs.

Abstract

A Comprehensive Workflow for Imaging Live Insulin Secretion Events and Granules in Intact Islets

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Myat, Phyu Sin M[1], Hudson McKinney[1], Guoqiang Gu[1], Irina Kaverina[1]

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Accurate detection of insulin secretion from pancreatic beta cells is crucial for understanding normal physiological insulin secretion and its pathophysiological counterpart in diabetic states. Traditional methods using fluorescently labeled insulin granules or dye labeling often struggle to distinguish secretion from insulin granule dynamics. We present an optimized protocol using the cell-impermeable Zn^{2+}-binding dye FluoZin-3, which fluoresces upon Zn^{2+} co-secretion with insulin outside of the islet, more accurately representing secretion. FluoZin-3 combined with intact islet attachment to vascular extracellular matrix and TIRF microscopy offers high spatial and temporal resolution as well as a high signal-to-noise ratio in a minimally perturbed system. Additionally, by integrating the cell-permeable Zn^{2+}-binding dye ZIGIR, we can track insulin granule dynamics alongside secretion events. Our approach generates large datasets, which we efficiently analyze using Ilastik machine learning software, enabling fast, accurate, and optionally supervised analysis. This technique builds on our group's previous protocols, detailing a streamlined workflow adaptable to high-resolution, live-cell microscopy for not just insulin but other secretory/granule systems as well. With this method, we investigated secretion behavior of different IG pools [Predocked (appear before HG stimulation and docked to the membrane), Docked (appear upon HG stimulation and docked to membrane) and Newcomer (appear upon HG stimulation but don't dock at the membrane)] in real time during the first phase of insulin secretion. The Predocked and New IGs (consist of Docked and newcomer IGs are equally secreted and newcomer IG dwell <1 second before secretion upon high glucose stimulation. The Predocked and docked IGs can stay longer on the membrane before secretion. This method is useful in the investigation of functional beta cell heterogeneity of IG secretion in space and time.

Abbreviations: Insulin granules (IGs), total internal reflection fluorescence (TIRF), low glucose (LG), high glucose (HG), Krebs-Ringer Bicarbonate (KRB)

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Organization of a cytoskeletal superstructure in the apical domain of intestinal tuft cells

Jennifer B. Silverman, Evan E. Krystofiak, Leah R. Caplan, Ken S. Lau, Matthew J. Tyska

Tuft cells, an epithelial cell type making up ~1% of the small intestine, play important roles in sensing and responding to luminal antigens. A defining morphological feature of this lineage is the actin-rich apical ‘tuft’, although details of the cytoskeletal ultrastructure underpinning the tuft, the molecules involved in building this structure, or how it supports tuft cell biology remain unclear. We found that tuft cell core bundles consist of F-actin crosslinked in a parallel and polarized configuration and contain a tuft cell-specific complement of actin-binding proteins that exhibit differential localization. Remarkably, the array of core actin bundles interdigitates and co-aligns with a highly ordered network of microtubules, resulting in a cytoskeletal superstructure that is well positioned to support subcellular transport and in turn, the dynamic sensing functions of the tuft cell that are critical for intestinal homeostasis.

Assessment of Reference Genes for RT-qPCR experiments in Retinal Pigment Epithelium of Sodium Iodate-Treated Mice

Yang Song, Sabine Fuhrmann

The sodium iodate (SI) injury mouse model mimics geographic atrophy and is widely used to study oxidative stress in dry age-related macular degeneration (AMD). Our project focuses on gene expression changes in retinal pigment epithelium (RPE) after SI injury. RT-qPCR, a cost-effective and precise method to measure mRNA expression, was used to examine genes of interest. We aimed to identify at least two stable reference genes for our SI model, since RT-qPCR accuracy heavily depends on reference gene stability, and one reference gene alone may affect RT-qPCR accuracy.

Swiss Webster and C57Bl/6J mice were intraperitoneally injected with 30 µg/g SI or normal saline (NS). After 7 days, eyes were dissected, and RPE was isolated in RNAprotect cell reagent. RNA was extracted from RPE and choroid separately. cDNA was synthesized via reverse transcription, following with RT-qPCR. RefFinder, an online tool, assessed the stability of four candidate genes (Rn18s, Ppia, Hprt, Gapdh) using gene stability algorithms: BestKeeper, ΔCq, GeNorm and NormFinder. Bulk RNA sequencing of RPE RNA with ribo-reduction validated gene expression.

Cq values and standard curves showed that all candidate reference genes were relatively stable with acceptable amplification efficiency. For RPE samples, GeNorm ranked Rn18s and Ppia as the most stable, while Gapdh was the least stable. Bulk RNA sequencing of RPE RNA after SI/NS injection validated that Rn18s and Ppia expression did not change significantly between both conditions. However, Gapdh was the least stable among four candidate genes (Log2FoldChange=0.66, padj=0.00014). Additionally, it was confirmed that there was no choroidal contamination during RPE isolation by comparing the expression of RPE (Rpe65, Otx2) and choroidal (Col6a1) genes, which were normalized to Rn18s and Gapdh (two top ranked by RefFinder for RPE and choroid samples).

This study showed how to assess gene stability utilizing RefFinder and validation via bulk RNA sequencing. We demonstrated that Gapdh, a commonly used RT-qPCR reference gene, is least stable among 4 candidates in RPE of SI/NS treated mice. RefFinder results also support utilizing two reference genes (Rn18s and Ppia) for better RT-qPCR accuracy in RPE samples. These findings provide a foundation for the more accurate use of RT-qPCR in the gene expression analysis of the SI injury mouse model.

Investigating the rearrangement of microtubules around epithelial wounds in *Drosophila* pupal notum.

Authors: Pankajam Thyagarajan, Junmin Hua, and Andrea Page-McCaw.

Abstract:

Epithelial cells at the wound edge undergo rapid reorganization in response to tissue damage. These cells undergo directed cell migration towards the wound in a coordinated manner. The key players mediating these cellular changes at the wound edge are the cytoskeletal components: Actomyosin-based contraction and its regulation around the wound is intensively studied but the regulation of the microtubule cytoskeleton is less understood in-vivo. Other than serving as tracks for transporting essential cargo, microtubules are known to contribute to cell elongation, directionality, and the formation of protrusions at the wound edge. Our broad goal is to identify signaling mechanisms involved in the regulation of microtubules around wounds. To address this, we initially aim to understand the precise spatiotemporal rearrangement of microtubule network post-wounding using the Drosophila pupal notum. This is a well-established wound model allowing us to study responses of epithelial cells using a combination of live-imaging and genetic tools. Using the UAS-Gal4 system that labels alpha-Tubulin in the pupal notum, we visualized the non-centrosomal microtubule arrangement in these epithelial cells conferring apical-basal polarity. Our preliminary results identified rearrangement of microtubules at the wound edge after laser wounding: there is increased enrichment of alpha-Tubulin in a subset of cell borders that point towards the wound. This rearrangement occurs around 5 to 10 minutes after wounding and lasts for ~20 minutes after which tubulin is enriched at the cellular protrusions formed at the wound edge. These observations suggest there is dynamic regulation of microtubules during tissue repair and its mechanism remains to be understood.

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Exosomes promote extracellular matrix assembly by breast cancer cells

Diego Torres Martinez, Bong Hwan Sung Ph.D., Alissa Weaver M.D., Ph.D.

Exosomes are small extracellular vesicles (SEVs) that are critical for intercellular communication and promote cancer progression and metastasis. Tumor cell-derived SEVs carry distinctive functional cargoes, including transmembrane adhesion receptors known to bind extracellular matrix (ECM) proteins. We previously found that the ECM protein fibronectin binds to cancer cell exosomes in an adhesive form and that the fibronectin-exosome complexes promote cell migration. Those data suggested that exosomes may, in fact, assemble soluble ECM into an insoluble adhesive form. To test whether cancer exosomes can promote ECM assembly, the exosome secretion regulator Rab27a was stably knocked down (KD) in 4T1 breast cancer cells. Rab27a KD cells were grown for 3 days, and cell-derived ECM samples were then collected for biochemical characterization and immunofluorescence analysis. Western Blot analysis of the cell-derived ECM deposited by Rab27a KD cells revealed lower levels of perlecan, nidogen, laminin gamma 1, and fibronectin compared to control cells. Moreover, immunofluorescence analysis of collagen IV and perlecan content of the cell-derived ECM demonstrated that Rab27a KD cells deposit less ECM compared to the control. Taken together, these studies suggest that exosome secretion may contribute to ECM assembly. Future studies will determine if SEVs isolated from parental 4T1 cells can rescue the ECM assembly defects of Rab27a KD cells and identify the EV cargoes that mediate exosomal ECM assembly.

A “non-muscle” alpha-actinin is an intrinsic component of the cardiac Z-disc and regulates sarcomere turnover, contractility, and heart remodeling

Authors: James B Hayes, Dylan Ritter, Abigail C Neininger-Castro, Alaina H Willet, Leah R Caplan, Yu Wang, Xiao Liu, Nilay Taneja, Zachary C Sanchez, Kyra Smart, Dharmendra Choudhary, Cynthia A Reinhart-King, Qi Liu, Matthew J Tyska, Erdem D Tabdanov, Quinn S Wells, Ela W Knapik, Dylan T Burnette

Abstract: Cardiac sarcomeres generate the fundamental forces behind each heartbeat and are thought to contain only muscle-specific cytoskeletal proteins. We show that a widely expressed actin cross-linking protein, alpha-actinin 4 (ACTN4), is a sarcomere component of the human, mouse, and zebrafish heart in vivo and in human iPSC-derived cardiac myocytes (CMs) in vitro. A confluence of biochemical experiments, immunofluorescence, and AI modeling suggest ACTN4 and muscle-specific ACTN2 form a heterodimeric complex at the cardiac Z-disc, the cardiac sarcomere border. ACTN4 depletion from human iPSC-CMs stabilizes canonical sarcomere proteins and drives contractility-dependent cellular hypertrophy while ACTN4 overexpression destabilizes sarcomeres. ACTN4 depletion from zebrafish embryos specifically increases ventricular contractility which drives atrial enlargement, suggesting biomechanically driven atrial remodeling. ACTN4-associated phenotypes in both model systems lack hallmarks of cardiac disease models and an ACTN4 variant in humans is associated with reduced risk for disease. Our findings suggest a “non-muscle” actinin regulates heart contractility and influences clinical outcomes related to heart failure.

Unraveling the role of polarity remodeling during cell intercalation

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Morphogenesis involves the constant remodeling of sheets of cells, which expand, elongate, invaginate, and fold to form an organ. Deciphering the mechanisms underlying these cellular rearrangements is critical to understanding morphogenesis. The mammary gland has been long appreciated as a model for studying epithelial behaviors during development. The key events of mammary gland development occur postnatally during puberty and throughout repeated cycles of pregnancy. The terminal end bud (TEB) orchestrates the formation of the ductal tree by giving rise to mature cell types. The TEB is a multilayer cluster of body cells that must resolve into a single luminal layer as the duct elongates and invades the surrounding fat pad. The precise mechanism of this process is poorly understood.

Previous work from the lab (Pfannenstein & Macara, *Dev Cell* 2023) identified a unique process of apical cell intercalation as the driving force behind resolving the multilayered TEB structure into the single luminal layer of the duct. An *in vitro* intercalation assay using either Eph4 mammary epithelial cells or primary luminal epithelial cells found that the tight junction (TJ) protein ZO-1 is critical for intercalation. ZO-1-depleted cells fail to intercalate into a WT epithelial monolayer, a result that was confirmed in vivo by mammary gland intraductal injections. Surprisingly, however, depleting ZO-1 in the monolayer enhances the in vitro intercalation of wild-type cells. Although ZO-1 is an abundant tight junction component, its depletion does not inhibit TJ formation, which suggests another role of ZO-1 during intercalation. Actin dynamics at the interface of the incoming cell and the monolayer was found to be essential for intercalation.

We are further investigating the function of ZO-1, other TJ proteins, and actin dynamics. We also address another aspect of the mechanism by examining how apicobasal polarity is remodeled during intercalation. Preliminary data indicate that the apical compartment of the incoming cell faces the apical surface of the monolayer and is reorganized as the incoming cell attaches and begins to penetrate at intercellular junctions.

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