2023 CELL DYNAMICS SYMPOSIUM

Hosted by Cell and Developmental Biology

MAY 18 -19

George Langford, Ph.D.
Syracuse University

Erika Matunis, Ph.D.
Johns Hopkins

Jin Zhang, Ph.D.
UC San Diego

Dyche Mullins, Ph.D.
UC San Francisco, HHMI

Daniel Colón-Ramos, Ph.D.
Yale University

The Company of Biologists

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

THURSDAY: FURMAN HALL
FRIDAY: WILSON HALL
POSTER SESSIONS: KISSAM

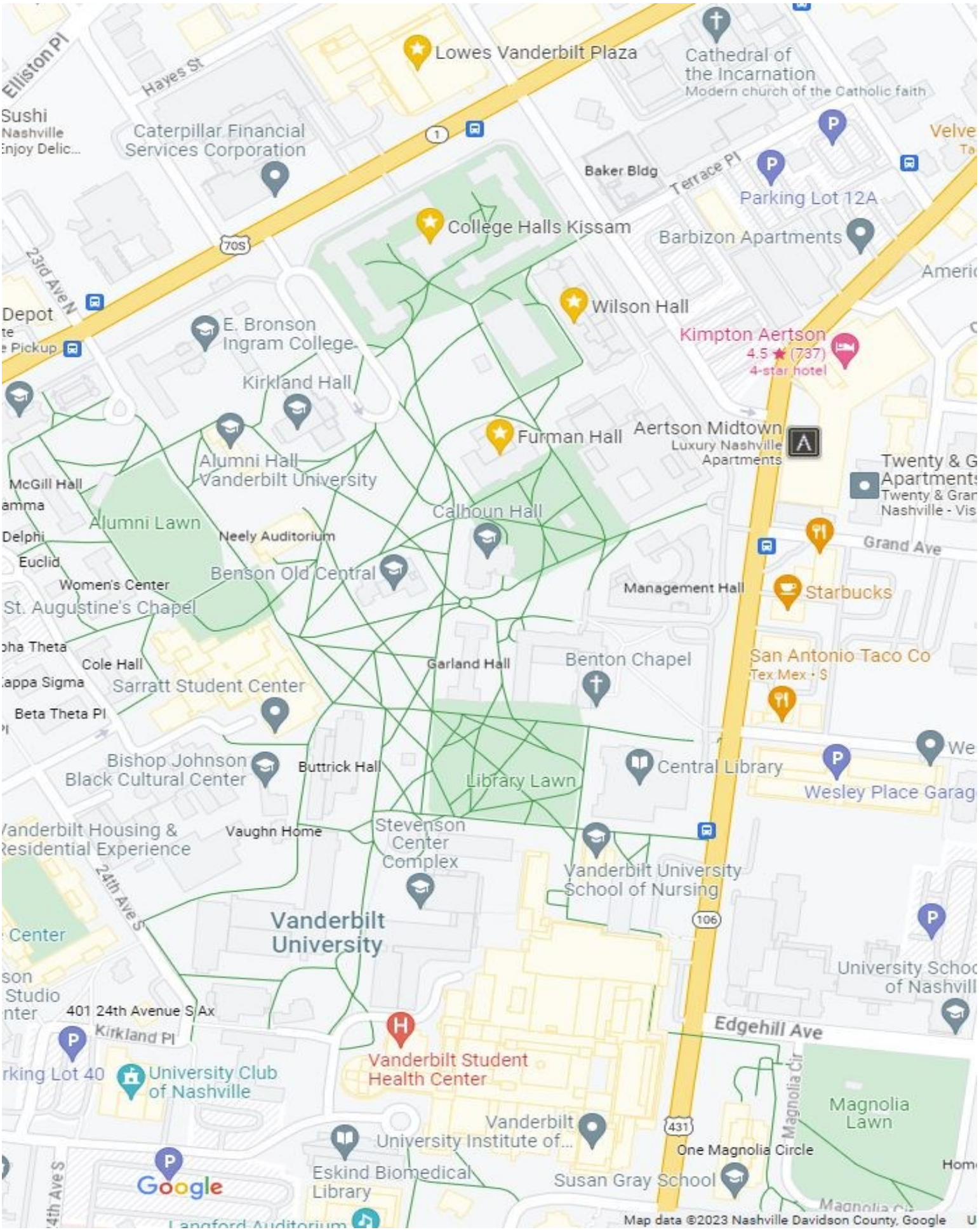
Hosted by: Cell and Developmental Biology, with support from the BRET Office, the Company of Biologists, HHMI Gilliam, EBC, Bio Sci, VBI

Furman Hall

Wilson Hall

Kissam Center

2023 Cell Dynamics Campus Map



2023 Cell Dynamics Symposium

Thursday May 18

3:30 pm Registration / Furman Hall Lobby
4:00 pm Welcome from Ian Macara and Provost Cybele Raver
4:20 pm Daniel Colón-Ramos / 114 Furman Hall
5:30 pm Reception & Poster Session / Kissam Lobby

Friday May 19

8:30 am Coffee & Conversation / Wilson Hall Lobby
9:00 am Welcome / 103 Wilson Hall
9:10 am Dyche Mullins / 103 Wilson Hall
10:00 am Presentations by Achievement Award Winners / 103 Wilson Hall

Caroline Cencer (Tyska lab)
Bhawik Kumar Hain, Ph.D. (Graham lab)
Indrayani Waghmare, Ph.D. (Page-McCaw lab)

10:45 am Coffee Break / Wilson Hall Lobby
11:00 am Diversifying Cell Biology / 103 Wilson Hall
Session moderated by André L. Churchwell, M.D., Vice Chancellor for Outreach, Inclusion and Belonging and Chief Diversity Officer for Vanderbilt University.
Presentations by George Langford and Daniel Colón-Ramos
12:30 pm Lunch / Moore Great Room
12:30 pm Roundtables with Speakers / Kissam 210
1:30 pm Poster Session / Kissam Lobby
2:45 pm Erika Matunis / 103 Wilson Hall
3:45 pm Coffee Break / Wilson Hall Lobby
4:10 pm Jin Zhang / 103 Wilson Hall
5:10 pm Conclusion of Symposium

Oral Presentations

“Molecular Mechanisms of synaptic assembly and function: Lesson from C.elegans”

Daniel Colón-Ramos, Ph.D

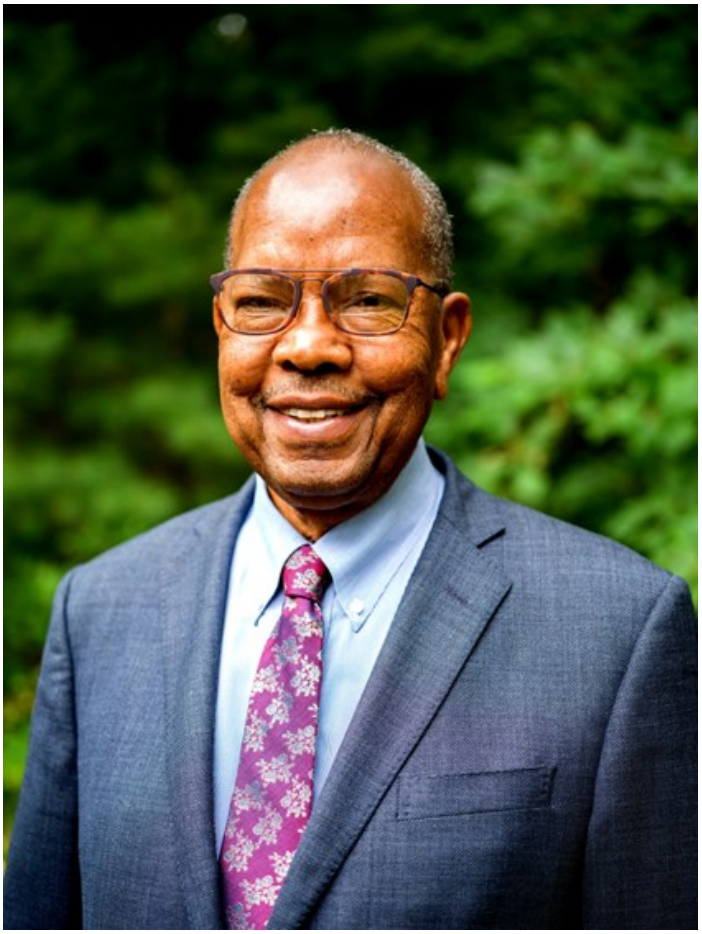
Dr. Colón-Ramos’ lab is interested in how synapses are precisely assembled to build the neuronal architecture that underlies behavior. To address this, they developed tools in the thermotaxis circuit of *C. elegans*.

“Prime Moves of Cell Biology: The most ancient molecular motors and how they work”

Dyche Mullins, Ph.D.

Dr. Mullins studies the assembly and regulation of cytoskeletal networks—collections of molecules used by living cells to move molecular cargo, establish polarity, and propel themselves forward. Understanding how cells construct their internal molecular "skeletons" is key to understanding a wide variety of biological processes and human diseases.

"Inclusivity in cell biology: cultural identity and the power of authenticity"



George Langford, Ph.D.

Research in the Langford lab is focused primarily on the cell and molecular biology of the actin cytoskeleton in health and disease. We study the function of the actin cytoskeleton in organelle/vesicle transport in axons of nerve cells, and cell migration of human epithelial cells.

Vice Chancellor for Outreach, Inclusion and Belonging and Chief Diversity Officer André Churchwell, M.D.

Andre Churchwell, M.D. Dr. André L. Churchwell is the Vice Chancellor for Outreach, Inclusion and Belonging and Chief Diversity Officer for Vanderbilt University. He is the inaugural Levi Watkins, Jr., M.D. Chair and previously served as the chief diversity officer for Vanderbilt University Medical Center and senior associate dean for diversity affairs in the Vanderbilt University School of Medicine. Churchwell is a Professor of Medicine (Cardiology), Professor of Radiology and Radiological Sciences, and Professor of Biomedical Engineering.

"Finding your niche - Stem Cell Dynamics in the Drosophila Testis"

Erika Matunis, Ph.D.

Dr. Matunis' research focuses on how stem cells renew and differentiate. She uses the fruit fly testis as a model system, studying the molecular signals that control whether germ line stem cells form more stem cells or become sperm. Her team recently discovered that cells transitioning into sperm can be redirected back in to stem cells.

"From variant to function in nervous system disorders: new approaches for deploying human stem cell models"

Jin Zhang, Ph.D.

Dr. Zhang's laboratory is interested in understanding the molecular mechanisms and functional roles of signaling enzymes such as protein kinases and phosphatases. They seek a fundamental understanding of spatiotemporal regulation of cell signaling.

Achievement Award Winners

Indrayani Waghmare, Ph.D. (*Page-McCaw)*
Indrayani joined the lab as a postdoctoral research fellow in 2016. She is studying how secreted ligands are distributed from the source cells to target cells in the extracellular space to orchestrate development. She received her bachelors of science degree in 2009 from Hislop College majoring in chemistry, zoology, and biotechnology. She earned a masters degree in molecular biology from Umea University, Sweden, in 2011. She earned her Ph.D. at the University of Dayton, Ohio, in 2016, working under the mentorship of Dr. Madhuri Kango-Singh where she studied alterations in inter-cellular signaling during tumor development using fly epithelial tumor models.

Bhawik Kumar Jain, Ph.D. *(Graham)*
Bhawik is from Mumbai, India. He received his Ph.D. from ACTREC, Mumbai in Life Sciences. His Ph.D. thesis focused on understanding the Golgi cisternal stacking mechanism in budding yeast Pichia Pastoris. Bhawik joined the Graham Lab in 2019 and exploring the structure function relationship of P4-ATPases. In his free time, he enjoys spending time with family, cooking, and playing cricket and tennis.

Caroline Cencer *(Tyska)*
PhD candidate studying what it takes to build a functional gut from the ground up. She is investigating the role of the calcium-dependent CDHR2 and CDHR5 adhesion proteins in the formation of the intestinal brush border. Investigating how actin-supported microvilli merge to form clusters through fixed and live cell imaging techniques will help elucidate early steps of brush border formation. She is passionate about translating science from the lab bench to the public, engaging more of the population via interpretable and relatable science communication methods.

Poster Presentations
Thursday May 18

1. Deronisha Arceneaux
2. Monica Brown
3. Alisa Cario
4. Anna Cassidy
5. Mary Chalkley
6. Clair Cross
7. Swapneeta Date
8. Andrew Dixson
9. Eric Donahue
10. Adam Ebert
11. Gaomin Feng
12. Loic Fort
13. Casey Gailey
14. Kakali Ghoshal
15. Sarah Glass
16. Junmin Hua
17. Jason Hughes
18. Oleg Kovtun
19. Paola Molina
20. Brandon Baer
21. Paige Vega

P1

Succinate driven Atoh1-independent tuft cells are derived from absorptive cell lineage

Deronisha Arceneaux[1], Amrita Banerjee, Joey Simmons[1], Yanwen Xu[1], Naila Tasneem[1], Mirazul Islam[1], Janney Wang, Yilin Yang[1], Bob Chen, Ken Lau[1]

[1]Cell and Developmental Biology

Atoh1 is known to be essential for the development of intestinal secretory cells such as goblet cells and enteroendocrine cells however, its role in tuft cell development remains unclear. Tuft cells are specialized epithelial cells that are found in the mucosal lining of various organs, including the respiratory tract, gallbladder, and intestine. These cells are characterized by apical tufts of microvilli and are involved in sensing and responding to different stimuli, with research indicating their roles in inflammation regulation, secretion of antimicrobial peptides, and modulation of the gut microbiota. Moreover, tuft cells have been implicated in the development and progression of intestinal diseases, including inflammatory bowel disease and colorectal cancer. Recent research from our lab has revealed the existence of a lineage of tuft cells, called induced tuft cells, which are triggered by the microbiome and are independent of Atoh1. Using immunofluorescence microscopy staining for DCLK1 on succinate-treated Atoh1-knockout mice, we have identified tuft cells, which were corroborated with single-cell RNA sequencing (scRNA-seq) data that identified two separate populations of tuft cells with distinct differentially represented gene programs. Furthermore, scRNA-seq analysis of cells obtained from CRISPR-cas9 barcoded mice revealed that tuft cells had identical mutations to enterocytes, suggesting shared lineage with enterocytes. In future research, we plan to investigate the link between induced tuft cells and the microenvironment by studying the anti-inflammatory properties of PGD_2 in ileitis.

Colonic precancerous organoids transcriptionally drift during routine passaging

Brown, M.E.[1,7], Simmons, A.J.[1,7], Bechard, M.E.[5], Tasneem, N.[1,7], Rolong, A.[1,7], Irudayam, M.J.[5], Ramirez-Solano, M.A.[6], Liu, Q.[6,8], Coffey, R.J.[5], Shrubsole, M.J.[3,4], Lau, K.S[1,2,3,7,8]

[1]Epithelial Biology Center, [2]Department of Surgery, [3]Vanderbilt-Ingram Cancer Center, [4]Department of Medicine, Division of Epidemiology, [5]Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, [6]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37232,[7]Department of Cell & Developmental Biology, [8]Center for Quantitative Sciences, Vanderbilt University School of Medicine, Nashville, TN 37232

Patient-derived organoids are a promising model used to study both homeostatic and disease states of different tissues, such as precancerous colon lesions. Using precancerous lesions to generate organoids has provided valuable insight into the mechanisms behind cancer development, but little study has been focused on whether organoids are truly able to recapitulate and maintain *in vivo* genetic, transcriptional, and morphological characteristics[1-3]. We utilized tissue from the three main precancerous subtypes – adenomatous, hyperplastic, and sessile serrated lesions (SSL), in addition to matched noncancerous tissue to develop organoids and analyze their transcriptional profiles using single cell RNA-sequencing. Adenomatous derived organoids maintained a more 'stem-like' expression profile, while the hyperplastic and SSL derived organoids maintained a more differentiated expression profile consistent with *in vivo* tissue findings[2]. In short-term culturing, the precancerous organoids were able to maintain their underlying tissue-derived identity, but as the organoids were further passaged, this identity drifted. Hyperplastic and SSL organoids began to converge in transcriptional similarities, while the non-cancerous matched tissue trended toward a hyperplastic or SSL profile during long-term passage. Altogether, this indicates that adenomatous, hyperplastic, and SSL organoids are able to maintain their identity during short-term culturing, but long-term culturing changes the profile of both pre-cancerous and noncancerous tissue-derived organoids indicating potential challenges in use of this model.

References

1. Leggett B, Whitehall V. (2010) Role of the Serrated Pathway in Colorectal Cancer Pathogenesis. Gastroenterology 138, 2088–2100. doi: 10.1053/j.gastro.2009.12.066. PMID: 20420948

2. Torlakovic EE, Gomez JD, Driman DK, Parfitt JR, Wang C, Benerjee T, Snover DC. (2008) Sessile Serrated Adenoma (SSA) vs. Traditional Serrated Adenoma (TSA). Am J Surg Pathol 32, 21. doi: 10.1097/PAS.0b013e318157f002. PMID: 18162766

3. Zhou YJ, Lu XF, Chen H, Wang XY, Cheng W, Zhang QW, Chen JN, Wang XY, Jin JZ, Yan FR, Chen H, Li XB. (2023) Single-cell Transcriptomics Reveals Early Molecular and Immune Alterations Underlying the Serrated Neoplasia Pathway Toward Colorectal Cancer. Cell Mol Gastroenterol and Hepatol 15, 393–424. doi: 10.1016/j.jcmgh.2022.10.001. PMID: 36216310

P3

Paralog-specific role of CLASP2 in the Golgi cargo export

Alisa Cario, Briahnah Streeter, Anneke Sanders, Irina Kaverina

CLIP-associated proteins (CLASPs) are microtubule associated proteins (MAPs) that belong to microtubule plus-end tracking proteins (+TIPs) and have known roles in cell division, cell motility, and microtubule stabilization. Interestingly, CLASPs are also known to localize to the Golgi and nucleate Golgi-derived microtubules. However, the role of CLASPs in microtubule-based cargo export from the Golgi remains unclear. Furthermore, there are two CLASP paralogs, CLASP1 and CLASP2, and their distinct functions are largely unknown. Here, we study the independent effects of CLASP1 and CLASP2 paralogs on cargo export through use of Erv29-dependent secretory cargo (ES-Cargo) assay using paralog specific knockout cell lines. We find that knockout of CLASP2 impedes cargo export while knockout of CLASP1 does not. Yet, we do not find large scale differences in the microtubule or Golgi morphology. We are currently investigating the mechanism behind this CLASP2 specific effect. Overall, our work reveals important distinctions between the functions of CLASP1 and CLASP2 as well as a role for CLASP2 in regulation of cargo export.

Investigating the role of the GTP-cap in the regulation of microtubule dynamics in mammalian cells

Anna Cassidy[1], Veronica Farmer[1], Göker Arpag[1] and Marija Zanic[1,2,3]
[1]Department of Cell and Developmental Biology
[2]Department of Chemical and Biomolecular Engineering
[3]Department of Biochemistry
Vanderbilt University, Nashville, TN USA

Microtubules are dynamic cytoskeletal polymers essential for cell division, cell motility, and intracellular transport. To execute a multitude of cellular functions, microtubule networks are remodeled via tight regulation of microtubule dynamics. An important feature of microtubule dynamics is dynamic instability—the ability of microtubules to rapidly transition 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. Loss of this stabilizing GTP-cap exposes the unstable GDP-tubulin lattice, leading to catastrophe. Although the GTP-cap is thought to protect the growing microtubule end, whether the GTP-cap size affects microtubule stability is not known. Notably, a family of microtubule end binding proteins, EBs, were recently found to recognize the nucleotide state of tubulin, and can thus be used as a proxy for the GTP-cap. Indeed, previous work from our lab demonstrated that EB comet size increases with increasing microtubule growth rates, achieved using microtubule polymerase XMAP215 in an *in vitro* reconstitution system using purified proteins. Surprisingly, the rate of microtubule catastrophe also increased, in spite of 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 growth rate in LLC-PK1 cells to that measured *in vitro*. We find that the scaling between the EB comet size and the microtubule growth rate is the same both in cells and *in vitro*. However, we observe more variability in microtubule growth rates measured in cells than *in vitro*. We also measure the loss of the EB comet intensity at the onset of catastrophe in cells. Our data reveal that, in spite of the increased complexity of the cellular vs. *in vitro* environment, the GTP-cap size is similarly modulated in response to microtubule growth rate in both contexts. Furthermore, our results emphasize the necessity to investigate the role of the GTP-cap in modulating microtubule stability in cells.

Early Neurodevelopment and Cytoarchitecture is Altered in Tuberous Sclerosis

Mary-Bronwen L. Chalkley[1], Lindsey Guerin[2], Samantha G. Mallahan[1], Asa A. Brockman[1], Laura C. Geben[3], Brittany P. Short[8], Mustafa Sahin[4], Emily Hodges[2,5], Rebecca A. Ihrie[1,6] & Kevin C. Ess[1,6,7]

Departments of (1) Cell & Developmental Biology, (2) Biochemistry, (3) Pharmacology, Vanderbilt University School of Medicine; (4) Department of Neurology, Harvard Medical School; (5) Vanderbilt Genetics Institute and Departments of (6) Neurological Surgery, (7) Neurology, and (8) Pediatrics, Vanderbilt University Medical Center

Tuberous Sclerosis Complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the heart, lungs, kidney, and brain are all hallmarks of the disease, often the most severe symptoms of TSC are neurological, including seizures, autism, psychiatric disorders, and intellectual disabilities. TSC is caused by a heterozygous loss of function mutation in the *TSC1* or *TSC2* genes, which encode the hamartin/tuberin proteins respectively. Hamartin/tuberin function as a heterodimer that negatively regulates mechanistic Target of Rapamycin Complex 1 (mTORC1). While TSC neurological phenotypes are well-documented, it is not yet known how early in neural development *TSC1/2*-mutant cells diverge from the typical developmental trajectory, and whether such phenotypes are seen in the heterozygous-mutant populations comprising the majority of cells in patients.

To examine early neurodevelopmental phenotypes, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) with a heterozygous microdeletion mutation in *TSC2*. Within the field, it is debated whether second hits are required. To model this state, CRISPR was used to create a similar deletion mutation in the other *TSC2* allele, producing a homozygous mutant line. The heterozygous mutant was also corrected to wild type, creating a set of isogenic lines. This isogenic series was compared to another allelic series with *TSC2* deleted.

Using immunofluorescent microscopy, immunoblotting, and flow cytometry, we observed aberrant early neurodevelopment in both sets of *TSC2* mutant iPSCs. Homozygous mutant neural progenitors exhibit altered behavior as *in vitro* differentiation proceeds, including changes in multicellular structures within the first 10 days with misexpression of key transcription factors associated with lineage commitment. Collectively, these data suggest that mutation or loss of *TSC2* has early effects on gene expression in proper neural development. Indeed, our preliminary studies have found that DNA methylation is changed with some key genes in neurodevelopment being hypermethylated in the wild type cells and hypomethylated in *TSC2* mutant cells. Understanding precisely when development is disrupted *in TSC1*/2- mutant brain will be essential to tailoring treatment and determining whether prenatal treatment should be pursued.

T cell metabolic profiling stratifies immunodeficiencies in patients with Inborn Errors of Immunity

Claire E. Cross, Todd Bartkowiak, Saara Kaviany, Madeline J. Hayes, James A. Connelly, Jeffrey C. Rathmell, Jonathan M. Irish

Background: Current cell clustering algorithms, such as FlowSOM and DBSCAN, seek to identify cell subsets based on density within a space defined by selected phenotypic markers, such as CD markers or transcription factors. Functional markers, such as metabolism, signaling, and proliferation markers, are typically excluded from phenotypic clustering and then analyzed after cell types are identified. However, this approach can overlook rare, mechanistically distinct cell subsets present within larger, well-established populations. Here, we tested the hypothesis that clustering based on metabolic profiles would reveal clinically relevant subpopulations of canonical T cell subsets.

Methods: To identify cells with a specified metabolism profile, we modified the T-REX algorithm (Barone et al., *eLife* 2021) to seek three metabolic (MET) signatures. Specifically, the algorithm identified cells with high glucose usage (MET1, based on GLUT1 and GLUT3 expression), high fatty acid oxidation (MET2, based on CPT1a expression), and/or high electron transport chain use (MET3, based on GRIM19, CYTOC, and ATP5a expression). These three MET profiles were sought within a t-SNE map based on measurements of 32 cell surface proteins that comprehensively defined canonical T cell subsets. Neighborhoods of T cells that were phenotypically similar to one or more of the three MET profiles were highlighted, assigned to a cluster, and characterized using marker enrichment modeling (MEM, Diggins et al., *Nature Methods* 2017). Patients were then classified based on the percentage of cells that were observed to be high for one of the three MET categories using the statistical method established in the RAPID algorithm (Leelatian et al., *eLife* 2020). This metabolic profiling approach was applied to mass cytometry data from samples of peripheral blood mononuclear cells from 45 patients diagnosed with an Inborn Error of Immunity (IEI) at Vanderbilt University Children's Hospital. Samples were collected by the Human Immunology Discovery Initiative (HIDI) team with IRB approval and informed consent in accordance with the Declaration of Helsinki.

Results: T cell clusters identified by metabolic profiling were compared to clusters identified with traditional FlowSOM or DBSCAN clustering. Clusters were evaluated for 1) whether the cluster contained a distinct metabolism profile and 2) whether the cluster stratified risk of positivity for any of six scored immune pathologies. Metabolically distinct subsets of canonical T cell populations were closely linked to clinical pathologies. For example, cells in clusters that were $MET1^{hi}$ expressed T cell activation markers ($CD44^{+10}$ $CD95^{+9}$ $CD38^{+9}$), and the associated patients were more often found to have immune deficiencies than other patients ($p<0.002$). The finding that $MET1^{hi}$ T cells were activated is consistent with observations of activated T cells highly expressing glycolytic proteins alone or in combination with other metabolism proteins (Palmer et al., *Frontiers* 2015; Levine et al., *Immunity* 2021). There was no association between patients with a high percentage of either $MET2^{hi}$ or $MET3^{hi}$ clusters and any of the 6 identified immune pathologies. Additionally, clustering patients based on a combination of $MET1^{hi}$, $MET2^{hi}$, and $MET3^{hi}$ status did not stratify immune pathology.

Conclusions: Neighborhoods of cells with elevated expression of proteins involved in glucose metabolism stratified immunodeficiencies in IEI patients. This approach can now be applied to myeloid and B cell mass cytometry data collected on the same patients. Going forward, this analysis approach could be adapted to locate rare cells using a pre-defined phenotype, such as healthy or malignant stem cells, regulatory immune cells, and cells with distinct phospho-protein signaling profiles.

P7

Vanderbilt Center for Technology Transfer and Commercialization: Driving innovations to market, promoting collaborations with industry, and supporting entrepreneurs in launching new ventures.

Presenter: Swapneeta Date, Ph.D. Assistant Director, Life Sciences Collaborations

The Vanderbilt Center for Technology Transfer and Commercialization's mission is to provide professional commercialization services to the Vanderbilt community, thus optimizing the flow of innovation to the marketplace and generating revenue that supports future research activities while having a positive impact on society. CTTC accomplishes this by serving as an efficient and effective conduit for the transfer of promising intellectual property to industry, contributing to regional economic development by licensing locally and supporting new venture creation, and encouraging greater translational research collaborations between academia and industry.

The Industry Collaborations team identifies, cultivates, and solicits partnerships between industry and life science academic departments.

In FY22, CTTC generated over $100 million in licensing revenue—quadruple its annual average—reviewed more than 1,000 material transfer agreements and facilitated nearly 300 U.S. patent applications.

CTTC continues to seek out new ways to bring services and support to Vanderbilt researchers and the Vanderbilt community.

Developing a cell culture model for detecting RNA-binding proteins inside endosomes

Andrew Dixson and Alissa Weaver

Exosomes are membrane-bound extracellular vesicles (EVs) that form within the endosomal network and are secreted into the extracellular space. Prior evidence suggests that RNA-binding proteins (RBPs) are packaged into EVs in conjunction with RNAs, and that RBP:RNA packaging depends on cell culture conditions. There is a need for a microscopy-based assay to assess entry of RNA-binding proteins (RBPs) into the lumen of endosomes, where they are presumably packaged inside of exosomal vesicles. Our approach is to localize RBPs in relation with endosomal markers in a variety of cell lines, with the goal of identifying culture conditions under which RBPs are present inside exosomes. Since prior research has found that stress granule RBPs are also found in EVs, we tested whether cell stress affects RBP localization to endosomal compartments. Our initial studies did not find a relationship between cell stress and RBP localization to endosomes marked by Rab7 or CD63. We then tested whether RBPs are present inside of endosomes artificially enlarged through expression of a constitutively active (GTPase-dead) Rab5 mutant protein. In HEK293FT cells, endosomes marked by this Rab5 mutant contained YBX1 and CD63; additionally, YBX1 and CD63 appeared to reside on different intralumenal vesicles within the same Rab5 mutant endosomes. Other RBPs, such as Ago2 and SafB, were detected but only at low abundance inside Rab5 mutant endosomes in HEK293FT cells. Future studies will identify culture conditions that alter YBX1 colocalization with endosomal compartments and ultimately identify factors required for inducible secretion of YBX1:RNA complexes in exosomes.

Longevity interventions protect the endoplasmic reticulum against age-related decline.

Eric KF Donahue, Alexandra G Mulligan, Kristopher J Burkewitz
Vanderbilt University, Nashville, TN, 37027

Cardiovascular disease, cancer, and neurodegeneration are the leading causes of morbidity and mortality in our aging population. Their frequent comorbidity and increasing prevalence emphasize the therapeutic value of understanding and targeting their shared risk factor—the aging process itself. Aging is driven by the progressive failure of processes such as proteostasis, mitochondrial function, and intercellular communication. These processes are coupled with endoplasmic reticulum (ER) subdomains, leading to our hypothesis that ER morphology regulates age-related decline. However, it is unknown whether the ER changes with age. Using TEM and *in vivo* imaging of *C. elegans*, we found that both intestinal and epidermal ER are depleted during early aging, and autophagy is necessary for this decline. Because many longevity interventions improve ER function, we hypothesized that longevity is associated with ER preservation. Accordingly, dietary restriction and inhibition of insulin/insulin-like signaling improved cellular ER content and protein expression, exhibiting structural and functional ER protection with age. Finally, we manipulated ER morphology to protect against age-related mitochondrial fragmentation, experimentally linking ER form and function with the aging process. Overall, we show that the ER is significantly depleted by autophagy with age, and long-lived animals are protected against this decline. These studies reveal a central role for the ER in early aging and establish ER structure as a potential target for aging interventions.

P10

Role of Sphingolipids in Age-Related Mitochondrial Dysfunction:

Adam Ebert, Nathaniel Hepowit, Robert Dickson, Jason MacGurn

Sphingolipids (SL) are an important structural component of cellular membranes. In addition, they function in cell signaling pathways and cell fate decisions. Despite their essential function a growing number of studies have linked sphingolipid accumulation to the pathology of cardiovascular diseases such as ischemic heart disease. Specifically, it was reported that ceramides, a type of sphingolipid, accumulate in the mitochondria of ischemic rat cardiomyocytes causing mitochondrial swelling and inflammation.

Myriocin is a potent inhibitor of sphingolipid biosynthesis that has been shown to increase the lifespan of various model organisms. However, the mechanism by which myriocin extents lifespan remains unknown. Herin, we have found that yeast mitochondria in aging cells undergo a transition from tubular mitochondria to an aberrant swelled phenotype, and furthermore that treatment of these cells with myriocin prevents mitochondrial swelling. To understand how myriocin may be preserving mitochondrial morphology and function we studied mitophagy as a mitochondrial quality control mechanism. We found that aging cells treated with myriocin have substantially higher levels of mitochondrial proteins in vacuole lumens suggesting that sphingolipid depletion may trigger an autophagic mechanism. Further, we observed that mitochondrial contact sites with vacuoles and the ER are elevated in myriocin treated cells. We hypothesize that sphingolipid accumulation may be responsible for the mitochondrial swelling phenotype, and that depletion of sphingolipids may restore mitochondrial quality control mechanisms lost in aging cells.

The InsP3R coordinates mitochondrial stress adaptation to promote longevity

Gaomin Feng, Erica Olfson, Elizabeth Ruark, Kristopher Burkewitz

Mitochondrial function plays a central role in aging and age-onset diseases. While mild inhibition of mitochondrial function can extend lifespan from yeast to mammals, more severe disruption is toxic. In order to therapeutically leverage the beneficial effects of mitochondrial reprogramming, we must therefore understand the mechanisms controlling this delicate balance between mitochondrial protection and dysfunction. Using a *C. elegans* model, we discover that lifespan extension via mild inhibition of electron transport chain (ETC) requires the inositol triphosphate receptor (InsP3R), a conserved calcium release channel on the endoplasmic reticulum (ER) surface. As a key component of inter-organelle communication between ER and mitochondria, the InsP3R mediates calcium flux into the mitochondrial matrix where calcium activates multiple bioenergetic enzymes. Although we find that the InsP3R indeed stimulates bioenergetic tone in invertebrates during both normal and stress contexts, surprisingly, mitochondrial calcium import is not required for longevity. This finding led us to hypothesize that the InsP3R promotes adaptation to mitochondrial stress via additional, matrix-independent pathways, and we have now identified multiple routes by which InsP3R function coordinates mitochondrial health and longevity. First, we have identified that the InsP3R plays novel roles in regulating retrograde transcriptional responses and expression of genes associated with mitochondrial metabolic functions. Secondly, the InsP3R regulates age-dependent mitochondrial dynamics in *C. elegans*, and we demonstrate that this latter role for the InsP3R is essential for its effects on mitochondrial-mediated longevity. Loss of InsP3R function leads to an increasingly hyperfused mitochondrial network in aging animals. Restoring the balance in mitochondrial fission/fusion dynamics by promoting fission is sufficient to rescue longevity in InsP3R mutants. Collectively, these results suggest that the InsP3R plays a central role in reprogramming mitochondrial functions and promoting longevity when ETC function is impaired.

Intracellular cell tension regulates stem cell conversion to the mesoderm lineage downstream of the apoptotic pathway

Loic Fort and Ian G Macara
Cell and Developmental Biology Department, Vanderbilt University, School of Medicine, Nashville, TN 37240 USA

The heart is the first functional organ to form during morphogenesis and failure to specify cardiac progenitors can result in severe congenital heart defects, affecting ~1% of all US newborns. Activation of the WNT pathway in human induced pluripotent stem cells (hiPSCs) recapitulates the initial stages of cardiac lineage commitment, driving sequential expression of primitive streak, mesoderm, and cardiac mesoderm markers. In parallel, cells undergo an epithelial-to-mesenchymal transition (EMT), similar to gastrulation in vivo, during which drastic morphological changes occur, including tension-dependent apical constriction. While the relations between EMT and lineage specification have been widely studied, our understanding of the connection between cell shape and lineage identity remains poor.

During development, changes in cell morphology can be the result cell division orientation, increase of cell number in a constrained space and active contractile forces, together driving morphogenesis. The acto-myosin cytoskeleton, a highly dynamic network of proteins that maintain cell morphology, is a crucial interface to translate physical signals into internal biochemical responses. While geometrical organization of "gastrulation-like" morphogenesis has been shown to affect WNT signaling and cell fate specification, it remains unclear how, mechanistically, cells communicate and integrate the state of their environment to coordinate developmental responses at the tissue level.

We hypothesize that mechanical forces, such as cell tension, regulate mesodermal identity in hiPSCs. Pharmaceutical inhibition of ROCK to reduce tension during differentiation, increases mesoderm gene expression. On the other hand, overexpression of a constitutively active ROCK2 to increase tension totally blocks the conversion of hiPSCs to the cardiac mesoderm. These cells also maintain their epithelial features, suggesting a blockage of the EMT, a crucial step during lineage specification. Probing for a mechanism, we investigated the effect of the apoptosis pathway. We previously published a crucial role for apoptosis during stem cell commitment to the mesoderm lineage. Surprisingly, while cells treated with apoptosis inhibitor fail to differentiate, adding ROCK inhibitor overcomes this blockage. This epistasis experiment suggests that contractility acts dominantly, downstream of the apoptotic pathway.

Together, our data suggest that apoptosis and contractility work together in balancing mesodermal identity. We are currently investigating the molecular mechanisms linking these two pathways to the genetic reprogramming occurring during mesoderm differentiation.

NCAM-1 promotes synaptic remodeling in developing GABAergic neurons

Casey Gailey[1,2], Leah Flautt[1], Andrea Cuentas-Condori[1], John Tipps[1], Siqi Chen[1], Eleanor Rodgers[4], Seth R. Taylor[1] and David M. Miller, III[1,2,3]

Department of Cell and Developmental Biology[1], Program in Developmental Biology[2], Program in Neuroscience[3], Vanderbilt University, Nashville, TN, USA. Saint Cecilia Academy[4], Nashville, TN, USA

Neural circuits are actively restructured during development as synapses are dismantled in some locations and assembled in others. To investigate the underlying cell biological mechanism, we are exploiting the DD-type GABAergic motor neurons which undergo synaptic remodeling during early larval development. In the newly hatched larva, DD presynaptic boutons are initially positioned on ventral body muscles but are then relocated over a ~5 hr period to connect with dorsal muscles. The conserved homeodomain protein, IRX-1/Iroquois orchestrates DD remodeling. An IRX-1 target, the sodium epithelial channel (ENaC), UNC-8, is upregulated in remodeling DD neurons to trigger a Ca^{2+}-dependent mechanism of presynaptic disassembly. Additional downstream effectors are likely required, however, because UNC-8 dismantles a subset of presynaptic components (RAB-3, v-SNARE, liprin-a, endophilin) whereas IRX-1 also acts in a parallel pathway to remove additional presynaptic proteins (UNC-13, ELKS, Clarinet).

To identify additional remodeling genes, we used single cell RNA-Seq (scRNA-Seq) to profile D-class GABAergic neurons at periodic intervals spanning the remodeling period. Analysis of this data set revealed 93 genes that are transiently expressed in remodeling DD neurons. An RNAi screen detected a necessary role for the neural cell adhesion protein, NCAM-1, in DD synaptic remodeling. A genetic mutant of *ncam-1* impairs both the removal of ventral GFP::RAB-3 and its reassembly at dorsal DD neurites. Interestingly, the *ncam-1* mutant also delays remodeling of CLA-1/Clarinet, an active zone component that is not regulated by UNC-8. Thus, our results suggest that NCAM-1 functions in parallel to UNC-8 to promote DD presynaptic remodeling. Because NCAM functions as a key regulator of synaptic plasticity in mammalian neurons, we are intrigued with the possibility that NCAM-1 drives synaptic remodeling in *C. elegans* in a conserved mechanism that also governs circuit refinement in the developing brain. NIH Funding: 5T32HD007502 (CG), R01NS10695 (DMM).

Epoxygenase *Cyp2c44* regulates hepatic lipid metabolism through improved insulin action

Kakali Ghoshal[1], David Wasserman[1], Suman Pakala[1], Ambra Pozzi[1,2]

[1] Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA;

[2] Department of Veterans Affairs, Nashville, TN, USA

We have previously shown that cytochrome P450 epoxygenase *Cyp2c44*, a major epoxyeicosatrienoic acid (EET)–producing enzyme in mice, leads to impaired hepatic insulin signaling by prolonging the retention of activated insulin receptor on plasma membrane. We observed that global deletion of *Cyp2c44* also contributes to impaired hepatic lipid metabolism. In this study we have shown that, in mice deletion of *Cyp2c44* leads to FATP2/FABP1 mediated fatty acid overload in hepatocytes and livers. Lipidomics data revealed different classes of fatty acids that were differentially regulated in *Cyp2c44* livers. RNA seq data identified >500 genes that are differentially expressed in *Cyp2c44* livers and a substantial of them are genes involved in various lipid metabolic pathways. Fatty acid overload in turn facilitates the accumulation of diacylglycerol (DAG) through various intermediate pathway. We have observed mice lacking *Cyp2c44* have increased hepatic DAG. Excess accumulation of DAG facilitates translocation of PKCδ on plasma membrane eventually attenuating insulin mediated IRS-1/Akt/GSK3β signaling. We observed DAG/ PKCδ mediated loss of IRS-1/Akt/GSK3β signaling in livers and hepatocytes of mice lacking *Cyp2c44*. This could possibly answer how epoxygenase *Cyp2c44* governs hepatic insulin signaling concurrently with lipid metabolism.

Evaluation of dipeptidase-1 as an extracellular vesicle-bound marker for colorectal cancer with informative localization patterns

Sarah E. Glass[1], Zheng Cao[1], Samuel T. Ellis[1], Qin Zhang[1], Dennis K. Jeppesen[1], James N. Higginbotham[1], Matthew E. Bechard[1], Jeffrey L. Franklin[1], Robert Coffey[1*]
[1]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[*]Corresponding author

Colorectal cancer is still the second leading cause of cancer death in the US, highlighting the need for early detection strategies. One promising marker is dipeptidase-1, DPEP1, as it is one of top upregulated genes in both adenomas and colorectal cancers, is not present in normal colonic epithelium, and has been shown to have roles in invasion, proliferation, and metastasis. We sought to characterize DPEP1's release in colorectal cancer for the purpose of developing an informative liquid biopsy modality. We identified DPEP1 as the most abundant protein in extracellular vesicles released from colorectal cancer cell lines by mass spectrometry and determined that it is present in a subset of EGFR+/CD81+ exosomes along with other clinically relevant biomarkers by FAVS analysis. Furthermore, DPEP1+ extracellular vesicle numbers were increased in the plasma from three colorectal cancer patients in comparison to normal controls, underscoring the clinical translatability of our findings. By immunofluorescence, colorectal cancer and adenoma organoids cultured in 3D display one of two distinct localization patterns: apical and luminal or diffuse cytoplasmic staining that is associated with a worse progression free and overall survival for colorectal cancer patients. These two localization patterns also correlate with two different forms of DPEP1 as seen by immunoblot, one of which has been previously uncharacterized. While future studies will involve defining this unexplored form of DPEP1 in terms of secretion and cancer progression, our current results suggest that DPEP1 is an informative marker of extracellular vesicles released from colorectal cancers that could be further developed for diagnostic assessments.

A New Model of Cell-Cell Fusion Mediated by Plasma Membrane Damage and Repair

Junmin Hua[1], Andrew D. Pumford[2], Lilia Nassar[2], James White[1], M. Shane Hutson[2,3], Andrea Page-McCaw[1,4]

[1] Department of Cell and Developmental Biology and Program in Developmental Biology, Vanderbilt University School of Medicine [2] Department of Physics and Astronomy, Vanderbilt University [3] Department of Biological Sciences, Vanderbilt University [4] Vanderbilt-Ingram Cancer Center, Vanderbilt University

Restoration of tissue integrity after injury is essential to the survival of all organisms. We use *Drosophila* pupal notum damaged by laser ablation as a model system to study the wound healing mechanisms. Starting about 10 minutes after laser ablation, mononuclear cells of the pupal epidermis fuse and form multinucleated syncytia to assist with re-epithelialization. The objective of this study is to investigate the mechanisms of wound-induced syncytia formation. Based on our data, we hypothesize that syncytia fuse in response to plasma membrane damage triggered by the wound. All trauma wounds generate a mixture of damage, but pulsed laser ablation creates a highly reproducible pattern of cellular damage and response. An expanding cavitation bubble is generated within microseconds after wounding, exerting shear mechanical force on cells under its footprint, damaging their plasma membranes and allowing an influx of extracellular calcium within milliseconds after ablation. Interestingly, syncytia form after this type of ablation, called single-shot ablation; but syncytia are absent after scanning ablation, when cells are destroyed by a low power laser scanning multiple times over a selected area of cells so that no damaged cells survive. Using Ca^{2+} entry as a proxy for plasma membrane damage in single-shot ablation, we found that approximately 96% of fused cell borders co-localize with sites of Ca^{2+} entry as early as 30 ms after wounding, and all fused cell borders are within the region of plasma membrane damage, as indicated by elevated level of Ca^{2+} within 60 ms after wounding. We thus conclude that plasma membrane damage primes wound-induced cell fusion.

Current models of plasma membrane repair are based on the finding that vesicles fuse with microtears and form membrane patches. We demonstrate that overexpressing the dominant negative form of dynamin, even at a low level, impairs wound-induced cell-cell fusion. Hence, we propose a new model of cell-cell fusion, which, rather than depending on fusogens, is mediated by the plasma membrane remodeling and the contact among membrane patches during the process.

Quantitative RNA-FISH Probe Design

Jason J. Hughes[1], Ben Kesler[1], Gregor Neuert[1]
[1]Department of Molecular Physiology and Biophysics, Vanderbilt University

RNA Fluorescent In-Situ Hybridization (RNA-FISH) is a standard method to detect RNA molecules in cells and tissue at high spatial resolution. Multiplex RNA-FISH has enabled the development of spatial transcriptomic experimental platforms that scientists can use to study the regulation of several RNA species simultaneously. These methods require designing specific probes that hybridize to RNA of interest while minimizing off-target binding that create false-negative or false-positive RNA spots, which is critical in using RNA-FISH to study gene regulatory mechanisms and identify different cell types. However, many factors can affect RNA-FISH probe efficacy, including genome sequence, genome size, secondary probe structure, hybridization temperature, and gene expression differences in cell types and cell lines. Several open-source and commercial probe design software packages aim to address this challenge. However, there has been no comprehensive computational and experimental comparison of their efficacy. Current RNA-FISH probe design software has limitations in designing specific probes due to their assumptions, incomplete consideration of off-target probe binding, and trial and error in the design process leading to use cases where programs may not create enough probes to develop a strong enough signal to detect RNA or make probes with low sensitivity and specificity. To address this, we developed probe design software that models computed genome-wide probe binding affinities to create application-specific high-specificity probe sets. Our software selects FISH probes to use experimentally based on their thermodynamic properties and the likelihood of on/off-target binding. Our software then predicts quantitative metrics for RNA-FISH probe sets, including the probability of observing on-target and off-target spots. By better quantifying off-targets and balancing probe on/off-target binding affinities, our probe designer outperforms existing software in several computational RNA-FISH probe design efficacy metrics to generate probe sets with fewer off-target RNAs and less likely to create off-target RNA spots. We seek to compare our software computationally and experimentally to published RNA-FISH probe design methods for multiple organisms. Our software allows end-users to design FISH probe sets to study various problems, including mature RNA, 5’ vs. 3’ end nascent RNA, intronic RNA, sense-antisense transcription, alternative splicing, allelic variation, and tissue/cell-type specificity.

Capturing and Annotating the Dynamics of Molecules, Organelles, and Cells at CISR: Classical, Feature-based, and Deep Learning Approaches

Oleg Kovtun, Sean Schaffer, Evan S. Krystofiak, Kari Seedle, Tegy J. Vadakkan, Rachel Hart, Maria Vinogradova, Jenny C. Schafer, W. Gray "Jay" Jerome, Matthew J. Tyska

The Vanderbilt Cell Imaging Shared Resource (CISR) is an institutional, fee-for-service, advanced microscopy resource. The CISR provides researchers with access to state-of-the-art imaging equipment and expert technical support for sophisticated microscopy and analysis of tissue and cellular anatomy and physiology. As of 2023, the CISR independently manages 17 advanced optical microscopes, 1 transmission electron microscope and 2 scanning electron microscopes. These instruments and the array of advanced capabilities offered by them are available to support any investigator with an appointment at Vanderbilt University or Vanderbilt University Medical Center. The CISR supports over 200 labs per year (over 400 individual users) for 12,000+ microscope hours per year and 100+ publications associated with these microscope hours. CISR staff offer microscope and image analysis training on both commercial and open-source software as well as support for optimizing imaging, image analysis, and sample preparation protocols.

Here, several case studies are discussed to demonstrate state-of-the-art imaging and image analysis CISR capabilities at the cellular and subcellular level. Case study #1 is an application of the Nikon Total Internal Reflection Fluorescence (TIRF) microscope for detection and 2D trajectory reconstruction of a cell surface protein tagged with monomeric green fluorescent protein (mGFP). Case study #2 features photoactivation localization microscopy (PALM) detection of nanoscopic organization of intracellular proteins tagged with photoconvertible mEos2 and mEos3.2 probes on the Nikon STORM microscope. Case study #3 demonstrates the application of the Nikon Spinning Disc Confocal (SDC) microscope to detect filamentous structure branching with subsequent 3D reconstruction in Imaris. Case study #4 shows the utility of the Nikon Structured Illumination Microscope (SIM) in super-resolution imaging of various organelles. Case study #5 establishes an advanced analysis workflow in Imaris and Arivis to reconstruct and annotate 3D trajectories of endocytic vesicles captured by the lattice light-sheet microscope (LLSM). Additionally, two new technologies will be introduced. The Zeiss Crossbeam 550 Focused-Ion-Beam Scanning Electron Microscope (FIB-SEM) allows volumetric EM of cells and tissue for the highest resolution cellular imaging. The Nikon Primo micropatterning instrument permits control of cell adhesion, spreading, and shape with micropatterning, hydrogel polymerization, and microfabrication.

P19

Genome-wide screens to identify genes that regulate epithelial integrity and extrusion

Paola Molina and Ian G. Macara
Dept of Cell and Developmental Biology, Vanderbilt University School of Medicine

Cell extrusion is a mechanism used by epithelial tissues to maintain homeostasis in the presence of oncogenic signals, cell death, or during overcrowding. However, the mechanisms that regulate extrusion of epithelial cells are not fully understood. Global approaches have not yet been employed to interrogate the collective behavior of cell extrusion. My ~~talk will~~poster will describe two approaches we have developed to better understand the regulation of cell extrusion, using mouse mammary epithelial cells as the model system. The first approach will use a pooled CRISPR library to conduct a genome-wide knockout screen that identifies genes required to maintain epithelial integrity. Loss of any such gene will disrupt cell-cell adhesion and promote escape of the cell harboring the gene deletion from the epithelial monolayer. These extruded cells can be captured and sequenced to identify the gRNA and its target gene. To validate the ~~approach~~approach, I have shown that knockout of E-cadherin results in efficient extrusion of Ecad-negative cells. In the second approach, I am studying the mechanism by which epithelial cell monolayers promote the extrusion of cancer cells. I have adapted a novel method called the G-baton (GFP-based touching nexus) system to identify genes that promote the extrusion from mammary epithelia of cells that harbor a mutant H-Ras oncogene. The system transfers a fluorescent tag from a "sender" cell specifically to neighboring cells with which it is in direct contact ("receiver" cells). This powerful new method will allow us to sort selectively for wild type cells that are touching Ras-transformed cells, then conduct RNAseq analysis to identify transcriptional changes in this subpopulation of wild type cells, as compared to wild type cells that have not been in contact with the cancer cells.

Overexpression of Alveolar Epithelial Tissue Factor Promotes Maintenance of Lung Barrier Integrity in ALI

Brandon Baer[1], Nathan D. Putz[1,2], Noo Ri Lee Han[2], Lorraine B. Ware[1,2], Julie A. Bastarache[1,2,3]

[1]Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, [2]Department of Cell and Developmental Biology, [3]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

Acute Respiratory Distress Syndrome (ARDS) is a common cause of acute respiratory failure. Despite extensive research in animal models, in which the syndrome is called Acute Lung Injury (ALI), no targeted therapy has been found to reduce its high mortality rate. Two major pathologic features of ARDS are loss of lung barrier integrity and activation of the Tissue Factor (TF) pathway of coagulation in the airspace. However, as an integral membrane protein TF also serves several non-coagulant functions including promotion of cell adhesion. All systemic anti-coagulants tested have failed to show clinical benefits in ARDS, with some trials of TF pathway inhibition showing increased mortality in ARDS patients. One explanation for these clinical results is that TF in the airspace is protective in ARDS. Supporting this concept, our previously published mouse work found that loss of alveolar epithelial cell TF caused increased loss of lung barrier integrity in models of ALI. As such, we hypothesize that epithelial TF is necessary for maintaining lung barrier integrity and that its overexpression will be protective in ALI. To determine whether supraphysiologic overexpression of TF in the lung can enhance barrier integrity we created a novel transgenic mouse in which TF was inducibly overexpressed in the lung epithelium (TF^{Epi+}). A human influenza hemagglutinin-tagged TF construct, driven by the CMV-TetO promoter and crossed with SPC-rtTA59 mice was used to produce inducible, lung epithelial-targeted TF overexpressing mice. High alveolar epithelial TF expression compared to wild-type littermates (WT) was confirmed through immunohistochemistry and western blot analysis after one week of doxycycline in drinking water. To induce ALI, mice were intranasally infected with 2000 colony forming units of Klebsiella pneumoniae or PBS. At 24-hours post infection, mice were euthanized, lung tissue was collected, and a bronchoalveolar lavage (BAL) was performed. Animal body weights were recorded pre-, and 24-hours post infection. BAL was analyzed to measure protein, clot time, and leukocyte influx. Lung tissue was utilized to calculate wet-to-dry weight ratios and bacterial burden. TF^{Epi+} mice infected with Klebsiella pneumonia showed lower BAL protein (411.74 vs 251.96 μg/ml; n=12; p=0.0121) and lung wet-to-dry weight ratios (5.22 vs 4.71; n=12; p=0.0575) compared to WT. However, weight loss, bacterial burden, BAL clot time, and BAL inflammatory cell counts did not differ between infected TF^{Epi+} and WT mice. These findings suggest that alveolar epithelial TF overexpression is protective for maintaining lung barrier integrity and a non-coagulate based mechanism, potentially linked to epithelial cell adhesion.

Epithelial, stromal, and microbial remodeling in a TNF-driven mouse model of Crohn's-like intestinal inflammation

Paige N. Vega, Alan J. Simmons, Jiawei Wang, Yanwen Xu, Yilin Yang, James Ro, Amrita Banerjee, Mariah H. Harned, Qi Liu, & Ken S. Lau

Crohn's disease is characterized by chronic inflammation of the gastrointestinal tract and can be modeled by TNF overexpression in the *Tnf*$^{\Delta ARE/+}$ mouse model. We use single-cell RNA-sequencing to profile epithelial and stromal cells in wildtype mice and *Tnf*$^{\Delta ARE/+}$ mice with mild to severe ileal and colonic inflammation. We found an emergence of innate immune cell types and transcriptomic changes in lymphocytes in both stages of inflammation in the ilea and colon of *Tnf*$^{\Delta ARE/+}$ mice. We identified a loss of normal fibroblast populations and an emergence of pro-inflammatory fibroblasts in *Tnf*$^{\Delta ARE/+}$ mice. Absorptive enterocytes, but not colonocytes, in *Tnf*$^{\Delta ARE/+}$ with severe inflammation displayed major transcriptomic changes when compared to normal or *Tnf*$^{\Delta ARE/+}$ mice with mild inflammation. Enterocyte transcriptomic changes reflected a switch from normal absorptive function toward an upregulation of inflammatory response genes. DNA sequencing on intestinal lavages revealed increased abundance of the obligate intracellular bacterium *Chlamydia muridarum* in *Tnf*$^{\Delta ARE/+}$ mice. While ileitis is spontaneous, colonic inflammation was dependent on the housing facility, where "clean" facility mice were free of colonic inflammation as well as *C. muridarum* colonization. We transferred and co-housed mice from the "clean" facility with those in the "dirty" facility and found *Tnf*$^{\Delta ARE/+}$ mice developed colitis, suggesting that TNF-induced colitis is microbiome-dependent. We also found that these transferred and co-housed *Tnf*$^{\Delta ARE/+}$ mice were colonized with *C. muridarum*, suggesting that this bacterial species may be colitic in the context of TNF overexpression. Here, we provide a full characterization of cell types and microbes in inflamed ilea and colon from the *Tnf*$^{\Delta ARE/+}$ model of Crohn's-like disease. Future studies aim to reveal the epithelial cell type that harbors *C. muridarum* and the role of Paneth cells and their antimicrobial products in persistent infection.

Poster Presentations
Friday May 19

1. Alexandra Mulligan
2. Simone Nevills
3. Julia Pinette
4. Laura Richardson
5. Gabriella Robertson
6. Nicole Rodgers
7. Elizabeth Ruark
8. Avishkar Sawant
9. Claire Scott
10. Fubiao Shi
11. Aubrie Stricker
12. Indrayani Waghmare
13. Jason Wang
14. Qian Yang
15. Deanna Bowman
16. David Gonzalez
17. Rincon Jagarlamudi
18. Bhawik Kumar Jain
19. Marianna Jimenez
20. Gregory Konar

Investigating organelle remodeling as a driver of aging

Alexandra G Mulligan, Eric KF Donahue, Sanja Sviben, James Fitzpatrick, and Kristopher Burkewitz

Maintenance of cellular metabolic homeostasis requires tight coordination between distinct subcellular compartments. One of the key ways by which cells achieve coordination between compartments is via signaling and metabolic flux at inter-organelle contact sites. However, age-related pathologies often correlate with aberrant morphology and organization of certain organelles, such as mitochondria. These observations led us to hypothesize that the interactions between organelles become dysregulated during aging and contribute to metabolic decline. To test this model in *C. elegans*, we first focused on the hub of inter-organelle contacts, the endoplasmic reticulum (ER). We generated strains with native fluorescent markers of distinct ER subdomains, including the translocon in rough ER and reticulon in tubulated ER. Both markers revealed significant alterations in ER morphology and a substantial loss of ER content during aging. To expand our analysis to all cellular organelles, we employed a combination of 2D transmission electron microscopy (TEM) and 3D focused ion beam-scanning electron microscopy (FIB-SEM), which both provide nanometer-level resolution of organelles without the bias of fluorescence labels. Despite the current focus of the aging field on aberrant mitochondrial dynamics, the mitochondrial networks showed only relatively modest swelling. In contrast, much more striking differences included a substantial loss of ER, supporting our optical imaging, as well as a dramatic increase in lipid droplets and their contact sites with ER and mitochondria. To begin determining whether these shifts in organelle interactions play causal roles in age-related metabolic dysfunction and physiological deterioration, we asked whether the lifespan extending paradigm of dietary restriction (DR) protects against age-onset remodeling of ER and lipid droplets, as it is known already to do for mitochondria. While this work is ongoing, we find that DR indeed reduces the loss of ER in aged animals. Intriguingly, we find evidence that DR impacts both ER and lipid droplet organization even in young animals. Given DR's established effects on mitochondrial dynamics, our emerging data suggest that DR may induce a unique subcellular architecture optimized for cellular metabolic health. Collectively, these ongoing experiments will provide insight into how the organelle interactome changes during aging, and its potential as a target to mitigate age-related dysfunction.

Maternal Over- and Undernutrition Predispose Offspring to β Cell Dysfunction Postnatally

Simone Nevills, Guoqiang Gu, Ph.D.

Previous studies have shown that intrauterine nutrient levels impact the risk of diabetes in offspring postnatally, but the molecular mechanisms underpinning this phenomenon remain poorly characterized. We hypothesize that maternal malnutrition during gestation may disrupt the fetus' epigenetic program during development, leading to insufficient functional beta-cell mass postnatally. To this end, we show that: 1) *de novo* DNA methyltransferases Dnmt3a and Dnmt3b are downregulated in embryonic endocrine progenitors exposed to a low-protein or high-fat diet *in utero*, while DNA demethyltransferases Tet1 and Tet2 are upregulated; 2) beta cells in offspring of dams fed a low-protein or high-fat diet during gestation demonstrate reduced expression of genes associated with beta cell maturation and function both during development and postnatally (e.g. Pdx1, MafA); 3) islets from adult mice exposed to maternal high-fat diet *in utero* have compromised insulin secretion when treated with sustained high glucose or free fatty acids *in vitro*. These findings are consistent with a model in which abnormal DNA methylation in islet progenitors is a key mechanism by which maternal nutrients mediate postnatal functional beta-cell mass and diabetes risk.

The Role of Nucleotide Biosynthesis in Adipose Development

Jacob W. Myers [1] *, Julia A. Pinette [1] *, Genesis A. Wilson [1], Elizabeth R. Nunn [1], Abhijit B. Shinde [1], Mathew T. Chvasta [1], Jessica B. Spinelli [4], Elma Zaganjor [1, 2, 3]

[1] Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA
[2] Vanderbilt Digestive Disease Research Center, Vanderbilt University Medical Center, Nashville, TN, USA
[3] Vanderbilt Diabetes Research Center, Vanderbilt University, Nashville, TN, USA
[4] Program in Molecular Medicine, University of Massachusetts Chan Medical School, Worcester, MA, USA

Obesity is a growing health concern worldwide because of its contribution to metabolic syndrome, type II diabetes, insulin resistance (IR), and numerous cancers. In obesity, white adipose tissue (WAT) expands through two mechanisms: increase in adipocyte cell number (hyperplasia) and increase in existing mature adipocyte cell size (hypertrophy). While hypertrophy is associated with the negative effects of obesity on metabolic health, such as inflammation and lipotoxicity, adipogenesis prevents obesity-mediated metabolic decline. Given that adipocytes are a reservoir for lipid stores and sense nutrient state to regulate organismal energy balance, metabolites themselves are significant regulators of adipogenesis. Although nucleotide biosynthesis has been well studied in the context of proliferation, in part due to its importance for DNA and RNA synthesis, little is known about the role of nucleotides in cell physiology and cell fate decision.

We found that nucleotide biosynthesis is a required pathway to stimulate adipogenesis, and that inhibition of nucleotide biosynthesis disrupts lipid accumulation and suppresses key adipogenic transcriptional regulators. Additionally, inhibition of de novo purine biosynthesis induces significant mitochondrial reprogramming. We hypothesize that the regulation of adipocyte differentiation may exist as a cyclic feedforward loop, rather than as a linear process. PPARy drives both adipogenic gene expression and mitochondrial biogenesis through the PGC1 family of cofactors. This is thought to be for the production of ATP and other metabolites for lipogenesis, but nutrient oxidation promotes PPARy suggesting that mitochondrial metabolism also contributes to the transcriptional regulation of adipogenesis. Temporal analysis further suggests that inhibition of purine biosynthesis affects transcriptional regulators prior to mitochondrial OXPHOS proteins, affirming PPARy's role in stimulating both adipogenic gene expression and mitochondrial biogenesis. In preliminary studies, we have identified mitochondrial metabolites that are altered by inhibition of nucleotide biosynthesis. Thus, our future studies will examine the role of mitochondrial metabolism in the regulation of PPARy transcription and adipogenesis.

Elucidating the Effect of SSNA1 on Microtubule Self-Repair

Laura Richardson[1], Beth Lawrence[2], Marija Zanic[1,2,3]
[1]Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN 37240, [2]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37240, [3]Department of Biochemistry, Vanderbilt University, Nashville, TN 37240

SSNA1 (Sjögren's Syndrome Nuclear Autoantigen 1) is a microtubule-associated protein (MAP) associated with Sjögren's Syndrome, an autoimmune disease prevalent in women over 40 years old. The role of SSNA1 in Sjögren's Syndrome remains unknown and little work has been done to investigate the function of SSNA1 in the context of the cellular cytoskeleton. Our lab has shown that *in vitro* SSNA1 can modulate microtubule dynamics, localize to microtubule lattice damage, and protect against lattice damage from a microtubule severing enzyme, spastin. Recently, great attention has been directed toward the ability of the microtubule lattice to undergo damage and "self-repair", a process by which GTP (guanosine triphosphate)-bound tubulin dimers incorporate into the damage sites. Intriguingly, certain MAPs have been shown to enhance the self-repair process by recruiting tubulin dimers to damage sites. However, the role of SSNA1 in microtubule self-repair is not known. We hypothesize that SSNA1 localization on lattice damage sites limits the efficiency of microtubule self-repair via steric hindrance. Given that SSNA1 forms fibrils along the microtubule lattice, we theorize that SSNA1 forms bridges across lattice damage sites and hence, strengthens the microtubule lattice. To test this hypothesis, we use an *in vitro* reconstitution approach with purified protein components and total internal reflection fluorescence (TIRF) microscopy. Our preliminary results suggest that the localization of SSNA1 at lattice damage sites does not inhibit the incorporation of free tubulin in the process self-repair. However, our data does not indicate that SSNA1 promotes microtubule self-repair, prompting further questions on the role of SSNA1 in the damage and repair mechanism. By understanding the influence of MAPs on microtubule self-repair, our work aims to provide insight into the mechanisms underlying the regulation of microtubules and the cytoskeletal networks in their essential cellular functions.

Defective mitochondrial & peroxisomal fission disrupts neurogenesis in the rare disease EMPF1

Gabriella L. Robertson[1], Caroline Bodnya[1], Mira Patel[1], Ram Prosad Chakrabarty[2], Ken Lau[1], Navdeep Chandel[2,3], Jason A. Mears[4], and Vivian Gama[1,5].

[1]Cell and Developmental Biology, Vanderbilt University.
[2]Northwestern University, Feinberg School of Medicine Department of Medicine Division of Pulmonary and Critical Care Medicine, Chicago, IL.
[3]Northwestern University, Feinberg School of Medicine Department of Biochemistry and Molecular Genetics, Chicago, IL.
[4]Case Western Reserve University, Department of Pharmacology and Center for Mitochondrial Diseases, Cleveland, OH.
[5]Vanderbilt Center for Stem Cell Biology, Vanderbilt University.

Mitochondria and peroxisomes are dynamic signaling organelles that constantly undergo fission, driven by the large GTPase DRP1 (dynamin-related protein 1). Patients with *de novo* heterozygous missense mutations in DRP1 present with encephalopathy due to defective mitochondrial and peroxisomal fission (EMPF1) – a devastating rare neurodevelopmental disease with no effective treatment. To interrogate the molecular mechanisms by which DRP1 mutations lead to developmental defects, we are using induced pluripotent stem cell (iPSC)-derived models from patients who present with clinically disparate conditions and have mutations in different domains of DRP1. Our studies with patient fibroblasts revealed that in addition to the hyperelongated mitochondrial morphology and lack of fission, patient-derived cells display lower coupling efficiency, increased proton leak, and upregulation of glycolysis. Mitochondrial hyperelongation is also associated with aberrant cristae structure and hyperpolarized mitochondrial membrane potential. Peroxisome morphology is severely elongated in patient cells and is associated with accumulation of very long chain fatty acids. Metabolomic analyses revealed impaired methionine cycle and synthesis of pyrimidine nucleotides. Given that EMPF1 patients present with a spectrum of neurodevelopmental abnormalities, we then explore the effects of DRP1 mutations on neural fate by introducing mutations found in EMPF patients into iPSCs, using CRISPR/Cas9. These iPSCs were differentiated into neural progenitor cells and forebrain organoids. We find that DRP1 mutant neural progenitor cells express lower levels of critical identity transcription factors, such as PAX6 and TBR2. Intriguingly, forebrain organoids with mutations in the stalk domain of DRP1 take on a choroid plexus identity instead of following a cortical development trajectory. Our results show that EMPF1 associated DRP1 mutations lead to metabolic dysregulation, which may cause changes in neural cell fate during early corticogenesis. We are currently investigating how dysregulated mitochondrial/peroxisomal fission and aberrant metabolic pathways may be central to the modulation of neural identity. Understanding these mechanisms will give insight into the role of mitochondrial and peroxisome dynamics in neurodevelopment, as well as the mechanisms underlying the rare EMPF1 disease.

CLASP2 facilitates dynamic actin filament organization along the microtubule lattice

Rodgers NC, Lawrence EJ, Sawant AV, Efimova N, Gonzalez-Vasquez G, Hickman TT, Kaverina I and Zanic M

Coordination between the microtubule and actin networks is essential for cell motility, neuronal growth cone guidance, and wound healing. Members of the CLASP (Cytoplasmic Linker-Associated Protein) family of proteins have been implicated in the cytoskeletal crosstalk between microtubules and actin networks, however, the molecular mechanisms underlying CLASPs role in cytoskeletal coordination are unclear. Here, we investigate CLASP2α's crosslinking function with microtubules and F-actin. Our results demonstrate that CLASP2α crosslinks F-actin to the microtubule lattice in vitro. We find that the crosslinking ability is retained by L-TOG2-S, a minimal construct containing the TOG2 domain and serine-arginine rich region of CLASP2α. Furthermore, CLASP2α promotes the accumulation of multiple actin filaments along the microtubule, supporting up to 11 F-actin landing events on a single microtubule lattice region. CLASP2α also facilitates dynamic organization of polymerizing actin filaments templated by the microtubule network, with F-actin forming bridges between individual microtubules. Finally, we find that depletion of CLASPs in vascular smooth muscle cells results in disorganized actin fibers and reduced co-alignment of actin fibers with microtubules, suggesting that CLASP and microtubules contribute to higher-order actin structures. Taken together, our results indicate that CLASP2α can directly crosslink F-actin to microtubules, and that this microtubule-CLASP-actin interaction may influence overall cytoskeletal organization in cells.

InsP3R coordinates transcriptional responses to promote mitochondrial homeostasis and longevity

Elizabeth M. Ruark, Gaomin Feng, Kristopher Burkewitz

Maintaining proper mitochondrial homeostasis is critical for health and longevity. Indeed, a variety of lifespan-extending interventions target different aspects of mitochondrial function. One such intervention is mild inhibition of the electron transport chain (ETC). One mechanism by which cells adapt to ETC inhibition is transcriptional remodeling, but our lab has recently shown that the inositol triphosphate receptor (InsP3R), an ER calcium efflux channel, is required for lifespan extension of the complex I mutant. While investigating how the InsP3R mediates longevity, we found that the InsP3R is required for full activation of the mitochondrial unfolded response target gene, *hsp*-6, during longevity. Thus, indicating that the InsP3R modulates transcriptional targets during longevity. We then utilized RNA-seq to identify further transcriptional targets regulated by the InsP3R during mitochondrial stress induced longevity. We identified that many transcripts related to ETC components and even actin cytoskeletal remodeling were differentially regulated during longevity. Altogether, these data suggest that the InsP3R coordinates adaptive response pathways during mitochondrial stress. I propose that the InsP3R coordinates transcriptional responses during mitochondrial stress to promote mitochondrial homeostasis and extend lifespan. To explore mechanisms by which the InsP3R mediates transcriptional responses during chronic mitochondrial stress, I will screen candidate transcription factors regulated by the InsP3R and identify downstream calcium targets. A recent study has shown that acute mitochondrial stress triggers a transient actin response around mitochondria, highlighting actin-mitochondrial interactions as an adaptive response to stress. The actin cytoskeleton has important roles in maintaining mitochondrial structural integrity and facilitating fission events, therefore, I will explore the role of mitochondrial homeostasis in longevity via the actin cytoskeleton. To determine the role of actin remodeling on mitochondrial homeostasis during longevity, I will test the necessity of actin remodeling to promote longevity and identify the effect on mitochondrial bioenergetics and fission/fusion events. Taken together, these experiments will reveal new insights into the cellular response to mitochondrial stress as well as establish further connections for ER-mitochondrial communication during aging.

A Cell-cycle-dependent Switch in Microtubule-dependent Molecular Motors Driving Golgi Positioning in Interphase Cells

Avishkar V. Sawant, John Lee, Claire F. Scott, Irina Kaverina

Department of Cell and Developmental Biology, Vanderbilt University

The Golgi complex undergoes dynamic morphological changes throughout the cell cycle. Numerous studies have shown the mechanisms involved in the changes in the Golgi complex during mitosis. However, there are limited studies which have explored the morphological transitions of Golgi during the interphase. A recently published work from our lab has shown that the Golgi transits between a compact configuration around the centrosomes in the G1 phase to an extended configuration around the nucleus in S and G2 phases. The data also indicated that the rearrangements rely on microtubules and microtubule-based transport. In the current study, we aim to dissect the mechanisms of this cell-cycle-dependent transition. We take advantage of the fact that microtubule depolymerization leads to a random scattering of Golgi fragments in the cytoplasm. We utilize the process of Golgi reassembly during microtubule regrowth as an assay to determine the destinations of microtubule-dependent Golgi transport. We observe that the reassembly of Golgi stacks in the G1 phase occurs around the centrosomes, whereas in the S/G2 phases, the reassembly occurs mainly around the nuclear envelope even though the radially organized microtubules produced by the centrosomes persist. Interestingly, our data suggest that the interplay between the minus-end and plus-end directed microtubule motors is essential for this distinction between G1 and S/G2. Inhibition of dynein, a minus-end directed motor, leads to fragmentation and dispersal of Golgi stacks in both G1 and S/G2 phases. In contrast, inhibition of kinesin-1, a plus-end directed motor, does not affect Golgi positioning in the G1 phase. However, in S/G2, kinesin-1 inhibition prevents its redistribution from the centrosomes to the nuclear equator. Subsequent washout of the kinesin inhibitor reverses Golgi configuration to the extended perinuclear configuration typical for G2. The importance of kinesin-1 for Golgi configuration in S/G2 was confirmed by the depletion of the kinesin light chain, which phenocopies the inhibition of kinesin-1. Based on these findings, we propose a model where dynein facilitates the connection of Golgi stacks into a continuous ribbon throughout the interphase. In contrast, kinesin-1 exerts a cell-cycle stage-specific action on the Golgi complex. In G1, kinesin-1 does not significantly contribute to Golgi complex positioning, allowing dynein to condense Golgi around the centrosome. Upon transition to S/G2, kinesin-1 overpowers dynein to move the Golgi away from the centrosomes and stretches the Golgi ribbon along the nuclear envelope, possibly utilizing specifically modified perinuclear microtubule tracks. Our data suggest a potential cell-cycle-dependent pathway tuning kinesin-1 capacity to transport Golgi stacks.

P9

Mapping the lipid landscape of murine and human brain tissue

Claire F Scott 1,2; Cody Marshall 1,2; Allison B Esselman 1,2; Martin Dufresne 1,2; Matthew S Schrag 1,3; Jeffrey M Spraggins 1,2

1 Vanderbilt University, Nashville, TN, 37235; 2 Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN 37235; 3 Cerebral Amyloid Angiopathy Clinic, Vanderbilt University Medical Center, Nashville, TN 37235

Uncovering the molecular underpinnings of Alzheimer's disease (AD)-associated brain tissue features will allow us to better understand disease progression and determine effective treatment strategies. Molecular imaging of AD tissues via targeted immunofluorescence (IF) microscopy can reveal the cellular neighborhoods present across tissue sections, including AD-associated features. MALDI IMS can be used for untargeted molecular mapping of human brain tissue sections. We establish a multimodal imaging workflow that combines IF and MALDI IMS to comprehensively study cellular and molecular landscapes of human brain tissue. Whole brain lipid levels are altered in those diagnosed with AD and here we will examine how lipids are impacted within AD-associated features in situ. AD and control human brain tissues were sectioned at 10µm thickness onto indium tin oxide (ITO) glass slides. Sections imaged using IF were photobleached prior to the application of antibodies and subsequent imaging. Prior to MALDI IMS, serial tissue sections were washed with 150 mM ammonium formate and sublimed with 5 mg of an aminated cinnamic acid analog using an in-house developed sublimation device. MALDI IMS data were acquired at 5 and 10µm spatial resolution in positive and negative ionization mode on a Bruker timsTOF FleX (Bruker Daltonics). Data analysis was performed using SCiLS, ZEN microscopy software, and in-house software. IF microscopy images give cellular context to molecular data acquired with MALDI IMS by marking AD-associated features. Initial studies were performed to optimize methods for high spatial resolution MALDI IMS. AD frontal lobe brain tissue samples from a 68-year-old female donor were used. Experiments were conducted at 5 µm and 10 µm spatial resolution with a scan range of m/z 400-2500. Various lipid species were found to localize to specifically the white matter or grey matter (e.g., m/z 885.552, m/z 734.562), and molecular heterogeneity within these regions was also detected (e.g., m/z 610.224). In future studies, we expect to correlate pathological cellular features with altered lipid landscapes and discover information about the molecular drivers of AD. In conclusion, combining MALDI IMS with IF microscopy allows for detailed mapping of the cellular and lipid landscape of Alzheimer's disease pathologies.

PKA phosphorylation of RAPTOR potentiates mTORC1 and controls adipose tissue homeostasis

Fubiao Shi[1#], Dianxin Liu[1#], Wei Zhang[1], Sheila Collins[1,2*]

[1]Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt University Medical Center,
[2]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA
[#] Co-first authors, *Corresponding author: sheila.collins@vumc.org

Norepinephrine stimulates the adipose tissue thermogenic program through a β-adrenergic receptor (βAR) - protein kinase A (PKA) signaling cascade. Previous work from our lab demonstrated that a noncanonical activation of the mechanistic target of rapamycin complex 1 (mTORC1) by PKA is required for the βAR-stimulation of adipose tissue browning. Here we report that PKA phosphorylation of RAPTOR at Ser791 (S791) is required for mTORC1 activation and essential to maintain adipose tissue function and metabolic homeostasis. To establish the *in vivo* function of S791 phosphorylation, we generated a mouse model with adipose tissue-specific knock-in of a phosphorylation-resistant Ser to Ala mutation in *Raptor* (*Raptor*^{AdS791A}). On chow diet, *Raptor*^{AdS791A} mice had normal body weight and glucose tolerance but showed a compromised thermogenic response when treated with β_3-AR selective agonist CL316,243 (CL), as indicated by reduced uncoupling protein 1 (UCP1) and mitochondrial protein in the subcutaneous inguinal white adipose tissue (iWAT). When fed with a high-fat diet (HFD), male *Raptor*^{AdS791A} mice gained similar body weight, but had smaller visceral epididymal WAT (eWAT) with increased tissue inflammation and enlarged liver with aggravated hepatic steatosis. Euglycemia-hyperinsulinemic clamp studies revealed that *Raptor*^{AdS791A} mice were more insulin resistant with reduced glucose infusion rate, increased hepatic glucose production, and decreased skeletal muscle glucose uptake. Mechanistically, in primary adipocytes that express phosphorylation-resistant RAPTOR S791A protein, CL-stimulated mTORC1 activity was impaired and the protein level of peroxisome proliferator-activated receptor gamma (PPARγ), the master regulator of adipocyte function, was significantly reduced. In contrast, in HIB-1B brown adipocytes that stably express phosphorylation-mimetic RAPTOR S791D protein, the PPAR signaling pathway is up-regulated, as indicated by RNA-seq transcriptomic analysis, and adipocyte thermogenic and mitochondrial gene programs are significantly enhanced. In line with these data from adipocyte models, PPARγ protein levels are also reduced in adipose tissues of *Raptor*^{AdS791A} mice, suggesting that RAPTOR S791 phosphorylation might act through a PPARγ-dependent mechanism to control the adipocyte metabolic program *in vivo*. Taken together, our study revealed the *in vivo* function and mechanistic insight of PKA phosphorylation of RAPTOR and demonstrated that PKA phosphorylation of RAPTOR at S791 potentiates mTORC1 activity and controls adipose tissue homeostasis through the PPARγ pathway.

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

Aubrie Stricker, Shane Hutson, Andrea Page-McCaw

Basement membranes are sheets of extracellular matrix that serve to separate tissue layers and provide mechanical support. Their mechanics are determined largely by collagen IV, the most abundant protein in basement membranes. Basement membranes are subject to mechanical damage and require repair, but repair must be highly regulated because collagen is a long-lived molecule, and too much causes fibrosis. Little is known about how repair occurs, and it is unknown how basement membrane damage is detected. Previously we found there are homeostatic mechanics of basement membrane replacement, raising the question of whether basement membrane damage is actively detected or passively repaired. Here we show that repair is an active process, requiring the mechanosensitive channel Piezo. To repair the basement membrane around *Drosophila* midgut, short-lived cells in the gut epithelium transcribe matrix components specifically after damage. Lineage tracing indicates these "matrix maker" cells are a subset of enteroblasts that die shortly after repair is complete. Piezo is activated by basement membrane damage and is required to upregulate collagen IV synthesis in the matrix makers. Further, most of the collagen IV in the gut originates from cells expressing Piezo, suggesting that mechanical sensitivity is a general property of collagen-producing cells.

Understanding glypican-based mechanisms of extracellular Wnt distribution

Indrayani Waghmare[1,2] and Andrea Page-McCaw[1, 2]

Wnts are evolutionarily conserved secreted ligands that form extracellular gradients to direct several cellular behaviors at short- and long-ranges. The *Drosophila* germarium, a tissue where oogenesis initiates, is an excellent model to study mechanisms of Wnt distribution as this tissue expresses several Wnts, which are required for proper egg production. My postdoctoral research shows that the distribution of extracellular Wnts is important for Wnt function and is primarily dependent on cell-surface localization of glypicans, which distribute Wnts by continual binding and release in the plane of the tissue. Further, because glypicans play an important role in modulating extracellular Wnt availability, their cell-surface levels are likely regulated by other factors. In the fly germarium, the glypican Dally-like protein (Dlp) promotes long-range distribution of extracellular Wg, the *Drosophila* ortholog of mammalian Wnt1, from Wg-producing cap cells to Wg-responsive follicle stem cells inducing their proliferation, required for egg development. Both knockdown of *dlp* or tethering Wg to cap cell membrane disrupts follicle stem cell proliferation. In genetic experiments, Matrix Metalloproteinase 2 (Mmp2) inhibits Dlp's long-range Wg distribution to restrict Wg signaling in follicle stem cells. Thus, Mmp2 acts as a molecular brake on Dlp's long-range function. Mechanistically, I discovered that in S2R+ insect cells, Mmp2 cleaves Dlp on the cell surface to induce a conformational change. Further cleavage of Dlp causes it to be destabilized and internalized from the cell surface, and cleaved Dlp sequesters more Wg than intact Dlp, suggesting that cleavage by Mmp2 alters Wg-Dlp binding affinities to inhibit Wg distribution. Taken together, my research identifies that Dlp modulates the extracellular availability of several Wnts and identifies the molecular basis of protease-mediated inhibition of cell-surface Dlp, which modulates Wnt ligand distribution and function.

Mechanistic Study of Ras Driven E-Cadherin Endocytosis

Wang, J. , de Caestecker, C. and Macara, I.

We used EpH4 cells and inducible oncogenes to model the earliest stages of Ras driven tumor formation, focusing on activation of the ERK signaling pathway and re-distribution of E-Cadherin, a major component of Adherens Junctions (AJ) and cell polarity. We found that E-Cadherin endocytosis was observed in both H-Ras and K-Ras transformed cells and that both forms of Ras activated the Map Kinase (MAPK) pathway leading to heightened levels of ERK phosphorylation. We subsequently showed that ERK activation was essential for E-Cadherin internalization yet not sufficient below a certain threshold. We determined that the process of E-Cadherin internalization must occur simultaneously with the dissociation of P120 and β-Catenin from the Cadherin complex. We conclude that ERK activation is required, but is not sufficient for E-Cadherin endocytosis, and hypothesize that sufficiently present ERK signaling phosphorylates p120 catenin and triggers its release from E-Cadherin, which enables E-Cadherin internalization.

Activity dependent Clustering of Neuronal L-Type Calcium Channels by CaMKII

Qian Yang[1], Lan Hu[1], Juliana Quay[1], Dorian Lawson-Qureshi[1], Roger J. Colbran[1,2,3]

1Department of Molecular Physiology and Biophysics
2Vanderbilt Brain Institute
3Vanderbilt-Kennedy Center for Research on Human Development,
724 Robinson Research Building, Vanderbilt University School of Medicine, Nashville, TN, USA 37232-0615

Abstract content

Excitation-transcription (E-T) coupling, linking membrane depolarization to nuclear gene transcription, plays a critical role in regulating long-term synaptic modifications and is involved in learning and memory, as well as various diseases, including autism spectrum disorders (ASD), Alzheimer's disease, Huntington's disease, and epilepsy. Activation of L-type voltage-gated calcium channels (LTCCs) induces several forms of E-T coupling by increasing the local concentration of calcium ions within an LTCC nanodomain. Formation of LTCC/Ca^{2+} nanodomains may require clustering of the primary neuronal LTCCα1 subunits ($Ca_V1.2$ and $Ca_V1.3$). However, the molecular mechanism of LTCC clustering remains unknown. Here, we found that a neuronal depolarization that induces CREB Ser133 phosphorylation and c-fos expression also increases $Ca_V1.3$ LTCC clustering in cultured hippocampal neurons. Our previous work showed that binding of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to an N-terminal RKR motif in $Ca_V1.3$ is required for LTCC-mediated E-T coupling. We co-expressed $Ca_V1.3$s containing two different epitope tags, with or without CaMKII in HEK cells. Co-immunoprecipitations (co-IPs) from the cell lysates revealed that CaMKII not only interacts with $Ca_V1.3$, but also assembles multimeric $Ca_V1.3$ LTCC complexes in an activity-dependent manner. Moreover, pharmacological activation of LTCCs in HEK293 cells co-expressing CaMKII increased the clustering of surface localized $Ca_V1.3$ channels. Notably, the N-terminal CaMKII-binding RKR motif is conserved in $Ca_V1.2$, and CaMKII also facilitates the activity-dependent co-clustering of $Ca_V1.3$ and $Ca_V1.2$ in HEK293 cells. Furthermore, we found that beta2a auxiliary subunits, which also directly bind to CaMKII, facilitate CaMKII-dependent $Ca_V1.2$-$Ca_V1.3$ co-clustering, relative to the beta3 auxiliary subunit which lacks the ability to bind CaMKII. Our ongoing studies are examining the role of CaMKII in $Ca_V1.3$ clustering and $Ca_V1.2$-$Ca_V1.3$ co-clustering in cultured neurons. Taken together, our work suggests that CaMKII mediates activity-dependent LTCC clustering, which may be crucial for initiating a specific long-range signal from LTCCs in the plasma membrane to the nucleus in various pathophysiological situations.

Deanna Bowman

Using MVID-Causing Mutations to Investigate MYO5B Motor Function

D.M. Bowman[1,2], M.J. Tyska[1,2], J.R. Goldenring[1,2,3]

[1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville TN, [2]Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, [3]Section of Surgical Science, Vanderbilt University Medical Center, Nashville, TN.

Myosin Vb (MYO5B) is a nonconventional myosin motor. Mutations in MYO5B lead to the autosomal recessive disorder microvilli inclusion disease (MVID) that causes life-threatening diarrhea in neonates due to the mis-trafficking of apical transporters and proteins. There are over 50 different disease-causing mutations in MYO5B comprised of truncations or frameshifts and point mutations in specific domains of the motor. It is unknown how each mutation is manifested in the function of MYO5B to translocate along actin. Understanding how different MVID-causing mutations in MYO5B disrupt the function of the motor functionality is critical to learning how MYO5B operates within the apical recycling and delivery pathways. I hypothesized that point mutations in the MYO5B motor head lead to defects in motor functionality by impacting the ability to bind actin, translocate, or hydrolyze ATP. To address my hypothesis, I used a live-cell assay to examine the functionality of the MYO5B motor and mutants independently of the cargo domain. A truncated MYO5B (1-1015 A.A) motor construct with a triple citrine tag was created, and constructs containing select patient MYO5B motor mutations. The MYO5B motor domain constructs were co-expressed with mCherry-espin in protrusion forming cell line, LLC-PK-CL4 cells. The localization of the MYO5B-motor at the tips of microvilli indicated a functional motor, while a lack of MYO5B-motor at the tips of microvilli indicated a dysfunctional motor. A tip to cytoplasm ratio was used to quantify this change in distribution. In accordance with previous literature, the wild-type MYO5B motor localized to the tips of microvilli with a ratio of 4.087, while the P660L mutation localized to the bases of microvilli and had a tip ratio of 0.1000. Patient mutations I408F and R824C did not accumulate at the tips of microvilli with a tip ratio of 0.1380 and 0.4963, respectively. Surprisingly some MVID-causing MYO5B motor mutations did not fully impair MYO5B motor function. G519R had a tip ratio of 1.870, and D492G had a tip ratio of 1.660. These partially functional motors indicate that while the MYO5B motor function is not impacted by the mutation, the entire protein is affected by the mutation. Unfortunately for patients with MVID, the prognosis is generally poor, and there are few options for treatment with no curative treatment. Understanding the subtle differences in the phenotype of separate mutations could lead to a better understanding of the disease pathology and lead to personalized medicine for patients with MVID.

Elucidating the role of Ubp10 on double-strand break repair pathways in *Saccharomyces cerevisiae*

David Gonzalez and Katherine Friedman

In the event of a double strand break (DSB), cells must accurately repair DNA to prevent sequence loss, mutagenesis, or cell death. If conservative repair pathways are unable to repair a DSB, such as in cancer cells, the DSB will persist and give rise to gross chromosomal rearrangements (GCRs). Telomerase is a reverse transcriptase that acts at the end of chromosomes to extend the 3' TG-rich overhang. If telomere-like sequences become exposed following resection after a DSB, telomerase can act at these sequences resulting in a *de novo* telomere addition. This phenomenon has been extensively studied in human diseases such as Phelan-McDermid syndrome and α-thalassemia. *Saccharomyces cerevisiae* is a well characterized model organism used to study telomeres and GCRs due its ease of genetic manipulation and conserved proteome. Because *de novo* telomere additions cause loss of genetic material, pathways have evolved to prevent telomerase action at a DSB; however, endogenous sequences in the *S. cerevisiae* genome stimulate *de novo* telomere addition ~200 fold higher than background. These sequences, termed Sites of Repair-associated Telomere Addition (SiRTAs), contain two TG-rich regions identified as the Stim and Core. Recruitment of Cdc13 to the Stim sequence dramatically increases *de novo* telomere addition at the site of a DSB. In an unpublished genetic screen for mutations that reduce *de novo* telomere addition, the Friedman lab identified Ubp10, a ubiquitin protease. Loss of *UBP10* function does not affect overall frequency of GCR events at a SiRTA but lowers the probability of *de novo* telomere addition such that only ~40-50% of GCR events observed at the SiRTA are *de novo* telomere additions (in a normal strain it would be ~100%). Interestingly, the probability of translocations observed at the SiRTA increases to ~50-60%. Sequence analysis shows loss of *UBP10* allows homologous sequences located in the SiRTA to be used to invade subtelomeric regions of donor chromosomes resulting in translocations. Thus, our data suggests that SiRTAs have the capacity to stimulate other conservative DNA repair mechanisms, such as break-induced replication (BIR), when *de novo* telomere addition is affected.

Title: Glial Phagocytosis of Brain Neurons Mediated by FMRP-Dependent JNK signaling for Filamin Control of the Actin Cytoskeleton

Authors: Rincon Jagarlamudi [1], Kendal Broadie [1,2]

Abstract:

Juvenile brain circuit remodeling is a crucial developmental process that is disrupted in a number of different neurological disease states, including Fragile X syndrome (FXS); the leading heritable cause of intellectual disability (ID) and autism spectrum disorder (ASD). Recent work suggests that the developmental clearance of neurons during circuit remodeling requires neuron-to-glia signaling that drives glial phagocytosis. We study these events in the *Drosophila* brain PDF clock circuit, which contains the developmentally transient PDF-Tri neurons. We have previously shown that the Fragile X Mental Retardation Protein (FMRP) is required in neurons to activate and recruit glia for the phagocytosis and developmental clearance of these PDF-Tri neurons.

Neuronal Fragile X Mental Retardation Protein (FMRP)-dependent signaling activates the glial engulfment receptor Draper (mammalian Megf10/Jedi) for the phagocytic clearance of developmentally transient PDF-Tri neurons in *Drosophila.* We have now found that glial Basket (mammalian Jun N-terminal kinase; JNK) signaling and the downstream AP-1 transcriptional pathway are required for the clearance of PDF-Tri neurons. We found that targeted neuronal FMRP and glial Draper RNAi mutants exhibit reduced Basket/JNK translocation into glia nuclei, indicating a disruption in JNK signaling to AP-1 transcriptional control. We discovered this JNK/AP-1 signaling pathway drives the glial expression of Cheerio (mammalian filamin A; FLNA), an F-actin cross-linking protein, which is required for the PDF-Tri neuron clearance. Null *cheerio* mutants and glia-targeted *cheerio* RNAi similarly prevent removal of the normally developmentally transient PDF-Tri neurons. We also discovered FMRP genetically interacts with Cheerio to impact glia at a cytoarchitectural and functional level. Transheterozygous animals with only one copy of the FMRP and Cheerio genes display an impaired glial actin cytoskeleton and complete blockade of PDF-Tri neuron removal. We discovered that the glial processes in both FMRP and Cheerio mutants have a diminished ability to infiltrate the PDF-Tri region and produce phagocytic markers. We found that neuronal FMRP drives Basket/JNK signaling principally in ensheathing glia, which use the Cheerio/FLNA actin crosslinker to migrate to and clear the PDF-Tri neurons.

Taken together, we conclude that neuronal FMRP drives glial Basket/JNK signaling to induce AP-1 transcription of Cheerio/FLNA to modulate the glial F-actin cytoskeleton to enable glial phagocytosis of the PDF-Tri neurons. Our work identifying structural actin defects in Fragile X and Cheerio mutants contributes to the hypothesis that downstream down-regulation of cheerio in Fragile X animals prevents glial process extension, via their actin cytoskeleton. This clearance pathway has important implications for the study of Fragile X syndrome, as it provides a new cellular mechanism for the disease state and new molecular targets for the development of new therapeutic treatments.

P18

A PI4P undercurrent in the secretory pathway is pumped to the cytosolic surface of the Golgi by a P4-ATPase

Bhawik K. Jain[1], H. Diessel Duan[2], Richard Zhang[1], Huilin Li[2], Todd R. Graham[1]

1. Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

2. Department of Structural Biology, Van Andel Institute, Grand Rapids, MI, USA

The plasma membrane of eukaryotic cells is characterized by transbilayer asymmetry of phosphatidylserine and phosphatidylethanolamine, which is established by P4-ATPases. Phospatidylinositol-4-phosphate (PI4P) is assumed to be restricted to the cytosolic leaflet of membranes by virtue of its synthesis on this leaflet of the Golgi and plasma membrane. After synthesis, PI4P is thought to be delivered by oxysterol-binding proteins to the ER where it is exchanged for sterol or PS and then degraded by the Sac1 PI4P phosphatase. However, lipids rapidly flip-flop rapidly between the luminal and cytosolic leaflet of the ER, and any PI4P in the luminal leaflet would be resistant to degradation by Sac1. PI4P in the ER lumen would be available for transport through the Golgi complex and potential exposure on the outer leaflet of the plasma membrane (PM). *NEO1* was first identified in a screen for overexpressed genes that confer resistance to the aminoglycoside neomycin, an antibiotic that specifically binds to PI4P. Here we test the hypothesis that Neo1 confers resistance to neomycin by flipping PI4P from the Golgi luminal leaflet to the cytosolic leaflet, thereby preventing PI4P exposure on the extracellular leaflet of the PM. The SidC-GFP biosensor for PI4P was purified from *E. coli* and used to probe the outer leaflet of WT and *neo1-ts* cells. Strikingly, temperature inactivation of *neo1-ts* causes exposure of PI4P on the PM outer leaflet of intact cells while very little SidC-GFP binds to WT cells. Structure-guided mutagenesis of residues in the substrate translocation path of Neo1 identified point mutations that cause PI4P exposure. These mutants are also extremely sensitive to neomycin while other *neo1* mutants that expose PS and/or PE, but not PI4P, are resistant to neomycin. In addition, the ATPase activity of purified Neo1 is stimulated by PI4P in the absence of other substrates. We also find that vesicle-mediated transport from the ER to the Golgi and from the Golgi to the cell surface is required for PI4P cell surface exposure in *neo1* mutants. Surprisingly, *neo1-ts* cells display a substantial loss of PI4P from the Golgi cytosolic leaflet. These observations suggest that Neo1 is a Golgi PI4P flippase that is required to transport PI4P on the cytosolic leaflet of the Golgi and prevent its exposure in the extracellular leaflet of the plasma membrane.

Escaping the endosomal system: The importance of P4-ATPases trafficking

Mariana Jimenez, Kateryna Nabukhotna, Davia Watkins, Claire Kyoung, Jordan T. Best, Todd R. Graham
Department of Biological Sciences, Vanderbilt University

The transport of proteins and lipids throughout the cell is crucial for diverse cellular processes including vesicular transport, signal transduction and apoptosis. One important aspect of cell functionality is an asymmetric membrane composition which is established by type-IV P-type ATPases (P4-ATPase), which uses the energy from ATP hydrolysis to transport lipids from the exofacial to the cytofacial leaflet of membranes. P4-ATPase deficiency disrupts vesicle-mediated protein transport from Golgi and endosomal membranes and can lead to a number of severe neurological defects. For proper P4-ATPase function, correct localization to appropriate membranes is essential. Of the five P4-ATPases in *Saccharomyces cerevisiae*, Dnf1 and Dnf2 localize to the plasma membrane (PM) while Neo1, Drs2, and Dnf3 localize to the Golgi, and each travels through the endocytic pathway as part of their trafficking itineraries. In this study, I seek to determine the retrograde trafficking pathways used to sort P4-ATPases from endosomes back to the Golgi, and how these flippases interact with components of those pathways. Key components of four major trafficking pathways between endosomes and the Golgi including Drs2/Rcy1/COPI, Snx4, retromer and AP-1/Clathrin, were deleted to determine the routes required for Dnf1/Dnf2 PM localization and Drs2/Neo1 Golgi localization. Deletion of retromer components including Vps35, Vps5, Vps17, and Snx3 led to mislocalization of Dnf1, Dnf2, and Drs2 to the vacuole, as described previously for Neo1. These data suggest a primary role for retromer in proper localization of P4-ATPases although a minor role for Rcy1 and Snx4 was detected. In dissecting th
e interactions between retromer and the P4-ATPases, it was found that Dnf1 contains retromer recognition motifs in the N-terminal tail and both Dnf1 and Dnf2 have a novel C-terminal tail motif. Finally, I test for changes in membrane composition of phosphatidylethanolamine and phosphatidylserine and find major changes to the plasma membrane when intracellular trafficking of the P4-ATPases is affected. Together, these results suggest the loss of retromer leads to a substantial loss of P4-ATPases at their primary membrane and therefore causes major changes in membrane organization. Because deficiencies in the human orthologs of Dnf1/Dnf2 and retromer have been linked to endosomal dysfunction leading to Alzheimer's and Parkinson's Disease, this study could help elucidate why mutations in these proteins cause severe neurodegenerative diseases.

Damage-induced senescent immune cells regulate regeneration of the zebrafish retina

Gregory Konar, Zachary Flickinger, Shivani Sharma, Kyle Vallone, Charles Lyon, Claire Doshier, William Lyon, James G. Patton

Zebrafish spontaneously regenerate their retina in response to damage through the action of Müller glia. Even though Müller glia (MG) are conserved in higher vertebrates, the capacity to regenerate retinal damage is lost. Recent work has focused on the regulation of inflammation during tissue regeneration with precise temporal roles for macrophages and microglia. Senescent cells that have withdrawn from the cell cycle have mostly been implicated in aging, but are still metabolically active, releasing pro-inflammatory signaling molecules as part of the Senescence Associated Secretory Phenotype (SASP). Here, we discover that in response to retinal damage, a subset of cells expressing markers of microglia/macrophages also express markers of senescence. These cells display a temporal pattern of appearance and clearance during retina regeneration. Premature removal of senescent cells by senolytic treatment led to a decrease in proliferation and incomplete repair of the ganglion cell layer after NMDA damage. Our results demonstrate a role for modulation of senescent cell responses to balance inflammation, regeneration, plasticity, and repair as opposed to fibrosis and scarring.

Many thanks to…..

financial contributions from these generous supporters:

Cellular, Biochemical and Molecular Sciences Training Program
Kathy Gould, Ph.D., Senior Associate Dean

The Company of Biologists

Department of Biological Sciences
Brandt Eichman, Ph.D., Chair

Epithelial Biology Center
Vanderbilt University

Howard Hughes Medical Institute Gilliam Program
Vivian Gama, Ph.D.

Vanderbilt Brain Institute
Lisa Monteggia, Ph.D., Director

Please respond to the Survey

QR Codes will be posted at the close of the Symposium and an email will be sent – we hope you will offer feedback

Enjoy the Symposium!