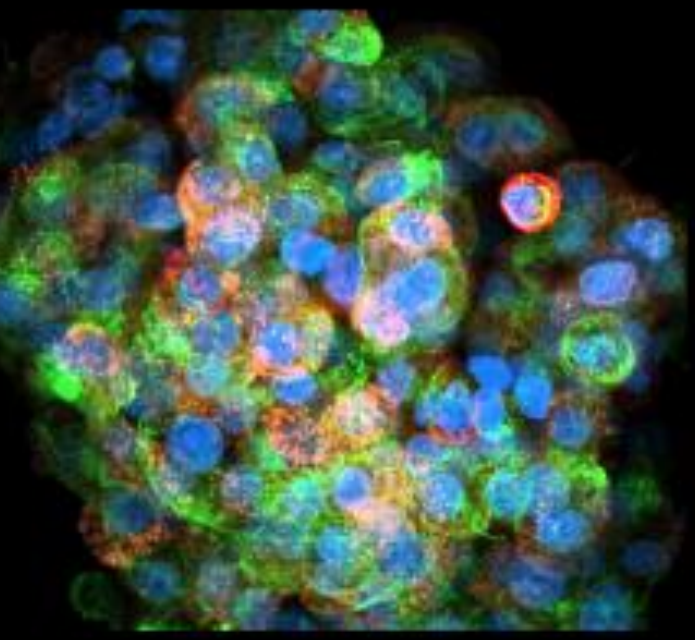
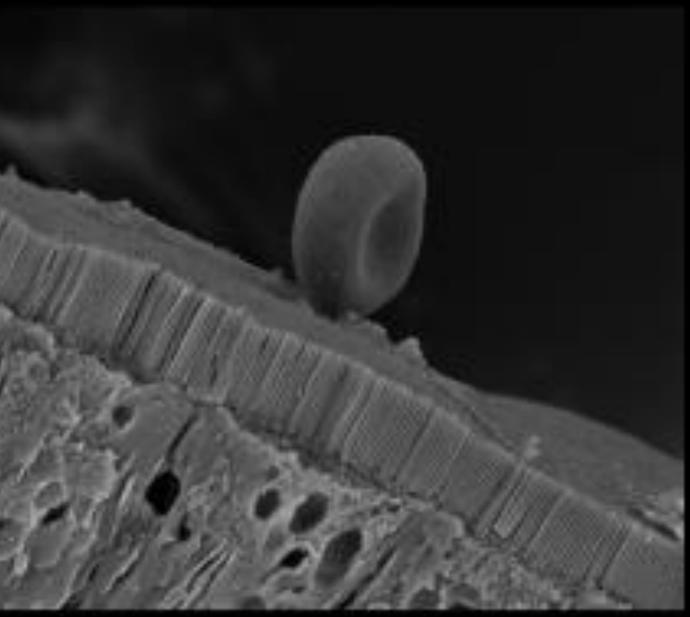
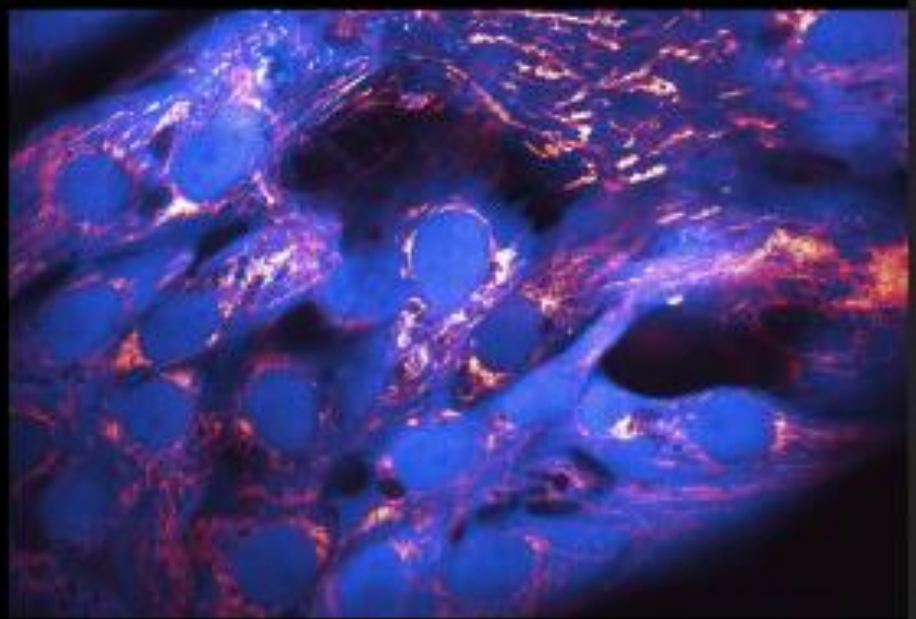
Department of Cell & Developmental Biology

22nd Annual Retreat

April 25, 2025





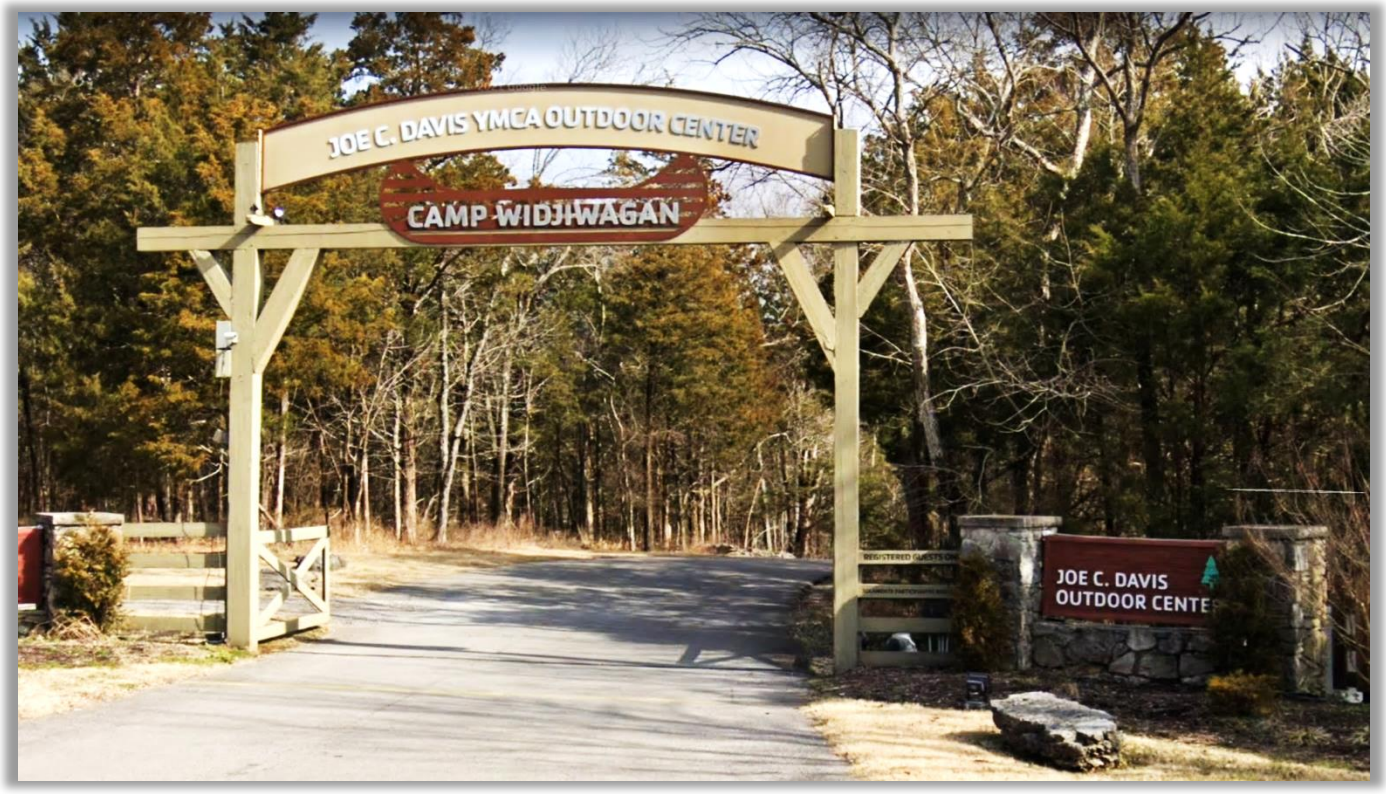


JOE C. DAVIS YMCA OUTDOOR CENTER
YMCA CAMP WIDJIWAGAN

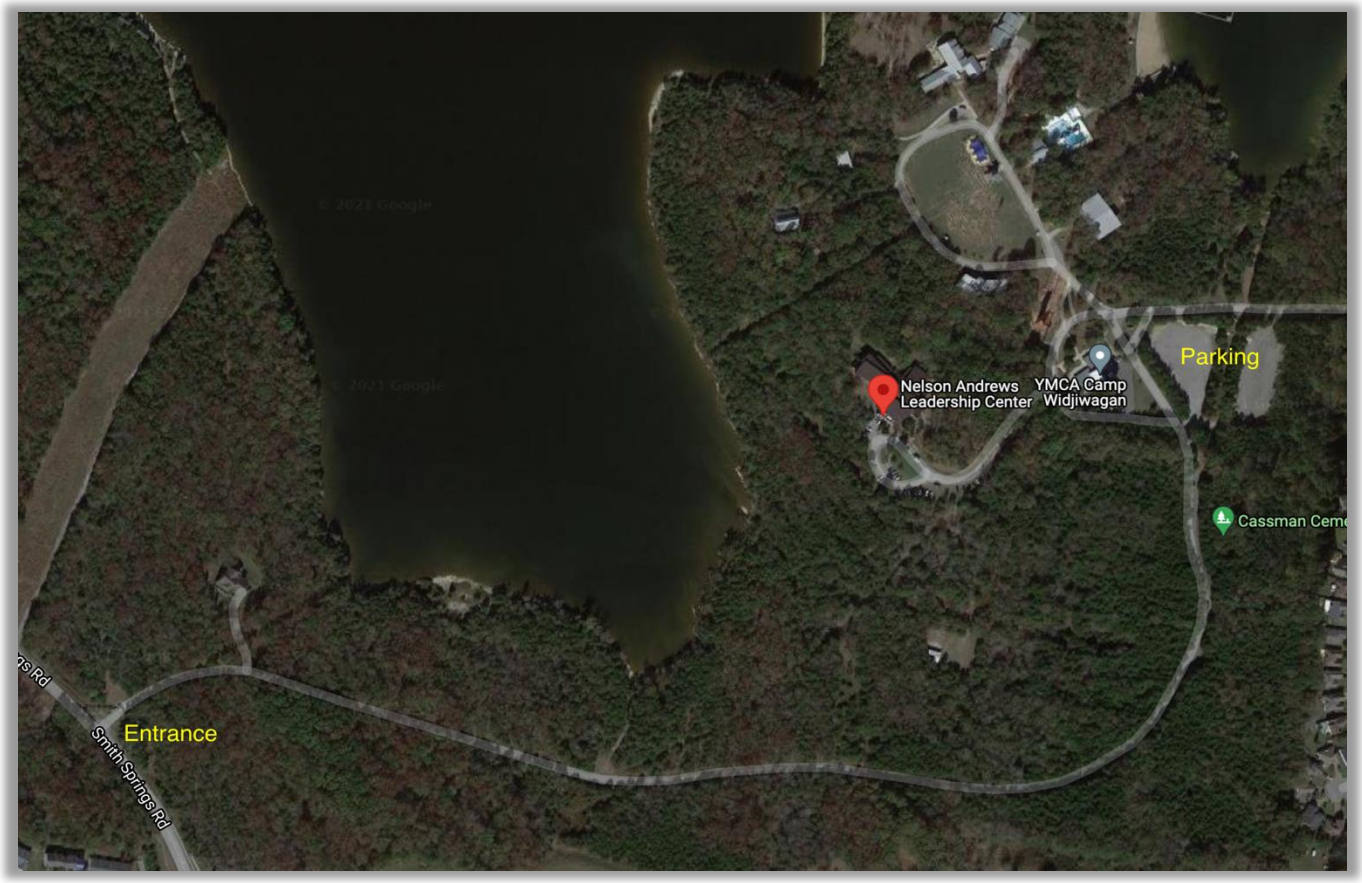
YMCA OF MIDDLE TENNESSEE
3088 Smith Springs Road, Nashville, TN 37013
P (615) 360-2267

https://www.campwidji.org/retreats-and-events/facilities/nelson-andrews-leadership-lodge-great-room

Street entrance to Camp Widjiwagan



Aerial view of Camp Widjiwagan and the Nelson Andrews Leadership Center



Front Entrance to the Nelson Andrews Leadership Center

Twenty Second Annual CDB

Retreat April 25, 2024
Nelson Andrews
Leadership Lodge

8:00-8:30	**BREAKFAST AND POSTER SESSION I SET UP** (Morning speakers- see Aaron Cooper)
8:30-9:15	**State of the Department Address by Ian Macara** **Image Awards -** Presented by Ian Macara **Staff Award Presentation -** Presented by Guoqiang Gu
9:20-10:05	**First Session**-Moderated by Jason MacGurn Anna Cassidy (Zanic) Bahnisikha Barman (Weaver) Ryan Finnel (Magnusen)
10:10-11:10	**Poster Session I** **Breakout Session** (Students/Postdocs only) Moderated by - Jack Trapani (Lee Lab) Elkie Peebles (Page-McCaw), Al-Borhan Bayazid (Bock Lab), Elizabeth Ruark (Burkewitz Lab), Sam Kang (Lau Lab), Syed Barmaver (Kaverina Lab)
11:15-12:45	**2nd Annual CDB Olympics**
12:45-2:15	**LUNCH** (Chang Noi Thai-Lao and Urban Cookhouse) **POSTER SESSION I TAKE DOWN AND SESSION II SETUP** (Afternoon speakers- see Aaron Cooper) **ACTIVITIES ON THE YMCA CAMPUS AND FREE TIME**
2:15- 2:45	**Graduate Student/Postdoc Award -** Presented by Andrea Page-McCaw - Steve Hann Award Winner presentation
2:50- 3:50	**Second Session**- Moderated by Krishna Mudumbi Caroline Bodnya (Gama Lab) Maggie Fye (Kaverina Lab) Casey Gailey (Miller Lab) James Allen (Zhou Lab)
3:50-4:50	**Poster Session II and Open bar**
4:50-5:00	**POSTER SESSION II TAKE DOWN**
5:00-8:00	**Reception** **Dream Events & Catering**

"I have not failed. I've just found 10,000 ways that won't work."

Thomas Edison

"Half of what we know is wrong, the purpose of science is to determine which half."

Arthur Kornberg

Oral Presentations

First Session- Speaker 1

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

Anna Cassidy, Veronica Farmer, Goker Arpağ, Marija Zanic

Vanderbilt University, Nashville, TN; Duke University School of Medicine, Durham, NC; Kadir Has University, Istanbul, Turkey

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.

First Session- Speaker 2

Cholesterol levels regulate the biogenesis of RNA-containing extracellular vesicles

Bahnisikha Barman, Elizabeth M Semler, Renee T Dwason, Kasey Vickers, Alissa M Weaver

Vanderbilt University School of Medicine, Nashville, TN-37204, USA

Objective: Extracellular vesicles (EVs) are small secreted nanoparticles that carry bioactive cargoes and mediate local and long-distance cell-to-cell communication. RNA is an EV cargo that can influence gene expression and phenotypes of recipient cells. We identified endoplasmic reticulum membrane contact sites (ER MCS) as key locations in the cell where RNA-containing EVs are formed. Furthermore, we found that the ER MCS linker protein VAP-A controls this process. As a significant function of ER MCS is to transport cholesterol and other lipids between organelles and VAP-A binds to cholesterol transporters, we hypothesized that cholesterol transfer between the ER and EV-forming organelles (e.g., endosomes and the plasma membrane) might regulate the biogenesis of RNA-containing EVs. Methods: We used QRT-PCR, confocal microscopy, and various biochemical techniques to analyze EV biogenesis and cargo content in colon cancer cell lines exposed to different dietary or cellular cholesterol levels or engineered for molecules that control cholesterol transport (ORP1L-KD). Results: Inhibition of either dietary (via LDL in the serum) or endogenously synthesized (in the ER) cholesterol altered the number and RNA content of EVs secreted from colon cancer cells. Interestingly, we observe the most significant decrease in RNA-containing EV (EV-RNA) secretion in dietary cholesterol-depleted cells. ORP1L is a key protein at the ER MCS that senses and transports cholesterol. We find that the knockdown of ORP1L led to a substantial defect in the biogenesis of RNA-containing EVs. Furthermore, our imaging data also showed a significant cholesterol decrease in CD63 positive endosomes in ORP1L-depleted conditions. Conclusion: These data suggest that cholesterol transfer and/or sensing at ER MCS via ORP1L drives the biogenesis of RNA-containing extracellular vesicles. These findings shed light on how the biogenesis of RNA-containing EVs is regulated and may be useful for future engineering of therapeutic EV

First Session- Speaker 3

Distal enhancers of Sox17 modulate the fate of pancreato-biliary progenitor cells

Ryan Finnel, Anna Osipovich, Jessica Musselman, Leesa Sampson, Isabella Silvestri, Maggie McFadden, Mark Magnuson

Vanderbilt University, Nashville, TN, 37232

SRY-box transcription factor 17 (Sox17) is essential for the formation of extra-embryonic and definitive endoderm, and the hemogenic endothelium. The hepato-pancreato-biliary system arises from ventral foregut endoderm and is exquisitely sensitive to perturbations of Sox17 expression, with discrete mutations within the promoter for transcription start site 2 (TSS2) of Sox17 causing marked alterations in pancreato-biliary formation. While proximal cis-regulatory elements within the TSS2 promoter have been shown to have a critical role in regulating Sox17, comparatively little is known about distal cis-regulatory elements. Topological associating domains (TADs) are large self-interacting chromatin structures that bring linearly distant DNA sequences, such as enhancers and promoters, into close spatial proximity, thereby segregating the genome into distinct regulatory units. TADs are often conserved across species, allowing for conservation of gene regulatory mechanisms. Using cross species homology between human and mouse, we identified orthologous TAD-defining CTCF binding sites thereby pinpointing endpoints for the murine Sox17 TAD. Similar to its human ortholog, Sox17 is the only protein coding gene present within its ~352 kb TAD. As TADs often define the spatial limits of enhancer-promoter interactions, we reasoned the murine Sox17 TAD contains conserved cis-regulatory elements that regulate Sox17 expression in a lineage specific manner. To test this possibility, we developed a strategy based on evolutionary conservation, epigenomic marks, and transcription factor binding that identified 38 regions of high sequence conservation within the Sox17 TAD that show dynamic epigenetic and transcription factor binding profiles in definitive endoderm and hemogenic endothelial cells. Our analysis identified two putative endodermal enhancers, Sox17e-13 and Sox17e-231, located -13 kb and -231kb upstream of Sox17, and are 391 bp and 315 bp in length, respectively. Both sites exhibit a permissive epigenetic state in endoderm and bind FOXA2 and GATA4. Individual deletions of these two regions in mice temporally reduce Sox17 and Pdx1 expression, resulting in abnormal embryonic pancreato-biliary bud morphology and a delay in the critical pancreato-biliary cell state transition. This delay alters progenitor cell allocation by increasing the number of biliary progenitor cells, and subsequently gallbladder size. As the individual mutations exhibit similar deletion phenotypes, we hypothesized that the enhancers function in a redundant manner to promote Sox17 expression during pancreato-biliary development. To test this hypothesis, we recently derived a Sox17Δe-13&e-231 dual deletion allele, thereby enabling us to determine whether the Sox17e-13 and Sox17e-231 enhancers act in an additive or synergistic manner.

Second Session- Speaker 1

Examining the Impact of Peroxisomal Metabolism on Cell Fate Decisions During Human Neurodevelopment

Caroline Bodnya1 and Vivian Gama1,2.

1Cell and Developmental Biology, Vanderbilt University, Nashville, TN. 2Vanderbilt 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.

Second Session- Speaker 2

Directed insulin secretion from beta cells occurs at cortical sites devoid of microtubules at the edges of ELKS/LL5β patches

Margret Fye, Pranoy Sangowdar, Anissa Jayathilake, Pi'ilani Regan, Guoqiang Gu, Irina Kaverina

Vanderbilt University, Nashville, TN 37212

To maintain normal blood glucose levels, pancreatic beta cells secrete insulin into the bloodstream at specialized regions at the cell periphery, often called secretion hot spots. While many secretory machinery components are located all over the cell membrane, directed secretion relies on distinct cortical patches of "active zone" proteins, mainly the scaffolding protein ELKS and the microtubule (MT)-anchoring protein LL5β. However, using TIRF microscopy of intact mouse islets to precisely localize secretion events within ELKS/LL5β patches, we now show that secretion is restricted to only 5% of ELKS/LL5β patch area, indicating strong heterogeneity amongst these patches. Moreover, the majority of secretion occurs at the margins of ELKS/LL5β patches, a novel finding in contrast to previous assumptions that secretion occurs at high-intensity regions of ELKS. These findings suggest that additional factor(s) must be responsible for hot spot definition. Because the MT cytoskeleton plays a regulatory role in the insulin secretion process via both delivery and removal of secretory granules from the secretion sites, we test whether local MT organization defines secretory activity at hot spots. We show three distinct configurations of MTs at ELKS patches, including end-on in which the MT end associates with ELKS, lattice-bound in which the MT lattice associates with ELKS, and N/A in which there is no MT association with some ELKS patches. We find that the majority of secretion events occur at these ELKS regions devoid of MTs. Based on our findings, we present a model in which local MT disassembly and optimal ELKS content are strong predictors of directed insulin secretion

Second Session- Speaker 3

NCAM (Neural Cell Adhesion Molecule) promotes synaptic remodeling in developing GABAergic neurons

Casey Gailey1,2, Robert Held1, Claudia Palacios1, Andrea Cuentas-Condori1, Leah Flautt1, John Tipps1, Siqi Chen1, Eleanor Rodgers4, Seth R. Taylor1, Engin Ozkan5, and David M. Miller, III1,2,3

Department of Cell and Developmental Biology1, Program in Developmental Biology2, Program in Neuroscience3, Vanderbilt University, Nashville, TN, USA. Saint Cecilia Academy4, Nashville, TN, USA. Department of Biochemistry and Molecular Biology5, University of Chicago, Chicago, IL, USA.

Neural circuits are actively remodeled during development as new synapses are assembled and others are removed. To investigate the underlying mechanism of synaptic remodeling, we are exploiting the DD-type GABAergic motor neurons. In young larvae, DD presynaptic boutons are initially positioned on ventral muscles but are then relocated to innervate dorsal muscles. We have shown that the transcription factor, IRX-1/Iroquois, activates expression of the sodium epithelial channel (ENaC), UNC-8, to trigger a Ca2+-dependent endocytic mechanism that promotes presynaptic disassembly and recycling to dorsal synapses1. To identify additional effectors of synaptic remodeling, we used single cell RNA-Seq to profile early larval stage DD neurons. A mutant of one of these genes, ncam-1, impairs removal of ventral GFP::RAB-3 and its reassembly at dorsal synapses. NCAM-1 localizes to DD synapses and is also required for normal remodeling of active zone proteins CLA-1 and ELKS-1, which are not affected by unc-8. Moreover, genetic epistasis experiments with GFP::RAB-3 suggest that NCAM-1 and UNC-8 function in parallel pathways. NCAM-1 is a conserved member of the Neural Cell Adhesion Molecule (NCAM) family with an extracellular domain composed of Ig and fibronectin repeats. We used CRISPR mutants to show that N-terminal Ig1 and Ig2 domains are dispensable for DD remodeling whereas the NCAM-1 intracellular domain (ICD) is required. These findings suggest that NCAM-1 homophilic interactions mediated by Ig1/Ig2 are not necessary for remodeling but that downstream signaling by the NCAM-1 ICD domain is important. We identified sequence motifs within the NCAM-1 ICD that are conserved in mammals. Strikingly, we showed that human NCAM can rescue synaptic remodeling in ncam-1 mutants, indicating that human and nematode NCAMs are functional homologs. Independent biochemical assays detected strong interaction of the extracellular domains of NCAM-1 with RIG-3, an Ig-domain cell adhesion protein with topological similarity to Drosophila Klingon. Genetic analysis shows that NCAM-1 and RIG-3 function in a common pathway, suggesting that the NCAM-1-RIG-3 complex may mediate synaptic remodeling. Because NCAM can regulate plasticity in mammalian neurons we suggest that NCAM-1 may drive synaptic remodeling in C. elegans in a mechanism that also governs circuit refinement in the developing brain. NIH Funding: T32HD007502, F31NS134292, R01NS10695.
1Cuentas-Condori et al., (2023) Cell Rep. doi.org/10.1016/j.celrep.2023.113327

Second Session- Speaker 4

Visualizing Dynamic Interactions of Cell Adhesion Molecules in Living Cells

James P. Allen, Richard Sando, Qiangjun Zhou

Vanderbilt University, Nashville, TN, 37232

Cell adhesion molecules (CAMs) are critical for diverse cellular processes. However, it is difficult to directly measure interactions between CAMs in living cells and organisms. Recent developments in split-HaloTag technology present a unique strategy for chemical labeling of protein interactions. Here, we apply this method to measure dynamic CAM interactions in living cells using split-HaloTag CAM fusion proteins. We visualized interactions between known adhesion pairs found in neuronal synapse, as well as immunological synapses, using HEK293T cells as a model system. Our split-HaloTag-CAM constructs show highly specific signal for CAM binding, as binding null mutants were sufficient to completely ablate signal, and constructs were not cross-compatible with other non-binding adhesion pairs. We retro-engineered the split-HaloTag cpHaloΔ for reversible dye exchange which allowed for rapid time lapse visualization of cell adhesion molecular interactions, which expands the utility of the original split-HaloTag system to measuring real time interaction of split-HaloTag components. These powerful tools are compatible with multiple fluorescent dyes with different spectral properties allowing for multiplexing and flexibility. These tools will enhance our understanding of the precise roles of cell adhesion molecular interactions in cellular and organismal function.

Morning Poster Session

Achey, Meredith	**P1**
Akizuki, Kazutoshi	**P2**
Ankenbauer, Katherine	**P3**
Arceneaux, Deronisha	**P4**
Barmaver, Syed	**See afternoon session**
Bayazid, Al Borhan	**See afternoon session**
Bekas, Caraline	**P5**
Biswas, Sushobhan	**P6**
Brown, Monica	**P7**
Buluwana, Savi	**P8**
Burgos, Julissa	**P9**
Caplan, Leah	**P10**
Cephas, Amelia	**P11**
Chalkley, Oliver	**P12**
Chen, Lei	**P13**
Copeland, Celina	**P14**
Costanzo, James	**P15**
Daniel, Mikiyas	**P16**
Dixson, Andrew	**P17**
Dong, Xinyu	**See afternoon session**
Dutta, Sarbajeet	**P18**
Eleuteri, Nicky	**P19**
Harmych, Sarah	**P20**
Hayes, Caleb	**P21**
Hua, Junmin	**P22**
Hurtado, Alan	**P23**
Jarvis, Brenda	**P24**
Jimenez, Lizandra	**P25**
Jung, Youn Jae	**P26**
Kennedy, Sean	**P27**
Lehmann, Zachary	**P28**
Leyhew, Brooks	**P29**
Mabry, Kelsey	**P30**
Mao, Zhuqing	**P31**
Martinez, Thyandra	**P32**

P1

Trans-mesenteric nerves are associated with multiple neuronal somata in the post-natal mouse

Meredith A. Achey, Kathleen R. Campbell, E. Michelle Southard-Smith

Vanderbilt University Medical Center, Nashville, TN, 37232

Introduction: The mesentery is a morphologically complex organ vital to the function of the mammalian digestive system, suspending the intestinal tract and digestive organs from the abdominal wall and providing a scaffold for the vasculature and nerves that support intestinal function in health and disease. Trans-mesenteric nerves supply the autonomic innervation to the enteric nervous system (ENS), a vast network of ganglia within the intestinal tract. The ENS is formed by migration of vagal neural crest cells along the developing intestine from oral to aboral, as well as via trans-mesenteric invasion of the hindgut. The presence of neuronal progenitor cells in the post-natal mesentery has been suggested by several prior studies but has not yet been definitively shown. Methods: We are mapping neuronal somata associated with mesenteric nerves throughout early postnatal development using two transgenic mouse lines, Phox2b-Histone2BCerulean (Phox2bCFP) and Sox10-Histone2BVenus (Sox10-YFP). These mice express fluorescent proteins under the control of the regulatory regions for Phox2b and Sox10, respectively. Phox2b is a master regulator of neuronal fate specification in migrating neural crest cells and is present at high levels in mature neurons, while Sox10 is a transcription factor active in migrating neural crest cells that becomes restricted to glia in post-natal animals. Using immunohistochemistry (IHC) in whole-mount specimens, we aim to gain insight into the neurochemical and structural makeup of these cells. Results: We have identified a population of sparsely distributed cells associated with mesenteric nerves in juvenile mice which express Phox2b and HuC/D, a pan-neuronal marker. These cells are present throughout the mesentery of the small bowel, with clusters at the root of the mesentery and near the bowel wall. Conclusions: While previous studies identified neuronal somata along mesenteric nerves in rats, cats, and humans, to our knowledge ours is the first to comprehensively map these populations in juvenile mice and investigate their neurochemistry. The function of these putative neurons remains unclear. Future experiments will enable more detailed analysis of their structure and function.

P2

Phosphorylation of Hhp2, a CK1 ortholog in Schizosaccharomyces pombe, regulates its functions in mitosis

Kazutoshi Akizuki, Sierra N. Cullati 1, Jun-Song Chen, Alyssa E. Johnson 2, Kathleen L. Gould

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA; 1 Current address: Department of Chemistry, Western Washington University, Bellingham, WA, USA; 2 Current address: Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA

CK1 enzymes are an evolutionarily conserved Ser/Thr kinase family that play important roles in many cellular processes such as circadian rhythm and Wnt signaling. In Schizosaccaromyces pombe, the CK1 orthologs of CK1δ and CK1ε, Hhp1 and Hhp2, contribute to a mitotic checkpoint that delays cytokinesis when the mitotic spindle is disrupted. This led us to be interested in how CK1 activity itself is regulated during mitosis. Generally, CK1 is inhibited through autophosphorylation of its C-terminal tail in vitro and there has been a report that endogenous CK1δ is hyperphosphorylated during mitosis in human cells although the physiological significance has never been determined. In this study, we observed that Hhp2 is also hyperphosphorylated during mitosis in S. pombe. Because Hhp2 is not an essential protein, we can easily assess the significance of mitotic phosphorylation via mutational analysis using Hhp2 as a model CK1 enzyme. We first identified the four Hhp2 autophosphorylation sites in vitro but found that they do not account for the majority of mitotic Hhp2 phosphorylation in vivo. We then determined that transphosphorylation by Cdc2/Cdk1 on three other residues accounted for the bulk of mitotic Hhp2 phosphorylation. An Hhp2-7A mutant, in which both auto- and trans- mitotic phosphorylation are eliminated, held the mitotic checkpoint arrest longer, indicating that Hhp2 phosphorylation of its C-terminal tail antagonizes its kinase activity to silence the mitotic checkpoint. On the other hand, in mitosis without spindle stress, the hhp2-7A mutant completes mitosis and cytokinesis faster. These results indicate that mitotic phosphorylation of Hhp2 has different effects on the progression of cell division depending on the intracellular situation.

P3

Osteopontin promotes epithelial plasticity in pancreatic tumorigenesis

Katherine Ankenbauer, Katherine Gell, Bob Chen, Danyvid Olivares-Villagomez, Ken Lau, Kathleen DelGiorno

Vanderbilt University, Nashville, TN, 37212; Vanderbilt University Medical Center, Nashville, TN, 37212

Background and Aims: Acinar to ductal metaplasia (ADM) is a process where pancreatic acinar cells transdifferentiate into ductal-like cells to support tissue repair. However, ADM can also initiate pancreatic intraepithelial neoplasia (PanIN), a precursor to pancreatic ductal adenocarcinoma (PDAC). Our lab previously used lineage tracing and single-cell RNA sequencing (scRNA-seq) to show that ADM generates diverse cell types, including tuft and enteroendocrine cells, rather than forming a uniform ductal population. Computational analyses revealed a progenitor-like population within ADM marked by high expression of osteopontin (Spp1). This study aimed to define the role of SPP1 in epithelial plasticity during ADM and PanIN development. Methods: We analyzed published scRNA-seq datasets from lineage-traced acinar cells under chronic injury or KrasG12D expression. Lineage trajectory and differential gene expression analyses were conducted to identify progenitor states. We generated mice with pancreas-specific Spp1 deletion in the presence (KCSpp1KO) or absence (CSpp1KO) of KrasG12D. ADM was induced using caerulein or by aging mice up to 6 months. Epithelial heterogeneity was assessed using cell-type-specific markers. Human pancreatic tissues were evaluated for SPP1 expression. Results: Trajectory analysis identified a transitional "uncommitted" cell population derived from acinar cells, preceding differentiation into tuft, enteroendocrine, and mucinous lineages. Spp1 was highly expressed in this uncommitted state. Pancreas-specific Spp1 deletion in CSpp1KO and KCSpp1KO mice led to reduced numbers of tuft, enteroendocrine, mucin-producing, and proliferative cells compared to controls. Despite more severe pancreatitis in CSpp1KO mice, Spp1 loss in the context of KrasG12D led to reduced PanIN formation and instead promoted cyst-like lesions. In human samples, SPP1 was elevated in both pancreatitis and PanIN. Conclusions: SPP1 supports epithelial plasticity during ADM and PanIN formation. Loss of SPP1 disrupts the generation of diverse epithelial cell types and shifts disease progression-worsening injury responses and favoring cystic lesions over PanIN in the KrasG12D setting. These findings highlight SPP1 as a key regulator of cell fate decisions during pancreatic remodeling. Ongoing studies using scRNA-seq and organoid models aim to further define SPP1's role in epithelial heterogeneity. Targeting molecular regulators of plasticity such as SPP1 may offer new strategies to intercept premalignant pancreatic disease before PDAC arises.

P4

A Non-Stem Cell Lineage Gives Rise to Atoh1-independent Tuft Cells

Deronisha Arceneaux, Amrita Banerjee, Joey Simmons, Yanwen Xu, Lucy Chen, Mirazul Islam, Ken Lau

Vanderbilt University, Nashville, TN

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.

P5

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

Caraline Bekas1,2*, Youn Jae Jung 1,2,3*, Bahnisikha Barman 1,2, Lizandra Jimenez1,2, T. Renee Dawson1, 2, Alissa M. Weaver 1,2

1Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee; 2Center for Extracellular Vesicle Research, Vanderbilt University School of Medicine, Nashville, Tennessee; 3Department 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.

P6

Single Cell Transcriptomics Reveals Damage Induced Mitotic Response in the Neonatal Mouse Utricle

Sushobhan Biswas, Ruiqi Zhuo, Macey Soltis, Sarah Easow, Taha A. Jan

Vanderbilt University Medical Center

Background: The utricle is an inner ear vestibular sensory organ that depends on mechanosensitive hair cells (HCs) for detecting linear acceleration. In newly born mice, hair cells are generated from supporting cells (SCs) and continue to be added for the first 7 postnatal days as part of the developmental process. Non-mammalian species regenerate hair cells, however, loss of hair cells in mammals is permanent because of limited regenerative capacity. There are currently no therapies that regenerate lost hair cells in mammals. Here, we elucidate the crucial molecular factors and pathways involved in the regeneration of murine utricular HCs following damage in vivo. Methods: We used an established in vivo hair cell ablation model (Pou4f3DTR) to understand the molecular mechanism of hair cell regeneration following injury using the utricle as a model. Damage was induced on postnatal day 1 (P1) to selectively ablate HCs. To address mitotic regeneration specifically, we utilized a triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) to fate map proliferating cells. Droplet-based cell capturing platform was used for single cell RNA seq (scRNAseq) following lineage tracing. Utricles were harvested at P2, P3, P4, P5, P6, and P9 for both histology and scRNAseq. Results: Early time point experiments confirm loss of HCs beginning at P2. Analysis of the damaged tissues shows a wave of proliferation at P3-P6 using Ki67 as a marker. Newly dividing cells begin to increase at P3 and peek at P6 ($p<0.0001$). Histologic data using our triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) show cells that undergo mitosis permanently switch color to GFP. We have collected scRNAseq data in both undamaged and triple-transgenic lineage traced damaged utricles at early postnatal ages (P3 through P9). Our preliminary analysis demonstrates high quality cells with expected clusters of hair cell sub-types, supporting cells, and transitional epithelial cells. A total of 28,809 cells passed all quality control metrics. A group of presumed damaged activated cells were identified from the scRNAseq data. Our sequenced scRNAseq data also contains fate mapped cells to infer trajectory of mitotic cells. Conclusion: Our results reveal insights into the molecular mechanism of post-injury mitosis within the inner ear, paving the way for in vitro and in vivo manipulation experiments for hair cell regeneration. Understanding mitotic regeneration within the neonatal mammalian utricle could lead to the discovery of new tools to regenerate hair cells for hearing and balance disorders.

P7

Magnifying the Structure of Tuft Cell Cytospinules

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

Vanderbilt University, Nashville, TN, 37232; Vanderbilt University Medical Center, Nashville, TN, 37232

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.

P8

The role of the Crumbs complex in tight junction dynamics

Savi Buluwana, Christian de Caestecker, Ian Macara

Vanderbilt University, Nashville, Tennessee, 37235

Epithelial cells are an important component of multicellular organisms have a variety of functions, including nutrient exchange, immunity, and development. They form continuous sheets in the tissues of organs facing the external environment. In these tissues, epithelial cells exist as sheets, connected by cell-cell junctions. A defining type of junction is the tight junction. Tight junctions form a semi-permeable seal between adjacent cells, which directly contributes to barrier function, and partitions the membrane domains into the apical and basolateral domains, with the apical side forming the lumen that faces the external environment. A determinant of the apical domain is the evolutionarily conserved Crumbs complex of proteins - made up of Crumb3, Patj, and Pals1 - which scaffolds tight junctions. Pals1 in particular has been shown to be essential for tight junction formation. Deletion of Pals1 leads to disjointed tight junctions in fixed in vitro experiments. In this project, we elucidate the mechanisms by which Pals1 influences tight junction assembly and dynamics. This is done through both fixed- and live-cell imaging of a mouse mammary epithelial EpH4 cell line edited with CRISPR/Cas9 to endogenously express fluorescently-labelled ZO-1. We show that when Pals1 is deleted using CRISPR, tight junctions eventually break down after initial formation. Additionally, when tight junctions are destroyed via an actin polymerization inhibitor, cells lacking Pals1 fail to re-form junctions in regular medium. The visual dynamics of breakdown and attempts at reformation of these junctions suggest that Pals1 regulates tight junctions through a tension-related mechanism. Additionally, deletion of Pals1 leads to mislocalization of actin to the apical surface, and colocalization of E-cadherin, a lateral adherens junction protein, to ZO-1. When Pals1 mutants that lack several binding domains are exogenously expressed in Pals1-deleted cells, we observe that the Pals1 mutant missing the Patj-binding domain can successfully rescue the normal ZO-1 phenotype, while the mutant missing the Crumbs3 binding domain cannot. Furthermore, endogenous deletion of just a portion of the Crumbs3 binding domain produces disjointed junctions nearly identical to full deletion of Pals1. These preliminary observations raise questions about how Pals1 interacts with Crumbs3, Patj, and other tight junction proteins to influence stability, and whether line tension along the junctions is involved in stabilization. We plan to further investigate these findings.

P9

Deciphering the role of IRTKS in EHEC bacterial attachment

Julissa Burgos-Rivera, Carina I. Roman-Aquino, Matthew J. Tyska

Dept of Cell and Dev Biology, Vanderbilt University School of Medicine, Nashville TN, 37212; Universidad de Puerto Rico Arecibo, Arecibo, Puerto Rico, 00612

Enterohemorrhagic Escherichia coli (EHEC) is a foodborne pathogen that breaches the intestinal epithelium and causes frequent outbreaks of bloody diarrhea and hemolytic uremic syndrome. Once in contact with enterocyte epithelial cells, EHEC secretes Translocated Intimin Receptor (Tir), a bacterial effector, into the host cytoplasm to initiate intimate bacterial attachment and a signaling pathway for cytoskeletal host protein recruitment. EHEC then reorganizes the host's cytoskeleton to form dynamic actin-rich structures known as "pedestals", which facilitate bacterial cell-to-cell spread and colonization of the intestine. Several gaps remain in our understanding of EHEC's unique infectious mechanism, particularly how Tir gets delivered to and inserted into the membrane for intimate bacterial attachment and subsequent pedestal assembly. During infection, Tir also binds to the inverse BAR (I-BAR) domain of Insulin Receptor Tyrosine Kinase Substrate (IRTKS), a host protein involved in cell membrane curvature and previously shown to play a role in EHEC pedestal assembly. Interestingly, using immunofluorescence microscopy, we found that overexpression of IRTKS leads to increased bacterial attachment and mislocalization (or spread) of Tir. In addition, the loss of IRTKS through CRISPR/Cas9 engineering resulted in decreased levels of Tir at the host-pathogen interface. Although IRTKS has previously been shown to play a role in EHEC pedestal assembly, together our results suggest that IRTKS may also contribute to bacterial attachment and to the enrichment of Tir in the apical plasma membrane. Future work will focus on loss of function experiments and live cell imaging to understand the dynamics of Tir's association with IRTKS in the host cell for bacterial attachment. Overall, this research will define how IRTKS promotes bacterial attachment and lead to a more comprehensive model of EHEC pathogenesis.

P10

Investigating a noncanonical role for cofilin in epithelial microvilli

Leah Caplan, Zach Sanchez, Matt Tyska

Vanderbilt University, Nashville, TN, 37240

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.

P11

The Role of Zfp800 in Intestinal Epithelial Cell Differentiation During Development

Amelia Cephas, Shreeya Moolamalla, Brenda Jarvis, Kathleen DelGiorno

Vanderbilt University, Nashville, TN, 37212

Background: The intestinal epithelium maintains homeostasis through a balanced composition of absorptive and secretory cell types, regulated on a tightly regulated network of transcription factors. The secretory lineages, which give rise to goblet, Paneth, enteroendocrine (EEC), and tuft cells arise from ATOH1+ progenitors. A subset of these progenitors express NEUROG3, which drives enteroendocrine cell (EEC) fate while also contributing to goblet and Paneth cell lineages. Zinc-finger transcription factor ZNF800 (ZFP800 in mice) fine-tunes differentiation of EECs by modulating NEUROG3. Its loss in human intestinal organoids leads to EEC subtype imbalances with an expansion of serotonin-(5-HT) producing enterochromaffin cells. These findings suggest ZNF800 may coordinate secretory lineage allocation in vivo, but its broader developmental role remains unclear. Unlike the fully developed human gut at birth, the murine intestine matures postnatally, making mouse models invaluable for studying developmental disorders that manifest clinically in premature infants or those with congenital enteropathies. Aims: Given the implications of ZFP800 in epithelial differentiation, this study aimed to investigate its role in regulating intestinal epithelial differentiation in vivo. Methods: We analyzed intestinal tissues from WT and Zfp800 -/- (KO) mice at embryonic (E18), neonatal (P1), and weaning (P20) stages. Tissues were processed for multiplex immunofluorescence staining of secretory lineages (EEC subtypes, tuft, Paneth, and goblet cells) and enterocytes, followed by quantitative analysis using QuPath. Additionally, qRT-PCR was performed on P20 tissues to assess transcriptional changes in lineage-specific genes (Neurog3, Muc2, Lyz1,etc). Intestinal organoids and tissues from proximal and distal segments of 20-day-old mice were analyzed by qRT-PCR to assess regional gene expression patterns. Results and Conclusions: Zfp800 deficiency resulted in consistent secretory lineage imbalances across all developmental stages examined. KO intestinal tissues from E18 to P20 showed (1) an expansion of serotonin-producing enterochromaffin cells (5-HT$^+$/CHGA$^+$, PAX4), (2) dysregulation of EEC hormones (GIP, SST, CCK), and (3) reduced numbers of goblet (Alcian blue$^+$) and Paneth (Lyz1$^+$) cells compared to WT mice. These findings were confirmed in P20-derived intestinal organoids, which exhibited decreased expression of Paneth (Lyz1) and tuft (Pou2f3) markers, alongside increased expression of EEC (ChgA) and serotonin-pathway (Tph1) genes, as measured by qRT-PCR. Collectively, these results suggest that Zfp800 plays a critical role in maintaining balanced differentiation of intestinal secretory lineages, with its loss favoring enterochromaffin cell fate at the expense of other secretory cell types. The consistency between in vivo and organoid phenotypes supports a cell-intrinsic role for Zfp800 in epithelial development.

P12

Developing a Cryo-Electron Tomography Tag

Oliver Chalkley, Robert McFadden, Feng Luo, Kevin McCarty, Ethan Zhao, Qiangun Zhou

Vanderbilt University, Nashville, Tennessee, 37235

Cryo-electron tomography (cryo-ET) has revolutionized the ability to visualize cells at near-native states with nanometer-scale resolution. Despite these advances, localizing specific proteins within the cell's dense molecular landscape remains a significant challenge because there is no universal cryo-ET tag that would be analogous to a GFP in fluorescent microscopy. The ongoing development of a genetically encoded cryo-ET tag is presented, enabling precise, unambiguous localization of a protein of interest with <10 nm spatial accuracy. The initial design, based on mini-ferritin, did not yield a sufficient signal-to-noise ratio for reliable detection. Although this design is not the final design, there is valuable data that will inform future designs: in vitro data using a combination of ferroxidase-competent and incompetent subunits produces 3-fold more iron granules than only ferroxidase-competent subunits. To improve the past design, maxi-ferritin is now employed because it can hold 9-fold more iron, allowing a more distinct, high-contrast signature from dense iron granules in tomograms while minimizing disruption to cellular architecture and preserving the native function of the fused protein. A common limitation of ferritin-based tagging approaches is that the ferritin cage is composed of 24 monomers, potentially resulting in multiple copies of the protein of interest per tag-thereby disrupting its native state. To address this, ferritin mutants are being screened to permit controlled cage assembly using a mixture of monomers that can or cannot form iron granules. In this design, the protein of interest is fused only to the iron granule-forming monomer, ensuring a clear tomographic signal while attempting to maintain a single copy of the fusion per cage and minimizing perturbation to the cellular environment. By integrating this tagging system with cryo-ET, protein localization could be directly visualized with complex cellular superstructures, such as membrane invaginations, cytoskeletal elements, or other cellular superstructures-providing critical insights into the spatial organization of molecular machines and structures in situ. This approach aims to bridge the gap between protein localization and high-resolution structural context, offering powerful new insights into cellular processes, protein complexes, and how superstructural organization within the cell contributes to biological function.

P13

Hierarchical regulation of cerebellar neurogenesis by Sin3A-mediated gene repression

Lei Chen, Ankita Roy, Chin Chiang

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232

Cerebellar granule cells (GCs) are the most abundant neurons in the central nervous system, originating from the rhombic lip (RL), which is a transient embryonic germinal zone located at the dorsal edge of the hindbrain. Lineage tracing has revealed a hierarchical developmental pattern of GCs. At the apex of this hierarchy is a group of Sox2-positive, stem cell-like quiescent cells derived from the RL, which undergo self-renewal with a relatively slow cell cycle. These cells subsequently cease Sox2 expression and become Atoh1-positive rapidly proliferating granule cell precursors (GCPs). After approximately eight rounds of cell division, GCPs differentiate into NeuN-positive mature GCs. The molecular mechanisms driving transitions between these hierarchical stages remain unclear. Here, we demonstrate that Sin3A, a component of the Histone deacetylase (Hdac) complex, plays an essential role at two developmental stages by repressing key genes involved in both stem cell-like progenitor activation and GCP differentiation. In Sox2-positive cells, Sin3A promotes their activation by inhibiting Sox2 expression; loss of Sin3A results in sustained Sox2 expression and delayed activation, leading to reduced numbers of GCPs in the external granular layer (EGL). In Atoh1-positive cells, Sin3A suppresses Atoh1 transcriptional activity through interaction with Neurod1, thereby promoting GCP differentiation. Sin3A null GCPs exhibit overexpression of Atoh1 and retention in a proliferative state. Additionally, Sin3A maintains survival and specification of GCPs by repressing non-lineage-specific gene expression. Our findings reveal that Sin3A functions as a pleiotropic co-repressor critical for regulating GC development.

P14

Effects of a high fat diet on the pancreatic plasticity of inbred and outbred mice

Celina Copeland, Kathleen DelGiorno

Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232

BACKGROUND & AIMS: A high fat diet (HFD) has been shown to contribute to the development of obesity and type 2 diabetes, increasing the risk of developing pancreatic cancer. Therefore, it is crucial to understand how HFD affects pancreatic plasticity. Typically, inbred mouse strains, such as the C57BL/6 strain, are used for diet-induced obesity and diabetes research. They are metabolically sensitive to HFD-induced obesity and have less genetic variability in comparison to outbred strains. CD-1 mice, a Swiss outbred strain, are also metabolically sensitive to HFD, but have more genetic variability, requiring use of larger cohorts of mice, due to the wider range of effects between cohorts. Outbred mice, however, can be used to reflect the heterogeneity within the human population, which is why it is important to evaluate disease progression in outbred mice as well. In this study, our aim was to investigate differences in the endocrine and exocrine pancreas between C57BL/6 and CD-1 mice on HFD. METHODS: C57BL/6 and CD-1 mice were put on HFD for 12 weeks. Immunohistochemistry and immunofluorescence tissue staining was used to probe for islet hormones (INS, GCG, SST), cholecystokinin, and proliferation markers (PCNA, STMN1). Images were imported into Qu-Path software and analyzed. RESULTS: In the endocrine compartment, HFD caused severe islet hyperplasia, indicative of hyperinsulinemia, but to a much larger degree in the CD-1 strain. This strain also showed a decrease in GCG and SST, even when compared to the obese C57BL/6 group. CD-1 mice also had more CCK+ cells in comparison to their obese inbred counterparts. In the exocrine pancreas, both obese strains showed a significant increase in pancreas weight. However, the pancreas-to-body weight ratio remained unchanged in the CD-1 mice, while dropping in the C57BL/6 mice, indicating that the outbred pancreas grew to the size of the mouse. To investigate the reason for this increase in size, proliferation markers such as PCNA and STMN1 were stained for. We found that many of the peri-islet acinar cells had high expression of Stmn1, which mimics a similar zonated phenotype that was found in diabetic mice (Egozi 2020). CONCLUSIONS: Both the inbred and outbred strain were shown to be metabolically sensitive to HFD, however, the CD-1 strain had larger increase in size in both the exocrine and endocrine pancreas. This could be an indicator that obese CD-1 mice display more plasticity in the pancreas when subjected to metabolic stressors such as HFD.

P15

Interrogating the effects of DRP-1 mediated fission on metabolic flux during neurogenesis

James Costanzo, Vivian Gama

Vanderbilt University, Nashville, TN, 37232

The primary function of mitochondria is coordinating metabolic energy output, which is vital for regulating cell differentiation. Mitochondrial morphology, which is dynamically regulated by mitochondrial fission and fusion mechanisms, has been shown to be a regulator of cell fate transitions during neural stem cell differentiation. If mitochondria in neural stem cells are elongated following mitosis, neural stem cells will self-renew, while mitochondrial fragmentation leads neural stem cells to commit to a neural progenitor cell fate. Therefore, mitochondrial dysfunction can lead to improper neural differentiation and developmental impairments, leading to neurodegenerative and neurodevelopmental disorders. Mutations in dynamin-related protein 1 (DRP-1), which governs mitochondrial fission, causes a rare neurodevelopmental disorder, encephalopathy due to defective mitochondrial and peroxisomal fission-1 (EMPF1). EMPF1 is an epileptic encephalopathy characterized by impaired neurogenesis and neurodevelopmental delay. However, how mitochondrial morphology affects cell fate in EMPF1 is poorly understood and may be explained by the metabolic functions of the mitochondria. Cellular metabolism is controlled in part by mitochondrial fission through regulation of the complexes involved in pyruvate metabolism and fatty acid oxidation. A key product of these mitochondrial metabolic processes is acetyl-CoA, the primary substrate used for the acetylation of proteins, including histones. Histone acetylation and methylation affect transcriptional regulation of genes, and thus, are critical during cellular differentiation. Our findings have identified changes in PAX6 expression, a neural progenitor transcription factor, in isogenic DRP-1 mutant and patient-derived neural progenitor cells. Moreover, studies have shown that mitochondrial and cytosolic acetyl-CoA levels are vital for neurogenesis, and slowing mitochondrial metabolism delays neuronal maturation. While mitochondrial metabolism influences epigenomic regulation via metabolites like acetyl-CoA, the interplay between DRP-1-mediated fission, metabolic flux, and epigenomics remains unclear. I hypothesize that DRP-1-mediated fission ensures metabolic-epigenomic coordination during neurogenesis by regulating acetyl-CoA availability. This study aims to investigate how DRP-1 dysfunction disrupts metabolic-epigenomic coordination, leading to neurodevelopmental deficits in EMPF1. Using human induced pluripotent stem cells (hiPSCs) from EMPF1 patients and isogenic lines with dominant-negative DRP-1 mutations, this study has identified several phenotypes in iPSC-derived neural progenitors and neurons. We have not only observed changes in PAX6 expression, but also CTIP2 expression, a transcription factor expressed in deep layer cortical neurons. Thus, our models show that defective fission causes impaired neurogenesis by this loss of PAX6 and CTIP2. Next steps aim to determine if acetyl-CoA depletion is a mechanistic link between mitochondrial dysfunction and epigenomic dysregulation.

P16

How Do New Cells Invade into Old Monolayers? - Investigating The Mechanism Of Apical Intercalation

Mikiyas Daniel & Ian G. Macara

Vanderbilt University School of Medicine Nashville, TN 37240

How Do New Cells Invade into Old Monolayers? - Investigating The Mechanism Of Apical Intercalation. Mikiyas Daniel & Ian Macara 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 efficiency 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 contest, 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 efficiency. A substantial reduction was observed for integration into the Pals1-KO monolayers, consistent with the loss of zippering between E-cadherin 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

P17

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

Andrew Dixson, Alissa Weaver

Vanderbilt University, Nashville, TN, 37240

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 or transfection of CD63-GFP. 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, but not with the processing body marker DCP1a. GW182 is a member of the RNA-induced silencing complex and is involved in translational repression of microRNAs. 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.

P18

Extracellular vesicles (EVs) mediated ECM deposition and assembly promote cancer cell invasion in head and neck squamous cell carcinomas (HNSCC)

Dr. Sarbajeet Dutta Prof. Alissa Weaver

Vanderbilt University, Nashville, TN 37235

The increased matrix deposition by cancer cells is often linked to the correct assembly of matrix components, which is crucial for cell adhesion and migration. While many extracellular matrix (ECM) proteins can self-assemble after secretion, certain ECM proteins require cell-derived factors such as extracellular vesicles (EVs). The release of EVs is primarily regulated by intracellular molecules like Rab27a. Notably, the mechanisms underlying ECM assembly facilitated by EVs remain inadequately understood. Our research employs human head and neck cancer cells, specifically Detroit 562, to investigate EV-mediated ECM assembly and the potential functions of Rab27a in this process.

P19

Recruitment of MYC to Target Genes by Chromatin-Resident Cofactors

Nicholas Eleuteri, Jing Wang, Brittany Matlock, David Flaherty, Qi Liu, William Tansey

Vanderbilt University, Nashville, TN, 37203

The MYC family of oncoprotein transcription factors (c-, N-, and L-MYC) are overexpressed in cancer and contribute to ~100,000 cancer-related deaths in the USA each year. MYC dysregulation promotes tumorigenesis through the aberrant expression of its target genes, which drives processes such as cell proliferation, metabolism, and ribosome biogenesis. Central to MYC function is its ability to recognize regulatory elements within its target genes, which it does by associating with its obligate partner MAX and binding DNA in a sequence-specific manner. Although this model for MYC function has been the accepted standard for decades, recent studies have revealed that target gene recognition by MYC can be an avidity-driven process involving interaction of MYC/MAX dimers with both DNA and chromatin-resident cofactors such as WDR5. This process of "facilitated recruitment" by WDR5 is important for binding of the c-MYC family member to a small cohort of genes (<100) required for protein synthesis. Importantly, mutations in c-MYC that disable interaction with WDR5 block its ability to initiate and maintain tumors, revealing that WDR5 can provide a therapeutic opportunity to block otherwise "undruggable" MYC function. Since the facilitated recruitment model was first proposed, studies have shown that a majority of chromatin targeting by MYC is likely to be a facilitated process, and both the histone demethylase LSD1 and the epigenetic reader BPTF have emerged as compelling MYC recruiters. If WDR5, LSD1, and BPTF are to open new inroads into targeting MYC in cancer, we must understand the gene networks that are controlled by facilitated recruitment through each of these factors. To achieve this objective, I will employ the "degron tag" (dTAG) system, which will allow me to acutely deplete WDR5, LSD1, and BPTF from cancer cell lines overexpressing c- and N-MYC. This work will lay the foundation for new ways to therapeutically target MYC.

P20

3D High Throughput Screen in Collagen Identifies Drugs That Induce Epithelial Polarity that Increase Response of Colorectal Cancer Cells to Chemotherapy

Sarah J. Harmych, Thomas P. Hasaka, Chelsie K. Sievers, Seung Woo Kang, Vivian Truong Jones, Zhiguo Zhao, Oleg Kovtun, Claudia C. Wahoski, Ken S. Lau, Robert J. Coffey, Joshua A. Bauer, and Bhuminder Singh

Department of Medicine, VUMC, Nashville, TN; Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; Department of Pharmacology, Vanderbilt University, Nashville, TN; Program in Cancer Biology, Vanderbilt University, Nashville, TN; Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN; Department of Chemistry, Vanderbilt University, Nashville, TN; Department of Biostatistics, VUMC, Nashville, TN; Epithelial Biology Center, VUMC, Nashville, TN; Center for Computational Systems Biology, Vanderbilt University, Nashville, TN; Department of Surgery, VUMC, Nashville, TN; Vanderbilt-Cancer Ingram Center, VUMC, Nashville, TN; High-Throughput Screen Facility, Vanderbilt University, Nashville, TN

The epithelial-to-mesenchymal transition (EMT), or the process through which cancer cells lose their epithelial identity and acquire characteristics of mesenchymal cells, is associated with worse prognosis and has been implicated in therapy resistance in colorectal cancer (CRC). We designed a high throughput drug screen using 3D type-1 collagen cultures of CRC cells that would allow us to measure morphological changes in colonies to assess re-epithelialization i.e. identify drugs which caused the colonies to form hollow, single-layered polarized cystic colonies instead of solid spiky masses. Our screen of an FDA-approved drug library identified six compounds that re-epithelialize the CRC cell line, SC. From these drugs, three were tested in the standard 24-well collagen culture format and confirmed the cystic conversion observed in the screen. Combination treatments demonstrated that one drug, azithromycin, enhanced the efficacy of SN-38, the active metabolite of the chemotherapeutic irinotecan. A retrospective analysis showed improved survival for CRC patients given azithromycin during irinotecan-based chemotherapy. Future studies aiming to identify mechanism of re-epithelialization by the hits from our screen will reveal a potential new target to enhance the effectiveness of current treatments for CRC.

P21

Interrogating the mechanisms of mitochondrial cristae remodeling during neurogenesis.

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

Vanderbilt University, Nashville, TN 37212

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.

P22

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

Junmin Hua, Andrew D. Pumford, James S. White, M. Shane Hutson, Andrea Page-McCaw

Department of Cell and Developmental Biology and Program in Developmental Biology, Vanderbilt University School of Medicine; Department of Physics and Astronomy, Vanderbilt University

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.

P23

Retinal progenitor cells show temporal changes in transcriptional maintenance of retinal identity regulated by Vsx2

Alan Hurtado, Xiaodong Li, Edward Levine

Vanderbilt University, Nashville, TN, 37235

During retinal development, retinal progenitor cells (RPCs) express Visual System Homeobox 2 (Vsx2) transcription factor which regulates neuroretina development and prevents ectopic expression of non-retinal genes. Work in Vsx2 germline mutants has identified Melanogenesis Associated Transcription Factor (Mitf) as a disruptor of retinal development and driver of non-retinal gene expression. Failure to prevent expression of Mitf and other non-retinal genes can result in lineage disruptions where RPCs acquire some properties of neighboring tissues like the retinal pigment epithelium (RPE). An unresolved question is how long through development RPCs require Vsx2 to prevent non-retinal gene expression that can lead to RPC lineage disruptions. We hypothesize that RPCs exit a critical period of non-retinal gene restriction by Vsx2. We used tamoxifen-inducible Cre recombination to inactivate Vsx2 in RPCs at specific embryonic ages and generate a Vsx2 CKO retina. Western blots of retinal lysates show a downregulation of Vsx2 expression within 24 hours of tamoxifen treatment. I used RT-qPCR to quantify ectopic gene expression in retinas that underwent Vsx2 inactivation at E9.5, E11.5, E13.5, and E15.5. In each case retinas were harvested three days after tamoxifen treatment. Mitf was upregulated after Vsx2 inactivation at every age tested, indicating that Vsx2 is required to prevent ectopic Mitf expression in the retina through embryonic development. Several genes predicted to be downstream of Mitf were also upregulated after Vsx2 inactivation at E9.5, but not at the later ages tested. This suggests that these genes no longer require repression by Vsx2 or that Mitf is losing activity. However, some non-retinal genes were upregulated after Vsx2 inactivation at the later ages, although not to the same extent as Mitf. This indicates that there is unlikely to be a full exit from Vsx2 mediated suppression of non-retinal gene expression but leaves a remaining question of whether ectopic Mitf is influencing retinal development? I am currently addressing the issue using genetics to block Mitf activity in the context of the Vsx2 CKO retina.

P24

Pcdh20 is a POU2F3 target gene required for proper microvillus formation in tuft cells

Katherine E. Ankenbauer1, Yilin Yang1, Chi-Yeh Chung2, Leonardo R. Andrade2, Sammy Weiser Novak, Brenda Jarvis1, Wahida H. Ali2, Jiayue Liu1, Claire E. O'Leary, Victoria Sarkisian, Neil Dani, Evan Krystofiak, Seham Ebrahim, Bechara Kachar, Qizhi Gong3, Ge

1Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA 2Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA 3University of California Davis, Davis, CA, USA 4University of California San Diego, San Diego, CA, USA 5Vanderbilt Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA 6Vanderbilt Digestive Disease Research Center, Vanderbilt University Medical Center, Nashville, TN, USA 8Center for Computational Systems Biology, Vanderbilt University, Nashville TN, 37232, USA

BACKGROUND & AIMS: Tuft cells are solitary chemosensory cells that play protective role(s) in infection, tissue injury, inflammation, and tumorigenesis through the synthesis and secretion of cytokines and eicosanoids. Tuft cells are known for their distinct tall, blunt microvilli, thought to be analogous to mechanosensory hair cell stereocilia, however a functional role for the microvillar apparatus is unknown. Transcription factor POU2F3 is the master regulator of tuft cell formation, however how POU2F3 drives formation of this unique cell structure is unknown. Here, we aimed to identify POU2F3 structural target genes and commonalities between tuft and hair cells to identify a possible role for tuft cells in mechanotransduction. METHODS: POU2F3 ChIP-seq was performed on isolated tuft cells and compared to the cochlear hair cell transcriptome. Tuft cell RNA-seq datasets were interrogated for hair cell structural and mechanosensory genes, which were validated at the protein level. Intestinal and gallbladder tuft cells were examined using multiple electron microscopy techniques. PCDH20 was knocked down in mouse models and ultrastructural analysis was performed on tuft cells. RESULTS: Structural and mechanosensory genes common to both tuft and hair cells, including protocadherin 20 (PCDH20), were identified. Immunogold labeling and imaging localized PCDH20 to extensive intermicrovillar linkages in tuft cells. Knockdown of PCDH20 in mice resulted in impaired microvilli formation and a disruption in structure. CONCLUSIONS: PCDH20 is a POU2F3 target gene in tuft cells critical to maintain the rigid microvillar apparatus, which may function in mechanotransduction.

P25

Evaluation of the impact of palmitic acid on induction of RNA-enriched small extracellular vesicles

Lizandra Jimenez1, 2, Bahnisikha Barman1, 2, Caraline Bekas1, 2, Marisol Ramirez-Solano3, Qi Liu3, and Alissa M. Weaver1, 2

1. Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee, 37240; 2. Center for Extracellular Vesicle Research, Vanderbilt University, Nashville, Tennessee, 37240; 3. Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee, 37203.

Extracellular vesicle (EV)-cargoes include proteins, RNAs, and lipids and regulate the gene expression and phenotypic properties of recipient cells. A previous paper from our laboratory found that the endoplasmic reticulum (ER)-endosome membrane contact site (MCS) linker proteins VAP-A and ceramide transporter protein CERT are important for the biogenesis of RNA-containing EVs. However, the upstream regulatory process by which the RNA-enriched EVs are generated is unclear. Palmitic acid (PA) is a fatty acid that is upregulated in the circulation in metabolic dysfunction syndromes, including obesity. PA is also an upstream substrate for ceramide synthesis in the ER. We hypothesize that PA acts upstream of VAP-A and CERT to induce the formation of RNA-enriched EVs (EVs). Using colorectal cancer (CRC) cells as a model system, we observed that PA treatment leads to a marked increase in the number of small EVs. PA treatment also increased the content of both total RNA and selected miRNAs in CRC small EVs, without changing the levels in cells. Western blot analysis revealed that PA treatment alters the levels of some RNA-binding proteins and select EV markers in small EVs. RNA-seq analysis of CRC small EVs also identified miRNAs that may be upregulated with PA treatment. An untargeted lipidomics analysis of small and large EVs also revealed lipids species that were altered with PA treatment. These data suggest that PA boosts EV-RNA biogenesis. Future studies will determine the role of ceramide and CERT in PA-driven EV-RNA biogenesis and further characterize the PA-driven EV population.

P26

Scalable Platform to Produce Extracellular Vesicles with Efficient RNA Loading

Youn Jae Jung1,2, John T. Wilson1* and Alissa M. Weaver2*

1. School of Chemical and Biomolecular engineering, Vanderbilt University, Nashville, TN 2. Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA

Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) have emerged as promising carriers for RNA-based therapeutics. However, challenges remain in achieving scalable production and efficient RNA loading. In this study, we present a tunable system for engineering MSCs to produce RNA-containing EVs through inducible overexpression of vesicle-associated membrane protein-associated protein A (VAP-A). Using a cumate-inducible expression system in hTERT-MSCs, we demonstrate dose- and time-dependent upregulation of VAP-A. Upon induction, VAP-A-overexpressing MSCs secrete significantly increased quantities of both small and large EVs, with a marked enhancement in RNA amount in EV compared to parental cells. EVs were isolated via iodixanol gradient ultracentrifugation and characterized by TEM, Western blotting for EV markers, and nanoparticle tracking analysis. Moreover, we employed a scalable platform, hollow fiber bioreactor system exhibited improved yield and RNA payload compared to 2D cultures. We will employ reporter system for EV mediated siRNA gene silencing in recipient cells, evaluating the therapeutic potential of these engineered EVs. Our findings establish VAP-A as a key modulator of EV biogenesis and RNA loading, providing a scalable strategy for the production of functional RNA-loaded EVs from MSCs.

P27

Cell-Specific Visualization of Electrical Synapses in the C. Elegans Nervous System

Sean Kennedy, Isabel Courtney, Tyler Amos, Becca Collings, and David M. Miller

Department of Cell and Developmental Biology, Vanderbilt University

Electrical synapses, or gap junctions, enable the exchange of small molecules and ions between neighboring cells. Gap junctions are made up of subunits called connexins in vertebrates, and innexins in invertebrates. While lacking sequence homology with one another, connexins and innexins have strikingly similar function and topology. Both innexins and connexins form oligomeric hemichannels. When aligned with the hemichannel of an adjacent cell, the two hemichannels create a functional junction. What remains largely unknown are the mechanisms that direct this gap junction assembly in the nervous system. We have used the motor circuit of C. elegans as a model to study these synapses. In this circuit, ventral A class (VA) motor neurons form electrical synapses with the interneuron AVA (VA →AVA), whereas ventral B class (VB) neurons establish electrical synapses with the interneuron AVB (VB →AVB). Previous work has shown that the specificity and placement of these synapses is regulated by the transcription factor UNC-4. For example, in an UNC-4 mutant VA electrical synapses are repositioned from the axon to the soma and are formed with AVB rather than AVA. One of the challenges of studying these gap junctions is visualizing them in a cell-specific manner and without perturbing function. Here, we present a robust approach to fluorescently label gap junctions within the motor circuit, as well as within the rest of the C. elegans nervous system. Using CRISPR/Cas-9, we endogenously tagged the C-terminus of the broadly expressed innexins UNC-7 and UNC-9 with a fragment of a split-fluorescent protein. To visualize the gap junctions in a cell-specific manner, we expressed the complementary fluorescent fragment under the control of targeted promoters, enabling reconstitution of fluorescence only at sites of co-expression. Using this new visualization method, we have confirmed the previous findings of miswiring in an UNC-4 mutant and are currently testing other UNC-4 targets (such as cAMP antagonists) to elucidate the mechanism by which this phenomenon occurs. Going forwards, we plan on using single cell RNA-Seq (scRNA-Seq) to identify more UNC-4 regulated genes that may have a roll in the specificity of these synapses.

P28

The junctional actin belt is a novel site of microvillar growth

Zachary J. Lehmann, Matthew J. Tyska

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37240

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.

P29

Mapping sex-specific gene expression in the nervous system

Brooks Leyhew1, Seth Taylor1,2, Claire Olson1, Rebecca McWhirter1, David M. Miller, III1

1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee, 37235; 2) Department of Cell Biology and Physiology, Brigham Young University, Provo, Utah, 84602

Male and hermaphrodite Caenorhabditis elegans display distinct behaviors (i.e. sex-specific mating behavior) likely arising from differences in neuronal function. Remarkably, neuron types that are shared between the two sexes show sexually dimorphic wiring and neurotransmitter usage. These differences likely result from sex-specific gene expression, but these molecular underpinnings have not been fully characterized. To profile molecular differences between sexes, we performed single cell RNA-seq on males and hermaphrodites. We captured gene expression in all 128 sex-shared neuron types and observed 26 cell types with separate clustering between males and hermaphrodites in UMAP space. Differential gene expression analysis for these 26 neuron types detected 1740 genes that were differentially expressed between male and hermaphrodite neurons. These genes were enriched for biological functions including binding and catalytic activity. Over 70% of the differentially expressed genes in the 26 neuron types were only differentially expressed in a single cell type, indicating that most sexually dimorphic expression is neuron-specific. Notably, mab-3 (transcription factor), lin-29 (transcription factor), and nlp-45 (neuropeptide) were male-upregulated in the most neuron types: mab-3 had elevated male expression levels in over 50% of the neuron types. Our ongoing research provides a comprehensive resource to investigate differentially expressed neuronal genes for sex-specific wiring and function across the nervous system.

P30
Investigating neuronal ER dynamics and discontinuities in vivo in C. elegans

Kelsey Mabry, Aaron Orgel, Eric Donahue, Brennen Keuchel, Kristopher Burkewitz

Vanderbilt University, Department of Cell and Developmental Biology

The endoplasmic reticulum (ER) is integral to cellular calcium, lipid, membrane, and protein homeostasis. In neurons, the morphology of the ER and several factors that regulate ER structure, including reticulons, spastin, and atlastin, are heavily implicated in multiple neurodegenerative pathologies. However, both how ER structure is altered in these conditions and how these aberrant structures drive pathogenesis remain open questions. Here we begin to address these questions in a C. elegans model. Through its genetic strengths, fully mapped and stereotyped nervous system, and transparency, C. elegans enables live, in vivo visualization of neuronal ER dynamics across neuron subtypes and subcellular compartments. We labeled the ER of worms via CRISPR/Cas9-mediated knock-in of fluorescent proteins to the sole C. elegans reticulon, RET-1, an ER membrane protein that maintain curved membranes for tubular ER. While the ER is traditionally believed to be fully continguous in cells, imaging of RET-1 in neurites revealed ER discontinuities to be surprisingly common, even under basal conditions. We generated new reporter lines to determine whether these gaps in the neurite ER network were related to anatomical defects in the neuron itself or gaps in RET-1 labeling along an intact ER tubule, and our results convincingly revealed these to be true discontinuities in the ER. We quantified the occurrence of these gaps across diverse neuronal subtypes, revealing discontinuities to be fairly ubiquitous, though longer neurites may be most susceptible. Timelapse imaging reveals ER tips to be highly motile in a fraction of cases and provides evidence that these gaps can be resolved. Moving forward, I aim to investigate the molecular mechanisms that cause ER gap formation and resolution., Based on our observations of ER dynamics and ER-shaping factors implicated in hereditary spastic paraplegia (HSP), I hypothesize that ER fusogens (e.g., atlastin) and microtubule linkers (e.g., spastin) likely play roles in regulating these discontinuities. Further ahead, we also aim to determine how these gaps may impact neuron function or neurite homeostasis by examining trafficking of synaptic cargoes, calcium-based neuronal activity, and the localization of other neuronal organelles in conjunction with these gaps. Altogether, this study will illuminate new forms of ER dynamics and provide a foundation for using C. elegans to understand how ER dynamics are linked to pathogenesis of neurodegenerative diseases.

P31

Molecular Mechanism of ferrosome formation

Rong Sun1,4, Zhuqing Mao1,4, Hualiang Pi2,4, Jia Mei1, Feng Luo1, Eric P. Skaar3, Qiangjun Zhou1

1 Department of Cell and Developmental Biology, Vanderbilt Brain Institute, Center for Structural Biology, Vanderbilt University, Nashville, TN, 37240, USA; 2 Department of Microbial Pathogenesis, Yale University, West haven, CT 06516, USA; 3 Vanderbilt Institute for Infection, Immunology, and Inflammation, Department of Pathology, Microbiology, & Immunology, Vanderbilt University Medical Center, Nashville, TN, USA; 4 These authors contributed equally.

The Gram-positive bacterium Clostridioides difficile (C. difficile) is the leading cause of nosocomial and antibiotic-associated infections in the USA1. To survive within their hosts, C. difficile has evolved iron uptake, storage, and detoxification strategies to maintain iron homeostasis. Our previous study demonstrated that C. difficile stores iron in membrane-bound ferrosome organelles, enabling the bacterium to combat nutritional immunity and support colonization and survival during infection2. However, the molecular mechanisms governing ferrosome formation remain unclear. Using mass spectrometry-based proteomic analysis of isolated ferrosomes, we identified two key membrane proteins, FezA and FezB, as essential to this process. Here, we successfully co-expressed FezA and FezB in E. coli, and cryo-electron tomography revealed the formation of abundant, uniformly sized ferrosome-like vesicles. Structured illumination microscopy (SIM) further demonstrated the colocalization of FezA and FezB. These findings elucidate the molecular mechanism underlying ferrosome formation.

P32

Sphingolipid metabolism drives mitochondria remodeling during aging and oxidative stress

Thyandra A. Martinez, Adam C. Ebert, Nathaniel L. Hepowit, Jason A. MacGurn

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN United States

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 C (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.

Afternoon Poster Session

Barmaver, Syed	**P1**
Bayazid, Al Borhan	**P2**
Dong, Xinyu	**P3**
Kang, Seung Woo	**P4**
Mehaffey, Thomas	**P5**
Melton, Nathan	**P6**
Merbouche, Lilia	**P7**
Mulligan, Alexandra	**P8**
Napoli, Francesca	**P9**
Norris, Alessandra	**P10**
Peebles, Elkie	**P11**
Peek, Jennifer	**P12**
Perkins, Olivia	**P13**
Richardson, Laura	**P14**
Robinson, Kianna	**P15**
Ruark, Elizabeth	**P16**
Ruelas, Amanda	**P17**
Sawant, Avishkar	**P18**
Schnitkey, Lauren	**P19**
Schwarzkopf, Anna	**P20**
Scott, Halee	**P21**
Shafiq, Adnan	**P22**
Sharma, Rahul	**P23**
Silverman, Jennifer	**P24**
Tan, Marcus	**P25**
Thyagarajan, Pankajam	**P26**
Torbit, Sabrina	**P27**
Torres Martinez, Diego	**P28**
Trapani, Jonathan	**P29**
Vamadevan, Vaishna	**P30**
Venkataravi, Aishwarya	**P31**
Wokasch, Anthony	**P32**
Yagan, Mahircan	**P33**
Yao, Vincent	**P34**

P1

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

Syed Nooruzuha Barmaver1, Gu Guoqiang1 and Irina Kaverina1

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. KIF21A, a kinesin-4 family member, is a good candidate for MT anchoring near the plasma membrane. It is known to stabilize peripheral MT plus ends by anchoring them to the cell cortex. It is also one of the highly expressed kinesin in β cells. 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). We also observed that KIF21A is heterogeneously expressed in beta cells. We hypothesize that KIF21A captures MTs at the cortex to organize tracks for IG transport and KIF21A-captured MTs are more stable, serving as remaining tracks for IG delivery by plus-end-directed motors. 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.

P2

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

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.

P3

Cytoplasmic α-Parvin Regulates Actin Dynamics and Cell Cycle Progression in Kidney Collecting Duct Epithelium

Xinyu Dong, Colton Miller, Hugo D. Arce-Santamaria, Fabian Bock, Shensen Li, Nada Bulus, Olga Viquez, Meiling Melzer, Roy Zent

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37203; Nephrology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, 37203; Aspirnaut program, Nephrology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, 37203

Kidney development requires coordination between cell movement and proliferation. We previously showed that α-parvin is essential for ureteric bud (UB) morphogenesis, in part through promoting 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 new function 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 block downstream or independent of mitogenic input. 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 NLS-mutant did not, indicating that nuclear localization is not required and that cytoplasmic α-parvin is necessary for proper cell cycle progression. Taken together, our findings suggest that α-parvin is required for cell cycle progression, potentially by maintaining the balance of nuclear and cytoplasmic G-actin required for proper gene expression.

P4

Leveraging Tools to Understand Distinct Early Onset Colorectal Cancer Microenvironment

Seung Woo Kang1,6,7, Alan J. Simmons1,6,7, Grayson A. Taber8, Harsimran Kaur1,6,7, Joseph T. Roland1,2, Tae Hyun Hwang1,2, M. Kay Washington4,9, Robert J. Coffey1,5, Jeffery M. Spraggins6, M. Shane Hutson8, Ken S. Lau1,2,3,6,7,10

1Epithelial Biology Centre; 2Department of Surgery; 3Vanderbilt-Ingram Cancer Center; 4Center for Mucosal inflammation and cancer; 5Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University Medical Center, Nashville, TN 37232; 6Department of Cell & Developmental Biology; 7Centre for Computational Systems Biology; 8Department of Physics and Astronomy; 9Program in Chemical and Physical Biology; 10Center for Quantitative Sciences, Vanderbilt University School of Medicine, Nashville, TN 37232

Both the absolute and relative number of colorectal cancer (CRC) cases under the age of 55, defined as early-onset colorectal cancer (EO-CRC), have been rapidly increasing. With almost identical molecular mechanisms behind average-onset CRC (AO-CRC), the aetiology of EO-CRC remains unknown. Interestingly, antibiotics usage was correlated with the EO-CRC, suggesting a potential connection between dysbiosis and a premature onset of CRC. Here, we used spatial transcriptomics to understand the tumour microenvironment (TME), encompassing fibroblasts, macrophages, and microbes, of EO-CRC compared to AO-CRC. We discovered a TME with intestinal stem & foetal-like transcriptome and a TME with secretory & differentiated intestinal epithelial-like transcriptome. We also fabricated a microfluidics device, a gut-on-a-chip, to culture patient-derived organoids in a colon-like environment alongside fibroblasts, immune cells, and microbes. In the CDB breakout session, I'd like to discuss two challenges pertaining to the aforementioned projects. One is to generate testable hypotheses by leveraging spatial multi-omic datasets. Another is the technical challenge of co-culturing organoids with rapidly proliferating fibroblasts in my system.

P5

Pax3 deficits in the neural crest alter differentiation and outgrowth of pelvic autonomic ganglia

Thomas Mehaffey (1), Ysabel Gomez (2), Zunyi Wang (3), Dale E. Bjorling (3), Michelle Southard-Smith (1,2)

(1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; (2) Department of Medicine, Division of Genetic Medicine, Vanderbilt University Medical Center, Nashville, TN; (3) Department of Surgical Sciences, University of Wisconsin, School of Veterinary Medicine, Madison, WI

The autonomic nervous system is necessary for regulating organ function, and therefore body homeostasis, in all vertebrates. In rodents, the autonomic neurons that provide motor control for bladder constriction reside in clusters known as the major pelvic ganglia. These structures, analogous to the human hypogastric plexus, function to control storage of urine and emptying of the bladder. Accordingly, damage to the pelvic ganglia caused by injury, infection, or congenital disorders can lead to urinary incontinence. The pelvic ganglia arise during fetal development in mice, when the sacral subpopulation of neural crest-derived progenitor cells migrates to the lower urinary tract and undergoes differentiation. Migration and differentiation of neural crest-derived progenitor cells to form the autonomic circuitry of other organs, including the heart, lungs, and gut, is well-defined. However, key molecular factors which control these processes in the sacral neural crest to establish lower urinary tract innervation remain unknown. We previously conducted exploratory studies of transcription factor expression in the developing pelvic ganglia and identified robust expression of Pax3, a gene encoding a protein with known regulatory involvement in neural crest development. This study introduces a novel mutant mouse strain carrying a deletion in Pax3 restricted to the neural crest. To determine whether Pax3 expression in the neural crest controls pelvic ganglia development and function, we utilized immunohistochemistry, confocal and light sheet fluorescence microscopy, and bladder voiding assays. With these approaches, we show that Pax3 expression in neural crest-derived progenitor cells which colonize the developing lower urinary tract is necessary for proper pelvic ganglia neurogenesis, neuronal outgrowth, and bladder function in mice.

P6

An inducible dominant negative allele of Sox10 alters enteric ganglia composition and gastrointestinal motility in mice

Meena Halaka, Jean-Marc L. DeKeyser, Nathan Melton, Noah J. Lawler, Anne Braun, Aris Economides, Karl-Herbert Schafer, Kelli Boyd, and E. Michelle Southard-Smith

Vanderbilt University Medical Center, Nashville, TN, 37232

The enteric nervous system (ENS) is an interconnected network of intrinsic ganglia in the intestinal wall that is essential for coordinated propulsion of luminal contents. Sox10 is a transcription factor that is essential for development of enteric ganglia. Mice carrying mutations in Sox10 recapitulate intestinal aganglionosis of Hirschsprung disease and present with variable loss of enteric ganglia in the distal bowl due to deficits in vagal neural crest progenitors that initially populate the fetal intestine. We have previously shown that Sox10 mutants also exhibit alterations in composition of enteric ganglia and motility of proximal ganglionated bowel. It has not been clear whether these defects were due to cell autonomous defects within progenitors that first populate the ENS or occur secondary to deficits in neuronal-glial signaling during differentiation phases. In order to distinguish these possibilities conditional alleles of Sox10 are needed. We have generated and characterized a COnditional INducible ("COIN") dominant negative allele of Sox10 in mice as a novel tool for temporal and cell type specific analysis of gene function. Genome targeting strategies in C57BL/6J ES cells were used to introduce a floxed splice acceptor cassette containing a red fluorescence reporter into the non-coding strand of intron 4 at the Sox10 locus. Chimeras were obtained and bred to establish lines of mice that appear normal until crossed with Cre. Whole mount acetylcholinesterase was applied to assess the patterning and distribution of ENS throughout the intestine complemented by immunohistochemical labeling of laminar myenteric muscle preparations to assess the composition of enteric ganglia. Ex vivo motility imaging was applied to assess motility patterns including intestinal contractile frequency, contractile response to feeding, and pharmacological stimulation. Our characterization of this new allele indicates that the COIN cassette functions in a dominant negative fashion and fluorescently labels neural crest derivatives following Cre action. Crosses of Sox10-COIN mice with neural crest specific Cre drivers produce mutant progeny (Sox10-COININV+) that exhibit spotting as well as gastrointestinal phenotypes. Sox10-COININV/+ mutants exhibit alterations in the composition of enteric ganglia in the small intestine and colon with accompanying deficits in bowel motility. Observations of the F1 produced from crossing two Sox10-COIN mice demonstrated the COIN/COIN exhibited the megacolon phenotype as well as spotting remarkably similar to the COININV/+, which suggests a reduction of expression without the Cre-induced inversion. This new allele will enable temporal and lineage specific studies of Sox10 function in the ENS and among other neural crest derivatives.

P7

Regulation of Ribosomal Biogenesis Gene Expression by the MYC-HCF-1 Interaction

Lilia Merbouche, Caleb Howard, Kristie Rose, Brittany Matlock, William Tansey

Vanderbilt University, Nashville, TN

MYC is a transcription factor that drives cell growth and metabolism, but how it activates these specific genes is still not fully understood. Our lab previously identified HCF-1 as an essential cofactor for MYC, particularly in regulating metabolic and ribosomal biogenesis gene expression. In this study, I investigate how MYC and HCF-1 work together to regulate transcription, and whether this mechanism is conserved across different cancer cell types. ChIP-seq in Ramos, SW620, and CHP-134 cells shows that MYC and HCF-1 co-bind a conserved set of genes involved in ribosome biogenesis and mitochondrial function. RNA-seq following HCF-1 degradation confirms that many of these co-bound genes are downregulated, suggesting that MYC and HCF-1 work together to promote their expression. To understand how this activation occurs, I focused on the NSL complex, a transcriptional coactivator complex known for its histone acetyltransferase activity. HCF-1 is a component of the NSL complex. Supporting this, proteomics from HALO-HCF-1 pull-downs show robust recovery of the full NSL complex. Comparing ChIP data various published NSL data sets with those generated in our lab we discovered key overlap at housekeeping genes including ribosomal biogenesis and mitochondrial function genes. To test whether the NSL complex is required for expression of MYC-HCF-1 target genes, I used dTAG degron cell lines to degrade HCF-1. RNA-seq and qPCR show that loss of HCF-1 reduces the expression of key NSL complex target genes. Together, these data support a model in which MYC may activate a conserved biosynthetic transcriptional program by recruiting the NSL complex through HCF-1. This work provides new insight into how MYC selectively activates gene expression and highlights the NSL complex as a key effector of this activity.

P8

Investigating the function of EPS8 in actin-based protrusions

Alexandra G Mulligan, Matthew J Tyska

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37240

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.

P9

Reassessing Conditions for Air-Liquid Interface Culture of ex vivo Adult Mouse Retina

Francesca R. Napoli1, Hannah Lee1, Megan Stone2, Edward M. Levine1,2*

1,Department of Ophthalmology and Visual Science, Nashville, TN, 37372; 2, Department of Cell and Developmental Biology, Nashville, TN, 37372

Three-dimensional (3D) culture of adult mammalian retina has the potential to retain the extracellular milieu and intercellular interactions between diverse cell types that occur in vivo, but long-term tissue preservation remains challenging. While many studies aim to promote long term viability, information on potential changes at the earliest stages of 3D cultures is lacking. Additionally, most studies rely on cross-sectional rather than longitudinal data, making culture optimization laborious and requiring many samples. To address these shortcomings, we used transcriptomics to identify gene expression changes in the adult mouse retina in air-liquid interface culture, developed a workflow to collect longitudinal data, and varied the medium, glucose, and oxygen concentrations for optimization of tissue viability and downstream applications. Whole retinal tissues from adult mice were cultured at the air-liquid interface on transwell filters. Bulk RNA sequencing was done after 0, 1, 3, 7, and 14 days ex vivo (DEV). Live imaging via widefield structured illumination microscopy was done throughout the culture period to track Muller glia and inner retinal neurons with fluorescent reporters. Conditions tested included two types of neuronal culture media (Neurobasal-A, Neurocult), varying concentrations of glucose (0, 5, 10, 25 mM), and oxygen (21%, 5%). Environmental monitoring of gases, ions, pH, glucose, and lactate was done on conditioned media with Chem8+ and CG4+ microfluidic cartridges using the iSTAT analyzer (Abbott). Retinal tissue and Muller glia persisted over 1 month in culture but with altered morphologies by 14 DEV. RNA sequencing revealed significant reductions in photoreceptor and RGC gene expression by 3 DEV, suggesting rapid loss of these cells. Muller glia gene expression persisted with increased expression of reactivity genes at 7 and 14 DEV. Live imaging of inner retinal neurons with Thy1-YFP revealed that Neurobasal-A, 10 mM glucose, and 21% oxygen best preserved the inner retinal neurons. Rapid changes in tissue integrity and cell survival likely impose limitations on long-term culture, negatively impacting applications such as drug testing, cell reprogramming, or gene therapy studies. Longitudinal measurements including live cell imaging and environmental monitoring should facilitate more efficient optimization of ex vivo culture conditions.

P10

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

Vanderbilt University, Nashville, TN, 37232

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.

P11

Controlling Collagen IV: How are Collagen IV levels regulated under homeostatic conditions?

K. Elkie Peebles, Aubrie Stricker, Junmin Hua, Andrea Page-McCaw

Vanderbilt University, Nashville, TN, 37235

The basement membrane is a sheet-like extracellular matrix that underlies epithelia and surrounds muscles providing specialized, crucial roles to tissues including structural support, filtration, and signaling. These roles are affected by the composition and structure of each specialized basement membrane. Basement membranes have four primary components (Collagen IV, Laminin, Nidogen, and Perlecan) as well as dozens of associated interactors, and the levels of each component will vary across distinct basement membranes. The levels of the most abundant component of basement membranes, Collagen IV, is known to affect many of these roles. The levels of Collagen IV change over time, but each basement membrane has distinct changes in levels with some increasing over time and some decreasing over time. Distinct changes in Collagen IV levels are also associated with many disease states. Although these specialized changes suggest a degree of control in the levels of Collagen IV, the regulation of Collagen IV turnover under homeostatic conditions in adults is not well understood. We seek to characterize this Collagen IV turnover in the gut basement membrane of Drosophila. In the Drosophila gut, basement membrane surrounds the peristalsis muscles to keep them flat and smooth. To quantify the levels of Collagen IV in the gut basement membrane, we use a Collagen IV-GFP fly in which a GFP exon is inserted into the endogenous locus. We describe a novel pipeline of analysis including modules of the interactive machine learning program ilastik and python coding to specifically quantify fluorescently tagged basement membrane components. Utilizing this methodology, we observe that Collagen IV-GFP accumulates in the gut basement membrane over time, indicating that incorporation of Collagen IV into the basement membrane is faster than the loss of Collagen IV. To specifically observe the loss of Collagen IV from the basement membrane, we start with a Collagen IV-GFP heterozygote with one labeled and one unlabeled allele. Then we conditionally turn off Collagen IV-GFP using genetic tools that initiate GFP knock down in adulthood. We find that the loss of Collagen IV-GFP from the gut basement membrane has a half-life around 68 days (the lifetime of a fly), highlighting the long-lived nature of Collagen IV under maintenance conditions. Future directions will be discussed including a screen to identify relevant proteins and signaling pathways that govern Collagen IV incorporation into and Collagen IV loss from the basement membrane.

P12

Proximal Tubule Targeted Gene Therapy for Cystinuria

Jennifer M. Peek, Alan Rosales, Lauren E. Woodard, Aravind Asokan, Matthew H. Wilson

Vanderbilt University, Nashville, TN, 37212; Vanderbilt University Medical Center, Nashville, TN, 37212; VA Medical Center, Nashville, TN, 37212; Duke University, Durham, NC, 27710

Gene therapy for kidney disease remains a challenge primarily due to lack of gene delivery. Cystinuria, the most common inherited kidney stone disorder, results from deficiency of cystine transport and reabsorption in the proximal tubule. Cystinuria patients suffer from cystine stones, obstruction, and CKD development; effective treatments are lacking for this lifelong disease. We have previously shown significant reductions in urinary cystine levels in murine models of type A (Slc3a1-/-) cystinuria through plasmid delivery of Slc3a1, which encodes rBAT. However, gene transfer was estimated to be <5% of proximal tubular epithelial cells (PTECs) and therefore did not affect stone formation. Recent innovations in viral vectors have improved kidney transduction. With novel adeno-associated viruses (AAV) including AAV.cc47, we have shown efficient kidney delivery in vivo, in vitro, and in human kidney organoids. We have used AAV.cc47 to deliver fluorescent reporters and therapeutic transgenes in both healthy and cystinuric mice. Immunofluorescence analysis and statistics were performed in QuPath and GraphPad Prism, respectively. We observed that AAV.cc47 consistently targeted the kidney, with >80% of PTECs transduced in all mice injected with at least 1x1011 viral genomes (p=0.0011). Further quantification revealed a dose-dependent increase in the % transduced PTECs and strength of transduction. We next identified AAV.cc47 specifically targets S1 and S2 PTECs in the kidney, as >90% of all transduced cells were SGLT2+ (p=0.0009). Finally, we have shown rBAT expression post-delivery of AAV.cc47-Slc3a1 to cystinuric mice and investigation of phenotypic changes is ongoing. We have shown Slc3a1 gene delivery for type A cystinuria gene therapy in vivo utilizing a proximal tubule-targeted vector, AAV.cc47. Phenotypic correction of a kidney disease remains a challenge, but our current efforts to optimize the delivery and stable expression of desired transgenes provide hope for overcoming the barriers to kidney gene therapy.

P13

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

Olivia L. Perkins1, Alexandra G. Mulligan1, Evan S. Krystofiak1, K. Elkie Peebles1, Rekha N. Nagarajan, Leslie M. Meenderink1,3, Bryan A. Millis1, 2, Matthew J. Tyska1

1Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232 2Department Biomedical Engineering, Vanderbilt University School of Engineering, Nashville, TN 37232 3United States Department of Veterans Affairs Tennessee Valley Health Care System, Nashville, TN, 37232

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.

P14

SSNA1 increases microtubule mechanical strength

Laura Richardson, Marija Zanic

Vanderbilt University, Nashville, TN 37240

SSNA1 (Sjögren's Syndrome Nuclear Autoantigen 1) is a microtubule-associated protein implicated in Sjögren's disease; an autoimmune disease prevalent in women over 40 years old. SSNA1's function on a cellular level has been sparsely studied; however, immunostaining reports show stark localization of SSNA1 at neuronal branch points and the basal bodies of cilia and flagella. Given this, we hypothesize that SSNA1 plays a mechanical role in stabilizing higher-order microtubule structures against forces within the cell. To test this hypothesis, we use an in vitro reconstitution approach with purified protein components and total internal reflection fluorescence (TIRF) microscopy. In this way, we can directly probe the effect of SSNA1 localization on the mechanical stiffness of microtubules on an individual polymer level. Our results reveal that SSNA1 increases microtubule rigidity, or resistance to bending, measured both by the application of hydrodynamic force via microfluidics assays and with gliding assays where adhered kinesin motors propel microtubules and induce local curvature. Additionally, we find that SSNA1 increases the amount of force required to rupture the polymer, as evidenced by a decreased frequency of microtubule breakage in our assays. Given this apparent mechanical reinforcement by SSNA1 and previous work by our lab that indicated SSNA1 recognizes damage sites on the microtubule lattice, we theorized that SSNA1 could further increase microtubule stability through promoting microtubule self-repair. However, our data indicates that SSNA1 limits the incorporation of tubulin into damage sites, prompting further investigation into its mechanism of binding to damage sites along the lattice. Overall, by understanding SSNA1's effect on microtubules in vitro, our research provides insight into the mechanisms underlying the regulation of microtubules in their essential cellular functions.

P15

Determining the mechanism of MYO7B motility

Kianna Robinson, Matthew Tyska

Vanderbilt University, Nashville, TN, 37235

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.

P16

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

Vanderbilt University, Nashville, TN, 37232

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 Ca2+ channel which promotes mitochondrial homeostasis through both direct matrix Ca2+ 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 Ca2+ 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 Ca2+ 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

P17

PSTAT3 AS A REGULATOR OF SPEM CELL LINEAGE PLASTICTY IN GASTRIC METAPLASIA

Ruelas, Amanda M ; Won, Yoonkyung ; Choi, Eunyoung

Cell and Developmental Biology, Vanderbilt University, Nashville, TN, United States

BACKGROUND: Gastric carcinogenesis progresses through a cascade, starting with pyloric metaplasia, characterized by the transdifferentiation of chief cells into mucin-secreting spasmolytic polypeptide expressing metaplastic (SPEM) cells. Through unclear mechanisms, SPEM cells can progress into dysplastic cells, which have the highest potential of developing into cancer cells. Our group recently designed a model of gastric carcinogenesis, a doxycycline-inducible transgenic model, the GCK (Gif-rtTA;TetO-Cre;LSL-Kras) mouse allele, which recapitulates histological features of carcinogenesis observed in human patient stomachs. The Choi lab has recently demonstrated that SCD1 is a key enzyme for fatty acid (FA) metabolism and desaturation required for dysplastic cell survival. The inhibition of SCD1 led to dysplastic cell death, but did not target SPEM cells. We have recently observed that STAT3 signaling is activated in dysplastic cells. However, it remains unclear how SCD1 expression in dysplastic cells is promoted or the molecular mechanisms that govern SPEM cell evolution into dysplastic cells and we therefore examined correlations between STAT3 signaling and SCD1 upregulation. METHODS: GCK mice at a metaplastic stage were treated with a SCD1 inhibitor (A939572) for 4 weeks, then withdrawn from the inhibition for 2 weeks. Immunostaining of pSTAT3 (Y705), Ki67, and SPEM cell marker, CD44v9, were performed in control and withdrawal stomachs. GCK untreated metaplastic and dysplastic tissue and organoids (derived from GCK mice) were used for immunostaining for SCD1, pSTAT3, CD44v9, and Trop2, a dysplastic cell marker. RESULTS: While metaplastic tissue had little expression of pSTAT3 in SPEM cells, the expression of pSTAT3 was observed in many SPEM and dysplastic cells. Also, dysplastic cells were co-positive for SCD1 and pSTAT3 in vitro and in vivo. Stomach tissues treated with A939572 induced dysplastic cell death, but stomachs withdrawn from A939572 treatment re-entered the carcinogenic cascade. The withdrawn stomachs exhibited expanded and widened gland bases and re-established the transitioning zone with emerging dysplastic cells and mucinous cells. Stomachs withdrawn from A939572 treatment had SPEM and dysplastic cells strongly positive for pSTAT3 compared to the stomachs treated with A939572. Additionally, SPEM cells were copositive for Ki67 and CD44v9, at the base of glands, indicating that the SPEM cells that survived SCD1 inhibition became more proliferative. CONCLUSIONS: Therefore, our study suggests that STAT3 pathway activation in SPEM cells may promote their lineage evolution into dysplastic cells and FA metabolism during metaplasia progression to dysplasia.

P18

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

Avishkar V. Sawant and Irina Kaverina

Vanderbilt University, Nashville, TN, 37203

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.

P19

Characterization of the role of the STK38 kinase in Wnt siganling and heart development

Lauren Schnitkey, Muhammad Taha, Yashi Ahmed, Ethan Lee

Department of Pharmacology, Vanderbilt University, Nashville, TN, 37232, USA; Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37232, USA; Department of Molecular and Systems Biology and the Dartmouth Cancer Center, Geisel School of Medicine at Dartmouth College, Hanover, NH, 03755, USA

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.

P20

Characterization of RNF111/Arkadia as a New Positive Regulator of Wnt/beta-catenin Signaling

Anna Schwarzkopf1, Ghalia Saad Siddiqui2, Hassina Benchabane2, Ganesh Pusapati3, Gemma Orton1, Mary Rockouski1, Rajat Rohatgi3, Yashi Ahmed2, Ethan Lee1

1Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37232, USA; 2Department of Molecular and Systems Biology and the Dartmouth Cancer Center, Geisel School of Medicine at Dartmouth College, Hanover, NH, 03755, USA; 3Departments of Biochemistry, Stanford University School of Medicine, Stanford, CA, 94305, USA

The canonical Wnt signaling pathway plays a critical role in developmental processes and is frequently dysregulated in human diseases, including cancer. Many of the proteins in the Wnt pathway are regulated by ubiquitylation, but only a subset of the E3 ligases mediating these ubiquitylation events have been identified. Thus, we sought to identify novel roles for known E3 ligases in regulating the Wnt pathway. Using CRISPR/Cas9 and RNAi screens in NIH-3T3 cells and Drosophila, respectively, we identified the E3 ligase RNF111/Arkadia as a new conserved positive regulator of Wnt signaling. RNF111 is a SUMO-targeted E3 ligase that is required for mesoderm patterning during embryonic development and positively regulates TGF-beta signaling, a process mediated by interaction with Axin, a component of the beta-catenin destruction complex. Through follow-up experiments in human cultured cells, we found that RNF111 knockdown and knockout significantly reduced the activity of the Wnt TOPFlash reporter and the levels of beta-catenin, the primary effector protein in canonical Wnt signaling. Further, our results suggest that RNF111 interacts with and ubiquitylates beta-catenin and stabilizes nuclear beta-catenin levels downstream of the beta-catenin destruction complex. Thus, RNF111 may provide a novel mechanism through which nuclear beta-catenin is regulated independently of the destruction complex and, upon further characterization, could be harnessed for targeting Wnt-driven diseases.

P21

Inhibited DNA methylation drives metaplastic cell lineage differentiation and progression

Halee Scott, Eunyoung Choi

Vanderbilt University, Nashville, TN 37235

Gastric cancer often arises following 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 examined Dnmt1 expression during SPEM cell proliferation, maturation and differentiation using in vitro and in vivo models. Finally, we determined the effects of inhibited 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 the SPEM cell population. Furthermore, Dnmt1 is 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 examine the impact of inhibiting 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. Treated organoids were analyzed using histology, IF and qPCR. Treatment with 5-AZA-CdR resulted in a decrease in SPEM cell lineage markers 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 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 altered DNA methylation contributes to both cellular differentiation and lineage evolution of metaplastic cells during gastric carcinogenesis. Additionally, this study provides novel insights into the biological roles of DNA methylation states in pyloric and intestinal metaplasia.

P22

Comparative proteomics analysis of small EVs-derived from mouse oral carcinoma cells

Adnan Shafiq, Shinya Sato & Alissa Weaver

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA; Department of Morphological Information Analysis Center, Kanagawa Cancer Center Research Institute, Yokohama, Kanagawa, Japan

Cancer metastasis is influenced by both tumor cell attributes and the microenvironment. Extracellular vesicles (EVs) play a key role in tumor metastasis by promoting cancer cell migration and invasion and influencing cells in the tumor microenvironment. To identify EV cargoes that drive head and neck squamous cell carcinoma (HNSCC) metastasis to lymph nodes and other organs, we performed a quantitative proteomics analysis of EVs purified from matched mouse oral cancer (MOC) cell lines with different metastatic abilities. Small EVs (SEVs) from MOC1 and MOC2 matched nonmetastatic and metastatic mouse oral cancer cell lines were purified by the cushion density gradient method. SEVs were analyzed by isobaric tagging for relative and absolute quantitation (iTRAQ) mass spectrometry. The proteome data was analysed using STRING and Gene Set Enrichment Analysis (GSEA) methods to identify enriched functional and interacting groups of proteins. Proteomics analysis of MOC1-and MOC2 cell-derived SEVs revealed that MOC1-SEVs contained factors involved in ribosome biogenesis/RNA processing, infectious diseases, and cell adhesion. In contrast, MOC2 cell-derived SEVs were enriched with factors related to nerve reprogramming and synaptic vesicles, cell migration, angiogenesis, and ECM organization. Interestingly, total RNA content per EV was significantly higher in indolent MOC1-SEVs than in aggressive MOC2-SEVs, although the total number of EVs secreted per MOC1 cells was lower. Significantly upregulated genes in the MOC2-SEV proteome were compared with the TCGA dataset of HNSCC patients, revealing a correlation of many genes with poor overall survival. Overall, we found a number of EV cargoes upregulated in MOC2 EVs related to HNSCC metastasis that could serve as potential biomarkers of aggressive behavior.

P23

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

Margret A. Fye1, Rahul Sharma1, Pi'ilani Regan1, Myat, Phyu Sin M1, Hudson McKinney1, Guoqiang Gu1, Irina Kaverina1

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

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 Zn2+ binding dye FluoZin-3, which fluoresces upon Zn2+ 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 Zn2+-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 do not dock at the membrane)] in real time during the first phase of insulin secretion. The Predocked and New IGs (New IG population consisting 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)

P24

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

Vanderbilt University, Nashville, TN, 37235

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.

P25

Tumor-derived extracellular vesicles modulate neural growth in premalignant pancreatic cystic neoplasms

Malli S. Turner, MS, Sabrina M. Torbit, BS, Katherine E. Ankenbauer, PhD, Alexandria M. Bontrager, MD, William H. McDonald, PhD, Vincent Q. Trinh, MD, Bruce D. Carter, PhD, Kathleen E. DelGiorno, PhD, Marcus C. Tan, MBBS

Vanderbilt University Medical Center, Division of Surgical Oncology and Endocrine Surgery, Nashville, TN, 37232; Vanderbilt University, Department of Cell and Developmental Biology, Nashville, TN, 37232; Vanderbilt School of Medicine, Nashville, TN, 37232; Vanderbilt University, Department of Biochemistry, Nashville, TN, 37232; University of Montreal, Centre de recherche du CHUM (CRCHUM), Montreal, Canada; University of Montreal, Department of Pathology, CHUM, Montreal, Canada; University of Montreal Institute for Research in Immunology and Cancer, Montreal, Canada; Vanderbilt Brain Institute, Nashville, TN, 37232; Vanderbilt-Ingram Cancer Center, Nashville, TN, 37232

Introduction: Intraductal papillary mucinous neoplasms (IPMNs) are precancerous lesions with the potential to progress to pancreatic ductal adenocarcinoma (PDAC), a malignancy associated with a 5-year survival rate of approximately 13%. We have previously shown that neural infiltration correlates with increasing dysplasia in IPMNs, suggesting that neural invasion may play a critical role in the malignant progression of these lesions. Extracellular vesicles (EVs) contribute to neurotropism, and recent neural research has highlighted the significance of ephrin receptors, such as EphB3, in neural growth. This study investigates the role of IPMN-derived EVs in modulating neural behavior, particularly their potential to enhance neural outgrowth and branching. Methods: EVs were isolated from two IPMN cell lines derived from genetically engineered mice harboring a constitutive KRAS mutation and a doxycycline (DOX)-inducible GNAS mutation. Dorsal root ganglia (DRG) were obtained from CD1 mouse embryos, and neurons were cultured in media containing either nerve growth factor (NGF, 0.1 ng/mL) or potassium chloride (KCl, 12.5 mM). DRG cultures were treated with EVs derived from IPMN cells with and without DOX induction, PDAC cells, and fibroblasts. Neurite outgrowth was assessed through Sholl analysis, which quantified peak neural density and proximal branching. Mass spectrometry was performed on one of the IPMN-derived EV lines, and human IPMN tissues were analyzed using Xenium spatial transcriptomics. Results: Sholl analysis revealed that neurons treated with DOX-induced IPMN-derived small EVs exhibited significantly higher peak neural density and increased proximal branching compared to untreated controls. Mass spectrometry of the IPMN-derived EVs showed a substantial increase in EphB3 protein expression in the DOX-induced samples. Furthermore, analysis of patient tissue samples revealed that low-grade IPMNs had high levels of EphB3 expression, whereas high-grade tumors showed a significant upregulation of EphB4, suggesting a shift in neural signaling pathways with tumor progression. Discussion: These findings demonstrate that small EVs derived from DOX-induced IPMN cell lines influence neuronal morphology by enhancing peak neurite density and promoting proximal branching, suggesting a role for intra-tumoral neurons in coordinating local tumor growth. Ongoing investigations aim to identify EphB3 as the key molecular mediator within these EVs responsible for this neural reprogramming, as well as to elucidate the underlying mechanisms through which they exert their effects.

P26

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

Pankajam Thyagarajan, Junmin Hua, and Andrea Page-McCaw

Dept. of Cell and Developmental biology, Vanderbilt University, Nashville, TN, 37212

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.

P27

Mutant GNAS drives a pyloric metaplasia with tumor suppressive glycans in intraductal papillary mucinous neoplasia

Vincent Quoc-Huy Trinh, Katherine Ankenbauer, Sabrina Torbit, Jiayue Liu, Maelle Batardiere, H. Carlo Maurer, Frank Revetta, Zhengyi Chen, Angela Kruse, Audra Judd, Celina Copeland, Jahg Wong, Olivia Ben-Levy, Brenda Jarvis, Monica Brown, Jeffrey Brown,

University of Montreal, Montreal, Quebec, Canada; Vanderbilt University, Nashville, TN, 37232; Vanderbilt University Medical Center, Nashville, TN, 37232; Klinikum Rechts der Isar, School of Medicine, Technical University of Munich, Germany; Washington University, St. Louis, MO, 63110

Intraductal Papillary Mucinous Neoplasms (IPMNs) are cystic lesions and bona fide precursors for pancreatic ductal adenocarcinoma (PDAC). Recent studies have shown that pancreatic precancer is characterized by a transcriptomic program similar to gastric metaplasia. The aims of this study were to assay IPMN for pyloric markers, to identify molecular drivers, and to determine a functional role for this program in the pancreas. Pyloric marker expression was evaluated by RNA-seq and multiplex immunostaining in patient samples. Cell lines and organoids expressing KrasG12D +/- GNASR201C underwent RNA sequencing. A PyScenic-based regulon analysis was performed to identify molecular drivers, and candidates were evaluated by RNA-seq, immunostaining, and small interfering RNA knockdown. Glycosylation profiling was performed to identify GNASR201C-driven changes. Glycan abundance was evaluated in patient samples. Pyloric markers were identified in human IPMN. GNASR201C drove expression of this program as well as an indolent phenotype characterized by distinct glycosyltransferase changes. Glycan profiling identified an increase in LacdiNAcs and loss of pro-tumorigenic Lewis antigens. Knockdown of transcription factors Spdef or Creb3l1 or chitinase treatment reduced LacdiNAc deposition and reversed the indolent phenotype. LacdiNAc and 3'-sulfoLeA/C abundance discriminated low from high grade IPMN. GNASR201C drives an indolent phenotype in IPMN by amplifying a differentiated, pyloric phenotype which is characterized by distinct glycans. Acting as a glycan rheostat, mutant GNAS elevates LacdiNAcs at the expense of pro-tumorigenic 3'-sulfoLeA/C, inhibiting cancer cell invasion and disease progression. LacdiNAc and 3'-sulfoLeA/C are mutually exclusive and may serve as markers of disease progression.

P28

Exosomes promote extracellular matrix assembly in breast cancer cells

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

Vanderbilt University, Nashville, TN, 37235

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 indeed 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 or 6 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 and nidogen compared to control cells. Moreover, immunofluorescence analysis of collagen IV 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.

P29

Using PROTACs to Target the Main Effector of the Wnt Pathway

Jonathan Trapani, Alex Waterson, Mary Rockouski, Gemma Orton, Jiqing Sai, Courtney Phifer, Christin Anthony, Carolina Cywiak, Vivian Weiss, Stephen Fesik, Ethan Lee

Vanderbilt University Nashville, TN, 37205; Vanderbilt University Medical Center, Nashville, TN 37205

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide, with mortality projected to nearly double by 2040. Over 90% of CRC cases exhibit mutations that aberrantly activate Wnt signaling, stabilizing cytoplasmic β-catenin-a transcriptional co-activator that drives expression of genes promoting cell proliferation and growth. No FDA-approved drugs currently target the Wnt pathway in the setting of cancer. Directly inhibiting β-catenin is challenging due to its lack of enzymatic small-molecule binding sites and its critical role in forming adherens junctions at the membrane, raising concerns about on-target toxicity. Proteolysis Targeting Chimeras (PROTACs) offer a promising solution by inducing degradation of target proteins via the ubiquitin-proteasome pathway. Unlike traditional inhibitors, PROTACs effectively target proteins without small-molecule binding pockets and selectively degrade cytoplasmic pools, making them ideal for addressing β-catenin. Recently, the Fesik Lab at Vanderbilt identified a novel β-catenin binding site, enabling the development of potent, drug-like β-catenin PROTACs. My preliminary studies with one such PROTAC, VU9132, demonstrate that it: 1) selectively degrades cytoplasmic β-catenin (Wnt pool) while sparing membrane-associated, cadherin-bound β-catenin (toxic pool), and 2) inhibits Wnt target gene transcription in HEK293T and SW480 (CRC) cells. In collaboration with the Vivian Weiss Lab at Vanderbilt University Medical Center, we further showed that VU9132 PROTACs reduce DLD1 CRC cell growth. Based on these findings, I hypothesize that β-catenin PROTACs will selectively degrade cytoplasmic β-catenin across multiple doses, time points, and cell types, and decrease CRC tumor growth through a Wnt dependent mechanism. In Aim 1, I will further characterize the selectivity of β-catenin PROTACs for cytoplasmic versus membrane-associated, cadherin-bound β-catenin using: 1) using engineered cell lines that allow for the quantification of cytoplasmic and cadherin-bound β-catenin through luminescence and 2) biochemical fractionation across a panel of six CRC cell lines with diverse Wnt and non-Wnt mutations. In Aim 2, I will evaluate the ability of β-catenin PROTACs to inhibit CRC growth using in vitro models, including CRC monolayers, spheroids, and patient-derived organoids from fine-needle aspirations. Wnt transcriptional activity will be measured by qPCR, and tumor growth will be assessed via cell viability, proliferation, and apoptosis assays. This study will be the first to characterize the ability for β-catenin degraders to selectively target cytoplasmic β-catenin over membrane-bound, cadherin-associated β-catenin-a critical step toward validating β-catenin-targeting drugs as viable therapeutics. Successful completion of these aims will highlight the potential of β-catenin PROTACs to inhibit Wnt signaling and reduce CRC tumor growth.

P30

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

Vaishna Vamadevan, Loic Fort, Jeremy Clements, Anna E Johnson, Scott W Hiebert, Ian G Macara

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA; Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA

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.

P31

Unraveling the role of polarity remodeling during cell intercalation

Aishwarya Venkataravi, Mikiyas Daniel, Dmitri S.Koklysh, Chung Yuen Hui and Ian G.Macara

Dept of Cell & Developmental Biology, Vanderbilt University School of Medicine, Nashville TN 37240, Vanderbilt Institute of Nanoscale Science & Engineering, Vanderbilt University School of Engineering, Nashville TN 37212 , Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca NY 14853

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 behaviours 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. Evidence from previous studies and preliminary data suggest that ZO-1-actin interactions might be crucial for intercalation. We are also addressing 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. This work was supported by NIH grant GM070902 to IM from the DHHS

P32

Investigating the roles of the Pdx1 C-terminal protein interaction domains during pancreas development

Anthony Wokasch, Jennifer Fuhr, Scott Soleimanpour, Doris Stoffers, Maureen Gannon

Vanderbilt University, Nashville, TN, 37232 ; University of Michigan, Ann Arbor, MI, 48109 ; University of Pennsylvania, Philadelphia, PA, 19104

Baseline beta-cell mass is established early in life through two key events: embryonic beta-cell differentiation and peri-natal beta-cell proliferation. A key member of the transcriptional network guiding these processes is the homeobox transcription factor pancreatic and duodenal homeobox 1 (Pdx1). Global loss of Pdx1 results in pancreatic agenesis while beta-cell specific Pdx1 inactivation during embryogenesis results in decreased beta-cell proliferation and thus decreased beta-cell mass at birth. Many transcription factors contain domains that mediate physical interactions with other transcriptional regulators and post-translational modifiers, that directly affect target gene selection, protein stability, or function. Pdx1 cooperates via its C-terminus with the onecut transcription factor Oc1 in multipotent pancreatic progenitor cells to promote endocrine specification and differentiation by activating expression of the master endocrine transcription factor neurogenin 3. Furthermore, the E3 ubiquitin ligase substrate adapter protein, speckle-type POZ protein (SPOP) also interacts with the Pdx1 C-terminus to promote proteasomal degradation of Pdx1. Importantly, we previously showed that a decrease in Pdx1 is required for beta-cell proliferation. Preliminary data indicate that SPOP and Oc1 interactions with the Pdx1 C-terminus are partially overlapping and potentially competitive, based on domain mapping studies and our observation that Oc1overexpression protects Pdx1 from SPOP-mediated degradation. Using unique in vivo Pdx1 C-terminal mutant mouse models, I am investigating the role of these Pdx1 C-terminal interaction domains at key stages of pancreas development. My preliminary data suggests that deletion of the Pdx1-Oc1 C-terminal interaction domain alone leads to severe pancreas hypoplasia and a significant decrease in endocrine differentiation and proliferation.

P33

Atf4 protects islet β-cell identity and function under acute glucose-induced stress but promotes b-cell failure in the presence of free fatty acid

Mahircan Yagan1,2, Sadia Najam1,2, Ruiying Hu1,2, Yu Wang3, Mathew Dickerson4, Prasanna Dadi4, Yanwen Xu1,2,5, Alan J. Simmons1,2,5, Roland Stein4, Christopher M. Adams6, David A. Jacobson4, Ken Lau1,2,5, Qi Liu3, and Guoqiang Gu1,2,*

1, Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. 2, Center for Stem Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. 3, Department of Biostatistics and Center for Quantitative Sciences, Vanderbilt Medical Center, Nashville, TN37232, USA. 4, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. 5, Epithelial Biology Center, Vanderbilt Medical Center, Nashville, TN 37232, USA. 6, Division of Endocrinology, Diabetes, Metabolism and Nutrition, Mayo Clinic, Rochester, MN 55902, USA.

Glucolipotoxicity, caused by combined hyperglycemia and hyperlipidemia, results in β-cell failure and type 2 diabetes via cellular stress-related mechanisms. Activating transcription factor 4 (Atf4) is an essential effector of stress response. We show here that Atf4 expression in β-cells is minimally required for glucose homeostasis in juvenile and adolescent mice but it is needed for β-cell function during aging and under obesity-related metabolic stress. Henceforth, Atf4-deficient β-cells older than 2 months after birth display compromised secretory function under acute hyperglycemia. In contrast, they are resistant to acute free fatty acid-induced dysfunction and reduced production of several factors essential for β-cell identity. Atf4-deficient β-cells down-regulate genes involved in protein translation. They also upregulate several lipid metabolism or signaling genes, likely contributing to their resistance to free fatty acid-induced dysfunction. These results suggest that Atf4 activation is required for β-cell identity and function under high glucose. But Atf4 activation paradoxically induces β-cell failure in high levels of free fatty acids. Different transcriptional targets of Atf4 could be manipulated to protect β-cells from metabolic stress-induced failure.

P34

The Interaction of WDR5 with MBIP is Necessary for the Formation of the Transcriptional Coactivator ATAC Complex

Vincent Yao, Soumita Goswami, Lauren Lucas, Gregory C Howard, Brian Grieb, and William P Tansey

Vanderbilt University, Nashville, TN 37235; Vanderbilt University Medical Center, Nashville, TN, 37232

WD40 repeat Domain 5 (WDR5) is a highly-conserved protein hub involved in gene regulation that has attracted significant interest as an anti-cancer drug discovery target. WDR5 participates in multiple tumorigenic processes such as scaffolding histone modifying complexes and recruiting the MYC oncoproteins to stimulate expression of half the ribosomal protein genes (RPGs). Small molecules targeted to block WDR5's arginine-binding cavity known as the WIN-site have been successful at slowing or killing cells of several cancer types. In collaboration with the Fesik Laboratory our laboratory has iteratively developed and validated WIN-site inhibitors (WINi) for the past decade to have picomolar affinity for WDR5, be orally bioavailable, and ready for IND-enabling safety studies. Despite the emerging potency, progress, and promise of WINi, these potential cancer therapies may be ready for clinical vetting before their mechanism of action is fully understood. While our laboratory has established that WINi rapidly evict WDR5 and MYC from chromatin at RPG loci, reduce target RPG expression two-fold, induce nucleolar stress, and activates p53-dependent cell death, these outcomes are still inadequate to describe the effects of WINi. We do not know how many of these changes are a direct result of disrupting WDR5 protein interactions or a secondary event. Also, we do not know what proteins tether WDR5 to chromatin at the RPG loci. One potential chromatin-bound home for WDR5 is the histone acetyltransferase ADA-two-A-containing (ATAC) complex which acts as a transcriptional co-activator. Subunits in the ATAC complex contain multiple chromatin recognition domains and co-localize to the same RPG loci where MYC interacts with WDR5 to express RPGs. We propose that an ATAC holocomplex with WDR5 is required to recruit MYC and express RPGs. Despite their colocalization, WDR5 may still facilitate MYC target gene expression through a different complex. Our goal is to determine whether an uninhibited WDR5 WIN site is required for ATAC complex formation and function. In silico Alphafold predictions identified MBIP as a high confidence WDR5 WIN site interactor within ATAC. The WIN site motif of MBIP is highly conserved in eukaryotes. Meanwhile YEATS2 and KAT14 of ATAC are also predicted to be a high confidence WDR5 interactors. Mutating the WIN motif of MBIP disrupts the interaction of WDR5 with MBIP. Rapid degradation of MBIP with a dTAG motif leads to the loss of WDR5 interacting with ATAC components in immunoprecipitations. Pharmacologically blocking the WIN site of WDR5 also disrupts WDR5 interactions with ATAC

"An expert is a person who has made all the mistakes that can be made in a very narrow field."

Niels Bohr

The End