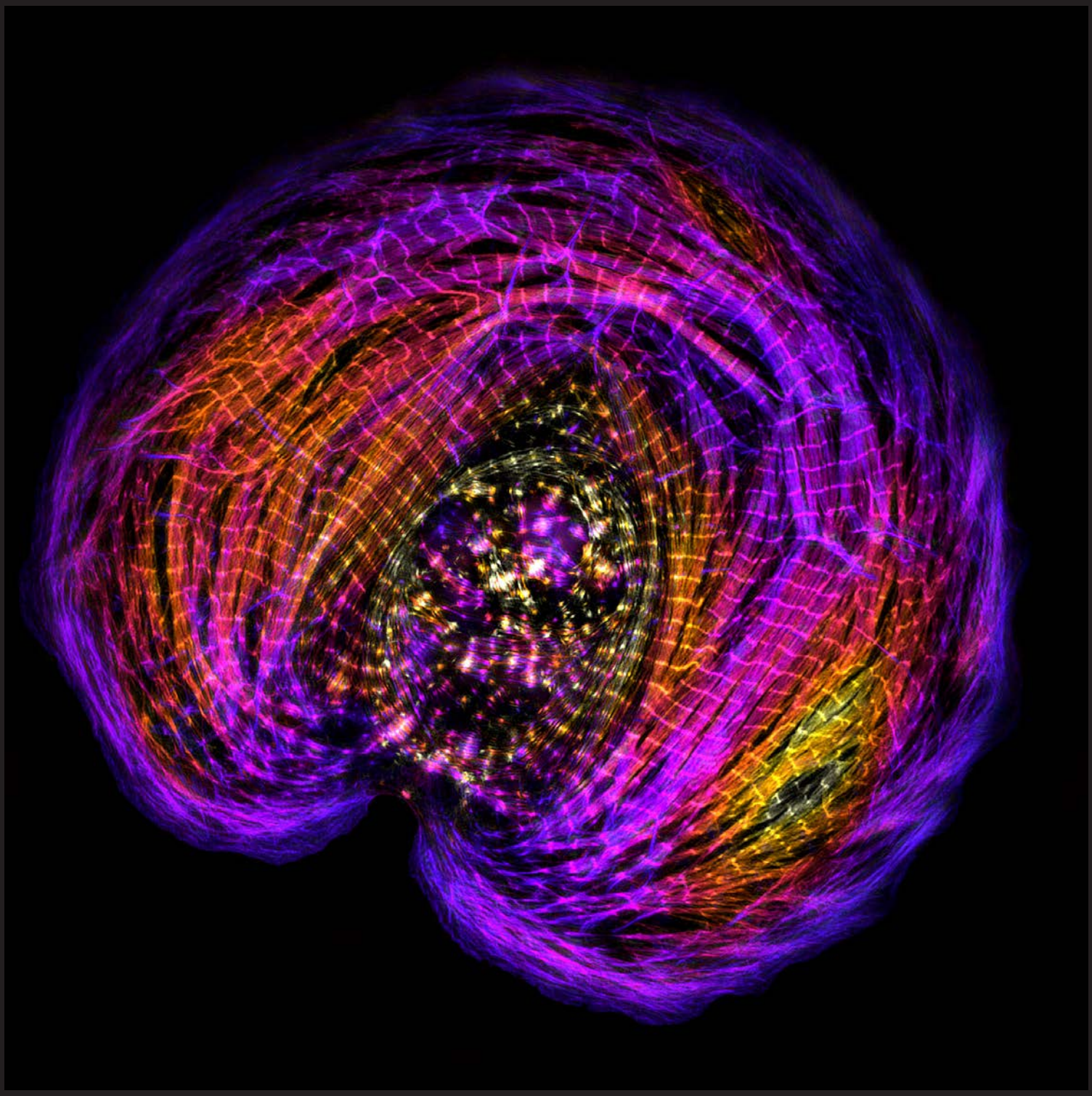
Department of Cell & Developmental Biology
Sixteenth Annual Retreat
May 18, 2018



Nelson Andrews Leadership Lodge

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Integrated Biological Systems Training in Oncology & The Program in Developmental Biology

Agenda

8:00 - 8:30 am	**BREAKFAST AND POSTER SESSION I SETUP**
8:30 - 8:45 am	**State of the Department Address by Ian Macara, Ph.D., Chair**
8:50 - 9:35 am	**First Session Talks- Moderated by Mark de Caestecker** Beth Lawrence (Zanic), Sierra Palumbos (Miller), Matt Zanotelli (Reinhart-King)
9:40 - 10:55 am	**Poster Session I (ODD NUMBERS)** **Breakout Session I (Students/Post Docs Only) Moderated by Meagan Postema** Alejandra Romero-Morales (Gama), Veronica Farmer (Zanic), Lauren Scarfe (de Caestecker), Angela Howard (Page-McCaw), Jessica Tumolo (MacGurn)
11:00- 11:45 am	**Second Session Talks - Moderated by Chin Chiang** Leslie Meenderink (Tyska), Sierra Cullati (Gould), April Weissmiller (Tansey)
11:45 am-12:30 pm	**LUNCH AND POSTER SESSION II SETUP**
12:30- 2:30 pm	**ACTIVITIES AND FREE TIME** Escorts will guide to sponsored activities
2:30 -3:15 pm	**Third Session Talks- Moderated by Ed Levine** Indrayani Waghmare (Page-McCaw), Merlyn Emmanuel (Weaver), Gabrielle Rushing (Ihrie)
3:20 - 4:35 pm	**Poster Session II (EVEN NUMBERS)** **Breakout Session II (Students/Post Docs Only) Moderated by Lindsey Seldin** Cherie Scurrah (Lau), Anneke Sanders (Kaverina), Chloe Snider (Gould), John Snow (Ess), Natalya Ortolano (Gama)
4:40 - 5:25 pm	**Fourth Session Talks- Moderated by Alissa Weaver** Zach Sandusky (Lannigan), Roslin Joseph Thoppil (Kaverina), Lindsey Seldin (Macara)
5:25 - 5:30 pm	**Award Presentations by Andrea Page-McCaw**
5:30 - 8:30	**RECEPTION**

Abbie Neininger
2018 Retreat Cover Art Winner

Abbie's Research Project Description

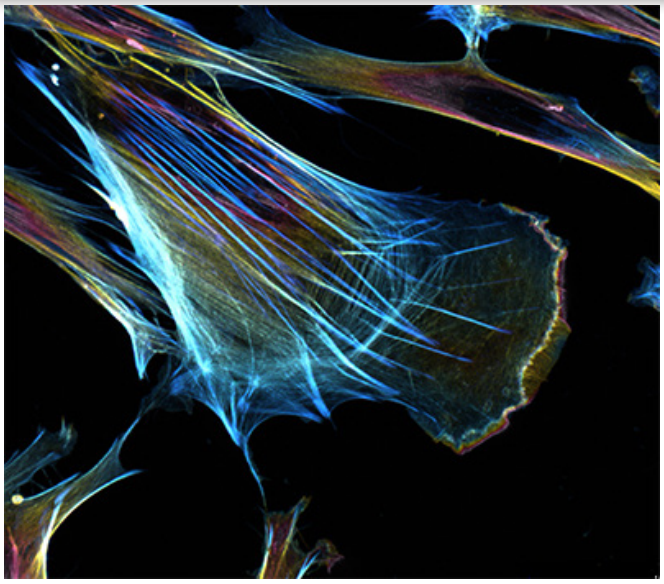
Abbie is working on how cardiomyocytes become bi-nucleated (i.e., obtain two nuclei). Bi-nucleation terminally blocks the re-entry of cardiomyocytes into the cell cycle and, thus, impedes the ability of heart muscle to regenerate after injury. Abbie has recently performed a small molecule screen using high content microscopy and identified 10 kinases required to prevent bi-nucleation. This image shows one of the cardiomyocytes used in her research.

Front Cover Art Caption

Spreading cardiomyocyte 24 hours post-plating stained for F-actin. Credit: Abigail Neininger

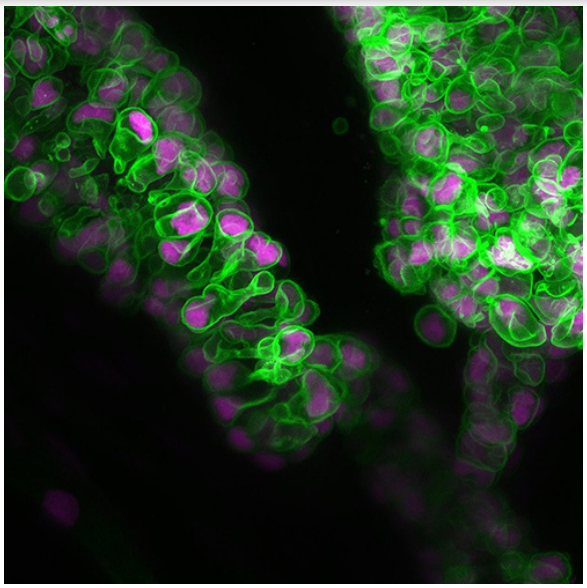
2nd Place - Shwetha Narasimhan

Actin stress fibers in a polarized lung fibroblast cell with temporal color coding; black- bottom of stack and white- top of stack



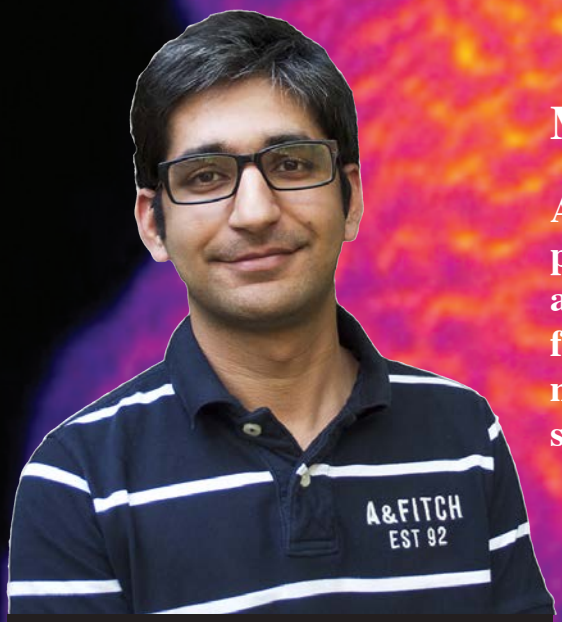
3rd Place - Gokhan Unlu

Maximum intensity projection of live zebrafish cartilage z-stacks from round mutant embryo. Transgenic labeling marks chondrocytes with cell membrane marker, caax-EGFP (green), and nucleus marker, H2A-mCherry (magenta).



Additional image submissions are at the end of this book.

First Place Movie Winners



First Place Movie Winner
Nilay Taneja

Movie Description

A human cardiomyocyte derived from induced pluripotent stem cells, expressing the protein alpha actinin that marks the Z-discs of sarcomeres, the fundamental unit of contraction in the heart. The movie shows a cardiomyocyte forming the Z-discs of sarcomeres over a time period of 36 hours.

Click Here To Watch Movies!

First Place Movie Winner
Meredith Weck

Movie Description

Spinning disk confocal imaging of a HeLa cell with induced heterodimerization between the motor domain of the filopodial myosin transporter, Myo10 (green), and a protocadherin adhesion protein, PCDH24 (magenta). 8 fps, 30 sec intervals between frames.

Watch these and other movie submissions on the CDB website via this link. You will be asked to use your VUnetID and Password to view these movies.

Oral Presentations - Nelson Andrews Leadership Lodge

ORAL PRESENTATIONS

Nelson Andrews Leadership Lodge

First Session 8:50 – 9:35 Moderated by Mark de Caestecker

Human CLASP2 specifically regulates microtubule catastrophe and rescue

Elizabeth J. Lawrence, Göker Arpağ, Stephen R. Norris, Marija Zanic

The Wnt modulator, SFRP-1, regulates gap junction specificity in the C. elegans motor circuit

Sierra Palumbos, Amanda Mitchell, Rebecca McWhirter, David M. Miller, III

Cellular energetic requirements during metastatic cell migration in complex microenvironments

Matthew R. Zanotelli, Aniqua Rahman-Zaman, Francois Bordeleau, Joseph P. Miller, Zachary E. Goldblatt, Paul V. Taufalele, Jacob A. Vanderburgh, Cynthia A. Reinhart-King

Second Session 11:00 – 11:45 Moderated by Chin Chiang

Microvillar motility is driven by actin assembly and facilitates intermicrovillar adhesion

L.M. Meenderink, B.A. Millis, M.J. Tyska

A tail of kinase regulation: how C-termini modulate CK1 substrate phosphorylation

Sierra N. Cullati, Zachary C. Elmore, Jun-Song Chen, and Kathleen L. Gould

Inhibition of MYC by the SMARCB1 tumor suppressor

April M. Weissmiller, Jing Wang, Shelly L. Lorey, Gregory C. Howard, Ernest Martinez, Qi Liu, William P. Tansey

Third Session 2:30 – 3:15 Moderated by Ed Levine

Dally-like differentially regulates Wnt ligands in the Drosophila germarium to promote germline stem cell maintenance and differentiation

Indrayani Waghmare, Xiaoxi Wang, Andrea Page-McCaw

Exosome secretion in stromal matrix assembly and organization

Merlyn Emmanuel, Bong Hwan Sung, Wanessa F. Alteiand Alissa M. Weaver

Subgroups of mouse neural stem cells have differential basal mTORC1 activity and distinct responses to TSC2 loss

Gabrielle V. Rushing, Asa A. Brockman, Nalin Leelatian, Madelyn K. Bollig, Bret C. Mobley, Jonathan M. Irish, Cary Fu, Kevin C. Ess, Rebecca A. Ihrie

Fourth Session 4:40 – 5:25 Moderated by Alissa Weaver

ERK1/2-RSK2 signaling is a developmental switch required for estrogen homeostasis

Katarzyna A. Ludwik, Zachary M. Sandusky*, Kimberly M. Stauffer, Kelli L. Boyd, Thomas P. Stricker and Deborah A. Lannigan*

PCLASP1 is required for CLASP2 localization and function at microtubules in interphase cells

Roslin J. Thoppil, Anna A.W.M Sanders, Elizabeth J. Lawrence, Kevin Chang, Shwetha Narasimhan, Marija Zanic and Irina Kaverina

Mammary myoepithelial cells respond to damage by altering cell fate in vivo

Lindsey Seldin and Ian Macara

Human CLASP2 specifically regulates microtubule catastrophe and rescue

Elizabeth J. Lawrence, Göker Arpağ, Stephen R. Norris and Marija Zanic

Cytoplasmic Linker-Associated Proteins (CLASPs) are microtubule-associated proteins essential for microtubule regulation in many cellular processes. However, the molecular mechanisms underlying CLASP activity are not understood. Here, we use purified protein components and Total Internal Reflection Fluorescence (TIRF) microscopy to investigate the effects of human CLASP2 on microtubule dynamics *in vitro*. We demonstrate that CLASP2 suppresses microtubule catastrophe and promotes rescue without affecting the rates of microtubule growth or shrinkage. Strikingly, when CLASP2 is combined with EB1, a known binding partner, the effects on microtubule dynamics are strongly enhanced. We show that synergy between CLASP2 and EB1 is dependent on a direct interaction, since a truncated EB1 protein that lacks the CLASP2-binding domain does not enhance CLASP2 activity. Further, we find that EB1 targets CLASP2 to microtubules and increases the dwell time of CLASP2 at microtubule tips. Although the temporally averaged microtubule growth rates are unaffected by CLASP2, we find that microtubules grown with CLASP2 display greater variability in growth rates. Our results provide insight into the regulation of microtubule dynamics by CLASP proteins and highlight the importance of the functional interplay between regulatory proteins at dynamic microtubule ends.

The Wnt modulator, SFRP-1, regulates gap junction specificity in the *C. elegans* motor circuit

Sierra Palumbos, Amanda Mitchell, Rebecca McWhirter and

David M. Miller, III

Gap junctions mediate intercellular communication in the nervous system where they function as conduits for ionic currents that electrically couple partner neurons. Although much has been learned about the cell biological pathways that govern gap junction assembly, little is known of the mechanisms that orchestrate this machinery to create gap junctions between specific neurons. We are addressing this question by exploiting a simple circuit in *C. elegans* in which adjacent motor neurons adopt gap junctions with different synaptic partners. VA and VB motor neurons arise as sister cells but are connected to specific presynaptic interneurons for either backward (AVA) or forward (AVB) locomotion. The UNC-4 transcription factor is selectively expressed in VA neurons to maintain AVA inputs. *unc-4* mutants are unable to crawl backward because VA neurons are miswired with gap junctions from AVB interneurons. We have previously shown that *unc-4* antagonizes an EGL-20/Wnt signaling pathway to prevent the formation of VB-type gap junctions but the UNC-4 targets that regulate this effect were unknown. To address this question, we used a new cell-specific profiling strategy, SeqCel (RNA-Seq of *C. elegans* cells) to identify ~300 candidate *unc-4*-regulated transcripts. Interestingly, the secreted frizzled receptor protein, SFRP-1, was detected in this data set as a downstream target that is positively regulated by *unc-4*. Because secreted frizzled receptor proteins have been shown to antagonize Wnt signaling, we hypothesized that *unc-4* promotes SFRP-1 expression to block Wnt-dependent miswiring of VA neurons. In support of this idea, fluorescence *in situ* hybridization (FISH) assays confirmed that *unc-4* is required for *sfrp-1* expression in VA neurons and a behavioral test showed that reduced *sfrp-1* function enhances the backward movement defect of a weak *unc-4* allele. In addition, we used immunostaining to detect AVB gap junctions with VA neurons in *sfrp-1* mutants. Thus, we propose that UNC-4 drives *sfrp-1* expression to prevent VA neurons from responding to an available EGL-20/Wnt cue. Interestingly, this effect is limited to a subset of VA neurons in the posterior region in which EGL-20/Wnt is expressed, thus, we expect that ongoing analysis of our SeqCeL data set will reveal UNC-4 targets that regulate additional downstream pathways in anterior VA neurons to control gap junction specificity.

Cellular energetic requirements during metastatic cell migration in complex microenvironments

Matthew R. Zanotelli, Aniqua Rahman-Zaman, Francois Bordeleau, Joseph P. Miller, Zachary E. Goldblatt, Paul V. Taufalele, Jacob A. Vanderburgh and Cynthia A. Reinhart-King

Cancer cell migration and invasion through the stromal extracellular matrix (ECM) is a critical aspect of cancer metastasis that demands cells to dynamically coordinate cellular machinery to overcome challenging physical barriers found in the tumor microenvironment. During solid tumor progression, the ECM commonly becomes deregulated, resulting in mechanically heterogeneous structures, organization, rigidity, and composition. To overcome these barriers and migrate, cancer cells need to reorganize their cytoskeleton, remodel the surrounding matrix fibers and/or squeeze between tight interstitial spaces between matrix fibers. Such migration events are energetically demanding. While significant research has been conducted to understand the molecular mechanisms guiding metastatic migration, less is known about cellular energy regulation and utilization during three-dimensional (3D) cell migration. Here, we examined the role of mechanical cues in the ECM on cellular energetic requirements during metastatic cell migration. Using the fluorescent ATP:ADP biosensor, PercevalHR, and the fluorescent glucose analog, 2-NBDG, we show that microenvironmental changes known to facilitate migration are linked to alterations in metabolic activity and cellular energy utilization. MDA-MB-231 highly metastatic breast cancer cells increased glucose uptake, intracellular ATP:ADP ratio, and ATP hydrolysis in denser collagen matrices that inhibit migration and are more challenging to transverse. Time-lapse studies demonstrated that energy utilization directly correlated with migration, as spikes in ATP consumption were correlated to increases in cell stepwise speed, and this relationship was dependent on matrix density. In aligned collagen matrices that facilitate migration, MDA-MB-231 energy consumption and ATP:ADP ratio decreased. Cell-scale microtracks in the interstitial matrix have also been observed as conduits for trafficking cells in vivo as they provide physical guidance and the path of least resistance for migration. The dimensions of these microtracks impose varying levels of confinement upon cells, which influences migration. To better understand the role of confinement in decision making during migration, we utilized micromolding to create Y-shaped bifurcated 3D collagen microtracks with channels of varying width at the bifurcation. We show that MDA-MB-231 cells preferentially migrate in the direction of least confinement and migrating into more confined tracks increased intracellular ATP:ADP ratio. Together, these findings demonstrate that one mechanism by which the mechanical properties of the local ECM affect 3D metastatic invasion is through altering the energetic requirements for cells to migrate.

Microvillar motility is driven by actin assembly and facilitates intermicrovillar adhesion

L.M. Meenderink, B.A. Millis and M.J. Tyska

The brush border is the sole site of nutrient absorption, but also provides a physical barrier preventing access of pathogens into the peripheral vasculature and tissues. Apically located, the brush border contains up to one thousand tightly packed microvilli, each containing a central core of 20-30 parallel actin filaments coated in membrane. During infection with A/E pathogens, normal apical architecture is destroyed followed by formation of motile actin pedestals at sites of bacterial attachment. Decreased microvillar density results in malabsorption and osmotic diarrhea, contributing to human disease. Though a properly formed brush border is critical for maintaining homeostasis at this key physiological interface with environmental bacteria, the spatiotemporal dynamics underlying early microvillar growth and initial stabilization are not known. Based on electron micrographs from our lab, a time series of polarized tissue culture cells (previously published) and mouse tissue with an intact crypt villus axis, early microvilli are sparse (individual or small clusters) with formation of larger clusters over time until optimal packing is achieved at maturation. We know clusters and packing are facilitated by cadherin-based intermicrovillar adhesion at microvillar tips, but the dynamic steps connecting these stages in maturation have not been visualized in live cells. We therefore used spinning disk confocal microscopy to image live epithelial cells; our time series observations revealed that that early, low density microvilli are highly dynamic with actin cores coated in membrane both protruding above and translocating across the cell surface. Only the distal tips of translocating microvilli are coated in membrane with a prominent internal actin rootlet. Microvillar translocation requires actin assembly but not myosin contractility. Microvillar motility is blocked by cytochalasin treatment, implicating actin assembly at the tips of microvilli powers movement. Finally, motility facilitates joining of individual microvilli into small clusters, as well as incorporation of small clusters into larger groups. We propose that this previously uncharacterized form of motility serves to distribute microvilli on the cell surface, and thus facilitates the packing of these protrusions during epithelial differentiation.

A tail of kinase regulation: How C-termini modulate CK1 substrate phosphorylation

Sierra N. Cullati, Zachary C. Elmore, Jun-Song Chen and Kathleen L. Gould

CK1 enzymes signal in a variety of cellular pathways, including DNA damage repair, mitotic checkpoint signaling, circadian rhythm, Wnt signaling, endocytosis, and neurodegenerative disease progression. Like other multifunctional kinases, CK1 must be regulated in space and time to target specific subsets of its substrates in each of the pathways it participates in. However, CK1 is generally regarded as a "rogue" kinase, which is constitutively active, ubiquitous throughout cells and tissues, and unregulated except by autoinhibition.

CK1 enzymes are known to autophosphorylate their C-terminal non-catalytic tails, which are proposed to inhibit their activity by acting as pseudosubstrates. This model would require a phosphorylation-dependent intramolecular interaction between the C-terminus and the kinase domain, but we are unaware of any evidence demonstrating such an event. Furthermore, this proposed mechanism of autoinhibition has not been tested *in vivo* in any organism.

We have identified six serine and threonine autophosphorylation sites on the C-terminus of *Schizosaccharomyces pombe* Hhp1, one of two soluble CK1 enzymes in this organism, and are testing candidate sites on Hhp2 and the human homologues CK1δ/ε. When these sites are specifically phosphorylated, the Hhp1 C-terminus binds the kinase domain via a low-affinity electrostatic interaction. At concentrations above the K_d of this interaction, the phosphorylated C-terminus inhibits Hhp1 kinase activity, while mutations that abolish phosphorylation increase the activity of the full-length kinase.

Structural studies have identified two conserved basic patches on the CK1 kinase domain that are hypothesized to interact with primed substrates and the phosphorylated C-terminus, respectively; however, we have found that mutation of either site abolishes tail binding and affects kinase activity in unexpected ways. As an alternative, we posit that substrates and the C-terminus compete for access to the same binding site, and the higher affinity of substrates is sufficient to displace the tail and activate the kinase. Because the C-termini of CK1 family members are responsible for nearly all of the sequence variability between isoforms, this mechanism can also explain variations in substrate specificities between CK1 enzymes. Current work is focused on identifying this binding site and determining whether different CK1 substrates require different levels of CK1 activity. We are also utilizing phosphorylation site and binding site mutants of *hhp1* to investigate the significance of autophosphorylation *in vivo*. With this work, we aim to determine the functional consequences of autophosphorylation and elucidate conserved mechanisms of regulation for the CK1 family of enzymes, which are apical mediators of cell signaling.

Inhibition of MYC by the SMARCB1 tumor suppressor

April M. Weissmiller, Jing Wang, Shelly L. Lorey, Gregory C. Howard, Ernest Martinez, Qi Liu and William P. Tansey

The SMARCB1 tumor suppressor (SNF5) is a known interaction partner of the oncoprotein c-MYC (MYC), and thought to serve as a co-activator of its transcriptional properties. This role of SNF5, however, is at odds with the tumor suppressor function of SNF5, and with findings that loss of SNF5 is associated with activation of MYC target gene signatures. We have used biochemical, genetic, and genome-wide approaches to reexamine the relationship of SNF5 with MYC within the context of malignant rhabdoid tumor (MRT), an aggressive childhood cancer in which SNF5 is lost. We find that, consistent with activation of MYC target gene signatures in tumors that lack SNF5, MYC function is important for multiple aspects of MRT biology. Surprisingly, SNF5 is capable of inhibiting MYC binding to E boxes *in vitro* and globally within the context of chromatin. Using ATAC-seq and PRO-Seq, we demonstrate that regulation of MYC binding by SNF5 is independent of the role of SNF5 in chromatin remodeling, but instead is responsible for controlling RNA polymerase pause release at MYC-regulated genes. These findings inform a novel model of MRT tumorigenesis in which loss of SNF5 derepresses MYC function, raising the possibility that MYC contributes to disease progression in this malignancy.

Dally-like differentially regulates Wnt ligands in the *Drosophila* germarium to promote germline stem cell maintenance and differentiation

Indrayani Waghmare, Xiaoxi Wang and Andrea Page-McCaw

The maintenance, proliferation, and survival of many cell types in the *Drosophila* germarium depend on coordinated activities of short and long-range signaling activities of several signaling pathways. The signaling pathways are activated by secreted ligands such as Hedgehog, Decapentaplegic, Unpaired, and several members of the Wnt family. Proper extracellular spreading of secreted ligands is important to ensure proper signaling activity and specificity in the target cells. The extracellular spread of ligands has been explained by three mechanisms in the *Drosophila* germarium. These include 1) cytoneme mediated spread of Hedgehog, 2) Dally mediated regulation of short-range Decapentaplegic ligand spread and activity to maintain germline stem cell niche, and 3) Dally-like mediated long-range spread of Wingless to follicle stem cells. Both Dally and Dally-like are cell-surface heparan sulfate proteoglycans that regulate the extracellular spread of Wingless, Hedgehog, Decapentaplegic, and Unpaired in other tissues. In this study, we investigate the role of Dally-like in regulation of different Wnt ligands in the germarium. The *Drosophila* germarium expresses Wingless, Wnt2, Wnt4 and Wnt6 ligands. Wingless and Wnt6 are required for maintenance of germline stem cell niche, whereas Wnt2 and Wnt4 maintain differentiation niche via several mechanisms. Mutations or genetic manipulation of Wnt signaling pathway components in the germarium disrupts early oogenesis. Here, we show that overexpression of Dally-like in the differentiation niche results in loss of germline differentiation (resulting in ovarian tumor phenotype) and loss of germline stem cells. Interestingly, while all four Wnts expressed in the germarium can bind to Dally-like in S2R+ cells, only Wingless and Wnt4 overexpression in the differentiation niche partially suppress the tumor phenotype, whereas overexpression of all four Wnt ligands—Wg, Wnt2, Wnt4, and Wnt6 suppresses the germline stem cell loss phenotype. Our results suggest that Dally-like has multiple distinct Wnt ligand binding sites, and differentially regulates the spatial distribution and activity of Wnt ligands to regulate the maintenance of germline stem cell and differentiation niches.

Exosome secretion in stromal matrix assembly and organization

Merlyn Emmanuel, Bong Hwan Sung, Wanessa F. Altei, Alissa M. Weaver

Fibrosis is a scarring process that develops when the body's natural wound-healing process becomes unregulated and there is excessive production and/or assembly of extracellular matrix proteins, such as fibronectin and collagen, by fibroblasts. Fibrosis contributes to many diseases including cardiac dysfunction and cancer progression. Therefore, understanding the fundamentals of how ECM is secreted and assembled by fibroblasts remains an important question. We recently made the finding that deposition of fibronectin (FN) by fibrosarcoma cells depends on the endolysosomal secretion of extracellular vesicles called "exosomes" (1). The goal of this study was to determine whether fibroblasts use exosomes to assemble FN. A preliminary proteomic study on exosomes isolated from hTERT immortalized human mammary fibroblasts identified ECM and ECM associated molecules. Using an improved density gradient purification protocol many of these factors copurify with exosomes. Further, inhibition of exosome secretion in fibroblasts led to a decrease in FN assembly in 2D and affected matrix alignment in 3D. Confocal immunofluorescence imaging revealed that integrin α5β1 regulates sorting of FN into the lumen of MVBs and possibly its association and secretion via exosomes. Finally, we developed an *in vitro* FN assembly assay using purified exosomes and purified FN. Interestingly, we observed *de novo* FN fibril formation is stimulated by exosomes. This assembly is enhanced by addition of $Mn^{2+}$to activate integrins and decreased when using exosomes purified from ITGA5-knockdown cells. Altogether our data suggest that exosomes are critical lipid-based platforms that promote ECM assembly. We expect that these studies will change the model for how ECM is assembled by cells and may identify new ways to treat fibrotic diseases.

Subgroups of mouse neural stem cells have differential basal mTORC1 activity and distinct responses to TSC2 loss

Gabrielle V. Rushing, Asa A. Brockman, Nalin Leelatian, Madelyn K. Bollig, Bret C. Mobley, Jonathan M. Irish, Cary Fu, Kevin C. Ess and Rebecca A. Ihrie

Subependymal giant cell astrocytomas (SEGAs) present in a subset of patients with Tuberous Sclerosis Complex (TSC), a neurodevelopmental disorder caused by mutations in TSC1/2. Interestingly, SEGAs are consistently located along the ventral portion of the ventricular-subventricular zone (V-SVZ) stem cell niche, suggesting a tight connection between anatomic location and disease pathogenesis. Neural stem/progenitor cells (NSPCs) in the V-SVZ are the proposed cell(s) of origin for these tumors. These NSPCs possess a positional identity that arises early in development, is defined by the expression of location-specific transcription factors (TFs), and predicts the type of progeny they produce. Since TSC is a disease of increased mTORC1 signaling, this suggested that signaling activity may be closely linked to positional identity. Therefore, we hypothesized that cells from the dorsal and ventral V-SVZ are differentially susceptible to mutations driving TSC pathogenesis, and that they harbor distinct mTORC1 signaling profiles. Upon removal of Tsc2 in the dorsal V-SVZ, mice died perinatally from seizures whereas in mice with Tsc2 removed in the ventral V-SVZ, nodular protrusions resembling patient tumors were observed. Dorsal and ventral lesions from TSC patients also displayed differential expression of location-specific TFs, consistent with findings in mouse. Higher basal mTORC1 signaling was observed in ventral V-SVZ cells compared to matched dorsal counterparts. This pattern was apparent in acutely isolated NSPCs, retained in cultured NSPCs, and visible in immunofluorescent staining of mouse and human tissue. This work reveals that positional identity also includes stereotypic basal signaling activity and that differing levels of growth pathway signaling in subregions of the V-SVZ are connected to distinct predispositions to disease phenotypes.

ERK1/2-RSK2 signaling is a developmental switch required for estrogen homeostasis

Katarzyna A. Ludwik, Zachary M. Sandusky, Kimberly M. Stauffer, Kelli L. Boyd, Thomas P. Stricker and Deborah A. Lannigan

Estrogen receptor alpha (ERa) is a critical regulator of adult homeostasis. In response to estrogens, ERa is degraded; however, despite the continued presence of estrogen prior to menopause, ERa protein levels are maintained in the adult by an unidentified mechanism. We discovered a negative feedback pathway in which estrogens increase the activity of ERK1/2-RSK2 in the adult mammary gland and uterus. Subsequently, activated RSK2 limits ERa-mediated transcription and reduces degradation of ERa through the 26S proteasome pathway. The ERK1/2-RSK2 pathway is temporally activated at the estrus phase during the estrous cycle. RSK2 is not required to maintain ERa protein levels in juvenile animals. Mammary gland regeneration demonstrated that the reduction in ERa in the RSK2 knockout (RSK2-KO) mice was intrinsic to the epithelium. Oophorectomy or inhibition of the 26S proteasome pathway restored ERa protein levels in the RSK2-KO. Transcriptomic analysis of the mature ER+ population in the mammary gland revealed enrichment for estrogen-responsive genes in the RSK2-KO compared to the wild type. This inappropriate estrogen response increased the number of double-strand DNA breaks, which resulted in retarded alveolar expansion during pregnancy and decreased fertility. These findings establish RSK2 as critical for estrogen homeostasis in the pre-menopausal adult.

CLASP1 is required for CLASP2 localization and function at microtubules in interphase cells

Roslin J. Thoppil, Anna A.W.M Sanders, Elizabeth J. Lawrence, Kevin Chang, Shwetha Narasimhan, Marija Zanic and Irina Kaverina

CLIP-associated proteins (CLASPs) are highly conserved microtubule (MT) plus-end tracking proteins (+TIPs) that regulate MT dynamics by promoting MT rescue and enhancing MT stability. Additionally, CLASPs are critical for nucleation of Golgi-derived microtubules (GDMTs). Human CLASPs consist of two paralogs: CLASP1 and CLASP2. Although both CLASPs have been structurally and functionally characterized in depth, mutual regulatory interactions between these two proteins have not been explored. In our in vitro reconstitution assays, CLASP2 by itself strongly promotes MT nucleation. However, decrease of GDMT nucleation in cells depleted of CLASP1 alone was as prominent as the effect of depletion of both proteins. Interestingly, we find that while CLASP2 depletion does not interfere with cellular CLASP1 localization, the loss of CLASP1 dramatically influences CLASP2 localization in interphase. In particular, CLASP2 is no longer associated with MT plus ends, while other MT +TIPs were not affected, and CLASP2 still retained at the Golgi. This effect suggests that CLASP1 specifically facilitates CLASP2 localization at the MT plus ends. Addressing potential underlying mechanisms, we tested if CLASP1 was involved in regulating CLASP2 phosphorylation by GSK3β, which is known to abolish CLASP2 binding to MTs. However, we found that CLASP1 did not modify CLASP2 phosphorylation levels. Moreover, when GSK3β was inhibited in CLASP1-depleted cells, CLASP2 localization to MTs was enforced. These observations indicate that CLASP1 is capable to localize CLASP2 to MTs despite its physiological phosphorylation level. We have further tested whether CLASP1 forms a complex with CLASP2 to promote its localization to MTs. Since prior evidence indicates that CLASP2 can homodimerize via a C-terminal coiled-coil region, it is possible that CLASP1 heterodimerizes with CLASP2 in order to target it to MTs. Indeed, our co-immunoprecipitation assays show that CLASP1 forms a complex with CLASP2 in cells and enforced targeting of CLASP2 to mitochondria facilitates relocation of CLASP1 to the same location. We therefore conclude that CLASP1 recruits CLASP2 to MTs by a complex formation. Our data further show that restoring CLASP2 localization to MTs can rescue MT nucleation in CLASP1-depleted cells and conclude that CLASP1-dependent localization of CLASP2 to MT ends is necessary for CLASP2 function in cells, and thus CLASP1 is upstream of CLASP2 in regulating MT nucleation and dynamics.

Mammary myoepithelial cells respond to damage by altering cell fate *in vivo*

Lindsey Seldin and Ian Macara

Metastatic breast cancer is the second leading cause of cancer-associated deaths in women. Nevertheless, the molecular mechanisms underlying breast cancer initiation, metastasis and recurrence following chemotherapy remain poorly understood. The mouse mammary gland provides a robust mammalian system for investigating how epithelial behavior impacts tumorigenesis. The mammary epithelium is composed primarily of two lineage-restricted unipotent cell populations, an inner layer of milk-producing luminal cells and an outer layer of contractile myoepithelial cells. Nevertheless, despite their unipotent nature in situ, myoepithelial cells that have been cultured and passaged demonstrate a remarkable capacity to generate de novo mammary glands upon transplantation. We hypothesize that this distinction in myoepithelial plasticity between in vivo and culture conditions is mediated by a damage response. To test this hypothesis, we established an inducible transgenic mouse model that allows for in vivo lineage tracing of myoepithelial cells in the presence or absence of two distinct types of damage: mechanical damage and DNA damage. We find that upon mechanical damage, myoepithelial cells remain unipotent. Strikingly, however, myoepithelial cells become multipotent in response to DNA damage, giving rise to hyperproliferative luminal cells that cause aberrant luminal filling reminiscent of ductal carcinoma in situ (DCIS). Notably, this damage response has no effect on the structural integrity of the myoepithelial monolayer. These findings not only reveal a myoepithelial-specific DNA damage response that triggers cellular reprogramming, but also provide important insight into a potential cause of breast cancer recurrence following chemotherapy, a treatment that exploits DNA-damaging agents. These studies highlight the remarkable plasticity of mammary cell states. To determine whether this damage response is specific to mammary epithelium, we utilized a similar approach to test whether DNA damage would elicit cellular reprogramming in mouse epidermis. Remarkably, mouse epidermal progenitors become hyperproliferative and exhibit lineage infidelity, akin to our findings in the mammary gland. Important goals are to determine the molecular mechanisms that underlie this cellular plasticity and the unusual epithelial tissue response to DNA damage.

Post Doc/ Graduate Student Breakout Sessions

Morning Session - 9:40 a.m. – 10:55 a.m. Moderator: Meagan Postema

Speaker	*Tagline*	*Lab*
Alejandra Romero-Morales	*The role of MCL-1 in early brain development*	Gama
Veronica Farmer	*The role of GTP hydrolysis in microtubule stability*	Zanic
Lauren Scarfe	*Use of a Phenotypic Screen to Identify Small Molecules that Selectively Enhance Repair in Certain Models of Acute Kidney Injury*	deCaestecker
Angela Howard	*Understanding basement membrane damage using dextran sodium sulfate*	
Jessica Tumolo	*Regulation of endocytic trafficking by a Snf1-related kinase*	MacGurn

Afternoon Session – 3:20 p.m. – 4:35 p.m. Moderator: Lindsey Seldin

Speaker	*Tagline*	*Lab*
Cherie Scurrah	*Non-stem cell-of-origin of colon cancer*	Lau
Anneke Sanders	*Anchoring of newly formed microtubules at the Golgi: potential roles of CLASP2 and CAMSAP*	Kaverina
Chloe Snider	*Mechanisms of cytokinetic ring anchoring to the plasma membrane*	Gould
John Snow	*Creating and investigating an induced pluripotent stem cell derived neuronal model for a neurodevelopmental disease, Alternating Hemiplegia of Childhood*	Ess
Natalya Ortolano	*Revealing the CUL9-APC/C connection during human cortical differentiation*	Gama

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Dynamics of exocyst-mediated vesicle tethering at the epithelial plasma membrane

Mukhtar Ahmed, Hisayo Fukuda, Ian Macara

Cargo is constantly delivered in a polarized fashion to the plasma membrane of epithelial cells. Incoming vesicles are docked and tethered to the plasma membrane by an octameric protein complex, known as the exocyst, before SNARE-mediated vesicle fusion can occur. While the exocyst complex has been intensively studied in yeast, less is known about the mechanism and dynamics of the exocyst complex in mammalian cells. To address this problem, we used CRISPR/Cas9-mediated gene-editing to incorporate fluorescent protein tags in frame with the coding sequence of five of the eight exocyst subunits at their endogenous loci. After validating functionality of the fusion proteins, we exploited these cell lines to investigate the arrival and departure itineraries of individual exocyst subunits at vesicle fusion sites on the basal surface of mammary epithelial cells, using total internal reflection microscopy (TIRFM) with high temporal resolution and single molecule sensitivity. Surprisingly, we found that while each of the five of subunits that were tested (Sec3-GFP, Sec5-GFP, Sec6-GFP, Sec8-GFP and Exo70-GFP) arrive at the vesicle fusion site at around the same time, before vesicle fusion, Sec3 frequently departs just prior to fusion, while the other 4 subunits leave slightly after fusion has occurred. We estimate that about 7 Sec3 subunits and ~9 of each of the other subunits are associated with each vesicle. Using TIRFM and quantitative proteomics, we found that the subunit connectivity is similar to the yeast exocyst complex, but that in mammals there are two individual subcomplexes, that arrive at and associate with the plasma membrane independently of each other. However, the complete octamer is a requirement for vesicle fusion. Our data indicate that the exocyst is not a stable complex in mammalian cells, and further lead us to suggest that only one of the subcomplexes is required for vesicle tethering, while the other may be involved in vesicle fusion. Based on these experiments we propose a new model for exocyst complex assembly and dynamics.

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WDR5 as an anti-leukemia target

Erin R. Aho, Caleb Howard, Jing Wang, Lance Thomas, Sabine Wenzel, Shelly Lorey, Pankaj Acharya, Scott Hiebert, Qi Lui, Rocco Gogliotti, Shaun Stauffer, Stephen W. Fesik, and William P. Tansey

MLL leukemia is a disease with extremely poor patient prognosis that is caused by expression of an MLL1-fusion protein. MLL1 is a histone methyltransferase capable of methylating lysine 4 of histone H3, an epigenetic mark associated with active gene transcription. One popular model for MLL leukemogenesis posits that a protein complex containing the WT MLL1 protein and a complex containing the MLL1-fusion protein cooperate to drive overexpression of genes sufficient for leukemogenesis, and the catalytic activity of the WT complex is dependent upon the interaction between the proteins WDR5 and MLL1. Based on this model, inhibiting the MLL1-binding site of WDR5 should have profound implications for the development of a highly sought-after targeted therapy for MLL leukemia. The anti-leukemogenic potential of inhibiting WDR5 has led to collaboration between the Tansey and Fesik laboratories at Vanderbilt University to discover small molecules that block the MLL1-binding site of WDR5. These novel WDR5 inhibitors are being employed as tool compounds to explore the utility of WDR5 inhibition in treating MLL leukemias and to challenge the current model for MLL leukemogenesis. Together, our approach will generate a comprehensive profile of the cellular phenotypic changes that occur after WDR5 inhibition, determine how WDR5 inhibition affects global gene expression and epigenetic regulation, and understand how these changes selectively reduce proliferation of certain cancer cell types.

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SAN1 – a senataxin associated nuclease required for the repair of interstrand-crosslinks

Alex Andrews, Heather McCartney, Tim Errington, Alan D'Andrea, Ian Macara

The DNA damage response (DDR) is a set of complex signaling pathways capable of sensing DNA damage, and activating a large number of enzymes involved in the remodeling and repair of the genome. Mutations in the genes involved in the DDR lead to DNA damage, genomic instability, and various cancers. One particularly dangerous type of DNA damage that can occur is an interstrand crosslink (ICL). ICLs can lead to the development of double strand breaks through the blockage of DNA replication and transcription. Although ICLs can arise endogenously from molecules such as aldehydes, most commonly they are induced from chemotherapeutic drugs such as Cisplatin and Mitomycin C (MMC). These drugs are commonly used in the treatment of breast and ovarian cancers. A better understanding of which proteins are involved in the repair of ICLs is critical for understanding resistance, toxicity, and response in patients treated with ICL inducing agents. The repair of ICLs requires the coordination of several DNA repair pathways including the Fanconi Anemia pathway, homologous recombination (HR), and nucleotide excision repair (NER). The Fanconi Anemia pathway is essential for the repair of these lesions as it is responsible for the recognition of the ICL lesion, as well as the recruitment of several nucleases responsible for unhooking and removal the cross-linked nucleotides. Recently, we identified an uncharacterized 5' nuclease that interacts with the RNA/DNA helicase Senataxin, which we have named senataxin-associated nuclease 1 (SAN1). Senataxin has been shown to act on R loops, RNA/DNA hybrids that are a source of endogenous DNA damage. Deletion of the SAN1 gene in HeLa cells or in mouse embryonic fibroblasts leads to the sensitization of cells to Cisplatin and Mitomycin C (MMC), but not to ionizing radiation that induces double strand breaks. Importantly, the defect in ICL repair can be restored using WT SAN1 but not with a mutant that is catalytically inactive. Treatment of SAN1 -/- HeLa cells with MMC also leads to radial chromosome formation, a characteristic of cells deficient in ICL repair. Additionally, treatment with MMC results in increased DNA damage and R loops in SAN1-/- cells. In conclusion, this study highlights the discovery of a novel nuclease involved in the repair ICLs, a process critical for understanding resistance and response to chemotherapies such as Cisplatin and MMC.

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Microtubule treadmilling revealed by *in vitro* reconstitution

Goker Arpag, Marija Zanic

Microtubules are cytoskeletal polymers composed of tubulin subunits that play essential roles during multiple cellular processes throughout the cell cycle. Tubulin subunits are kinetically added and removed from microtubule ends with different rates, such that the net rate results in either growth or shrinkage of the polymer. Microtubule polymers stochastically switch between the growing and shrinking phases, behavior known as microtubule dynamic instability. The net polymer assembly/disassembly rate at a given end can be calculated at a population level using the mean rates of growth, shrinkage and transition frequencies. If the assembly rate at one end is equal to the disassembly rate at the other end, the polymer will move its center of mass in the direction of the growing end, while keeping its length constant. This phenomenon is called treadmilling, and is frequently observed for polymers such as actin. In earlier analytical and theoretical studies, microtubules were also predicted to exhibit treadmilling and treadmilling-like behavior at a population level through modulation of the dynamic rates. Indeed, treadmilling-like behaviors were observed in plant and animal cells, as well as *in vitro* using a number of perturbations. Here, we investigate *in vitro* conditions for transition from microtubule dynamic instability to microtubule treadmilling of individual polymers by modulating tubulin concentration in the reaction solution. Our *in vitro* observations reveal treadmilling-like behavior with leading microtubule minus ends for tubulin concentrations between 6 -7 μM when the microtubule population reaches steady-state number and polymer mass. Our results suggest that actin-like treadmilling can be observed in microtubule reconstitution systems with controlled modulation of microtubule end dynamics, having potential implications for regulation of microtubule cytoskeleton in cells.

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Defining the role of the microbiome in small intestinal tuft cell specification

Amrita Banerjee, Chuck Herring, Alan J. Simmons, Eliot T. McKinley, Qi Liu, Robert J. Coffey, and Ken S. Lau

Parasitic helminth infection presents a significant global health burden. Recent studies have revealed that small intestinal tuft cells orchestrate a type 2 immune response against intestinal eukaryotic infection. Dclk1+ intestinal tuft cells are a morphologically unique cell type, best characterized by striking microvilli that form an apical "tuft" and represent approximately 0.5% of gut epithelial cells depending on location. While much remains to be understood about tuft cell function, previous work from our group has demonstrated that specification of small intestinal tuft cells proceeds independently of *Atoh1*, originally thought to be necessary for tuft cell differentiation. We used a novel $Lrig^{CreERT2/+}; Atoh1^{fl/fl}$ mouse model to demonstrate a significant increase in Dclk1+ tuft cell number following *Atoh1* recombination and loss of other intestinal secretory cell types, including Muc2+ goblet and lysozyme+ Paneth cells. We confirmed these results using both immunohistochemistry of tuft cell-specific protein markers and single-cell RNA sequencing (scRNA-Seq) of known tuft cell gene signatures. We hypothesized that the loss of barrier-regulating goblet and Paneth cells may alter the luminal microbiome in such a way as to promote tuft cell hyperplasia. In order to test this hypothesis, $Lrig^{CreERT2/+}; Atoh1^{fl/fl}$ animals were given a broad spectrum antibiotic cocktail (Ampicillin, Neomycin sulfate, Metronidazole, and Vancomycin) in their drinking water to target gram-negative and gram-positive bacterial species in the intestinal microbiome. Antibiotic-treated $Lrig^{CreERT2/+}; Atoh1^{fl/fl}$ mice lacked small intestinal tuft cells following tamoxifen treatment, compared to the tuft cell hyperplasia observed in glucose water-fed controls. Similarly, $Lrig^{CreERT2/+}; Atoh1^{fl/fl}$ animals treated with a second antibiotic regimen of Kanamycin, Gentamicin sulfate, Colistin sulfate, Metronidazole, and Vancomycin lacked tuft cells entirely following tamoxifen administration. This suggests a model by which, upon *Atoh1* loss and deletion of microbiome-regulating intestinal cell types, perturbations in the microbiome drive tuft cell hyperplasia. We will query the microbiome using direct 16s rRNA sequencing and utilize *ex vivo* enteroid culture to investigate whether microbial-derived compounds are responsible for driving tuft cell hyperplasia. Understanding tuft cell function and specification could enable us to better leverage this rare and elusive cell type in orchestrating immune responses during parasitic worm infections.

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ER-membrane contacts promote small RNA trafficking to extracellular vesicles (EVs)

Bahnisikha Barman, Alissa Weaver

Transfer of RNA between cells via extracellular vesicles (EVs) is a newly recognized form of cellular communication that can affect gene expression and phenotypes of recipient cells. Although a few RNA-binding proteins (RBPs) have been shown to mediate transfer of RNAs into EVs, how these RBPs are themselves trafficked and sorted is unknown. In this study, we report that endoplasmic reticulum (ER) membrane contact sites (MCS) with multivesicular bodies (MVB) and the plasma membrane affect RNA-RBP transfer into EVs. Inhibition of MCS by knockdown (KD) of VAP-A led to a decrease in the miRNA and RBP content of exosomes and microvesicles. Conversely, enhancement of MCS with MVB by cholesterol manipulation led to an increase in the miRNA and RBP content of exosomes. Functionally, inhibition of ER MCS leads to a decrease in cell-cell transfer of miRNAs via EVs. Altogether, we find that contact with the ER is a major mechanism that controls the miRNA content of EVs.

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Tissue Transglutaminase 2 Regulation of Tumor Cell Tensional Homeostasis

Francois Bordeleau, Wenjun Wang, Marc A. Antonyak, Richard A. Cerione and Cynthia A. Reinhart-King

Cell contractility is increasingly seen as a critical regulator of cell behavior. Notably, cell contractility can modulate how cells respond to growth factors or their ability to properly adapt to change in the physical properties of the underlaying extracellular matrix (ECM). Conversely, the effects of ECM stiffness on growth factor signaling is dependent on cell contractility. Several pathological conditions are characterized by increased ECM stiffness levels or cell contractility, most notably in tumor cells where both stiffness and cell contractility are correlated to metastatic potential. Tissue transglutaminase (TG2) is a protein that possesses GTPase and acyl transferase activity with both extracellular and intracellular functions. Overexpression of TG2 is known to increase migration and invasion of tumor cells. Outside of the cell, TG2 acyl transferase activity can crosslink the ECM, potentially increasing ECM stiffness of different type tumors. In addition, TG2 can affect several proteins involved in focal adhesion (FA) regulation, such as FAK and Src, as well as the cell response to epidermal growth factor (EGF) stimulation. However, the relevance of these TG2-mediated events in regulating tumor cell tensional state and metastasis remains unclear. Here, we show that TG2 plays a pivotal role in regulating cell tensional homeostasis in both invasive and non-invasive cells. Specifically, measurement of cell contractility by traction force microscopy or 3D collagen compaction, using either inhibitors or TG2 knockdown cells, reveals that TG2 positively contributes to the tumor cell contractile phenotype. In fact, TG2 inhibition led to decreased activation level and altered spatial localization of the small GTPase RhoA. Moreover, expression of TG2 in non-invasive MCF10a cells was sufficient to increase their contractility to similar levels to those observed in MDA-MB-231 invasive carcinomas. Our results also show that TG2 modulates FAK activation in response to changes in ECM stiffness. Interestingly, the difference between the mechanical state of control versus TG2 knockdown cells, both in terms of cell contractility and FA assembly, could readily be abrogated following inhibition of the EGF receptor. This last result suggests that TG2-mediated modulation of cell mechanics could occur though a regulation of the FAs/growth factor receptor signaling cross-talk. Together, our results uncover a novel mechanism that can explain the altered tensional homeostasis observed in tumor cells. By understanding the mechanisms that control the altered state of tumor cell contractility, our findings will likely reveal an entirely novel strategy in designing cancer therapeutics.

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The Role of Microtubule Sliding in Regulation of Insulin Secretion

Kai Bracey, Irina Kaverina

Diabetes mellitus is a major metabolic disorder currently affecting 5–10% of the population in the western societies. In type-2 diabetes, which accounts for 90% of all diabetes, insulin is not released into the bloodstream in sufficient amounts. Insulin secretion is a function of pancreatic beta cells. Beta cells have to secrete restricted doses of insulin, in order to reduce blood sugar to normal levels but do not completely deplete it; this requires tight coordination between intracellular insulin storage and secretion. Our data indicate that this coordination is regulated by cytoskeletal polymers microtubules (MTs), which are known to serve as intracellular highways; molecular motors move along MTs to transport and park membrane organelles and insulin granules at specific cellular locations. Our lab has shown that the dense MTs in pancreatic beta cells restrain insulin granules in "cages", restricting insulin granule availability for glucose-stimulated insulin secretion. Interestingly, I have found that glucose stimulation promotes the novel MT sliding process in beta cells. Through application of high-end approaches for high- and super-resolution biological imaging I examine whether MT sliding resolves "caged" insulin granules and/or impacts MT network configuration in pancreatic beta cells.

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Investigating the role of non-muscle myosin II in the development and maintenance of the intestinal brush border

Colbie R. Chinowsky and Matthew J. Tyska

Within the intestine, nutrient absorption occurs at the brush border, a region of densely packed actin based protrusions on the apical surface of enterocytes. These protrusions, known as microvilli, also form the first line of defense against luminal pathogens. Proper formation of the brush border is dependent on the correct formation and clustering of microvilli. Microvilli are membrane covered protrusions, each containing a bundle of 20-30 actin filaments, with the plus ends located at the distal tips, and the minus ends anchored in a region of the cell known as the terminal web. However, little is known about the mechanism(s) that anchor microvilli in the terminal web.

Within the terminal web, there are a variety of proteins, including non-muscle myosin II (NM-II), a conventional myosin motor expressed in all eukaryotic cells. Within the human intestine, three different heavy chains of NM-II are expressed; A, B and C, with NM-IIA and NM-IIC dominating expression in enterocytes. Mutations in the NM-II genes have been linked to a dysfunction in a variety of organs, including the intestine. In some cases, single point mutations have been associated with gastrointestinal distress, suggesting that NM-II plays an important role in proper maintenance of this tissue. Classical electron micrographs of the brush border show short "thick" filaments spanning the space between adjacent microvillar actin core bundles. These putative "thick" filaments are reminiscent of the bipolar filaments formed by NM-II. Super-resolution images from our laboratory indicate that NM-IIC forms novel sarcomere-like assemblies across the apical surface of enterocytes, which may represent the short filaments seen in early electron micrographs. It is likely that NM-II forms short filaments that stretch across the terminal web of the cell, fill the space between microvillar actin bundles, and link adjacent protrusions. In live-cell imaging experiments using Blebbistatin, a myosin II inhibitor, nascent microvilli get noticeably longer, and appear to lose their upright orientation, falling onto the surface of the cell. Our experiments indicate that non-muscle myosin II may play a novel role as a regulator of actin bundle orientation and turnover at the apical surface.

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Bulk endocytosis removes presynaptic terminals in remodeling GABA neurons

Andrea Cuentas-Condori, Ben Mulcahy, Mei Zhen, David M. Miller, III

Presynaptic boutons are dynamic structures that can be actively dismantled for reassembly at new locations during development. Although this phenomenon has been widely observed, the underlying mechanisms are poorly understood. To address this question, we have exploited an example of synaptic remodeling in the *Caenorhabditis elegans* motor circuit. Our findings suggest a model in which the conserved phosphatase calcineurin drives presynaptic disassembly by activating a mechanism similar to that of Activity-Dependent Bulk Endocytosis (ADBE).

During early larval development, GABAergic motor neurons eliminate presynaptic terminals with ventral muscles and reassemble them with dorsal muscles. We have previously proposed that neural activity and calcium, regulated by the DEG/ENaC cation channel, UNC-8 and calcineurin/TAX-6, drive the removal of ventral GABAergic presynaptic terminals. Here we show that branched actin polymerization, mediated by Arp2/3, and syndapin activity are also required in this pathway to promote presynaptic disassembly. Consistent with this finding, the WAVE Regulatory Complex (WRC) and the F-BAR protein, TOCA-1, two upstream regulators of Arp2/3, are necessary and function cell autonomously. Several of these components including TOCA-1, calcineurin/TAX-6 and UNC-8/DEG/ENaC are localized at presynaptic terminals of remodeling neurons.

ADBE has been previously shown to function in highly active neurons to accelerate recycling of synaptic vesicle membrane. This mechanism depends on presynaptic CaN, which triggers assembly of the dynamin-syndapin complex to modulate actin polymerization and drive the formation of bulk endosomes. Calcineurin, is required for ABDE and a related mechanism of bulk endocytosis in yeast. Bulk endosome formation and presynaptic elimination in remodeling D-GABAergic motor neurons share several characteristics: Both are clathrin-independent, are regulated by calcineurin, are driven by neural activity and require branched-actin polymerization and syndapin. We used EM reconstruction to confirm that large (70-100 nm), clear spherical structures resembling bulk endosomes are most abundant in GABA motor neurons during presynaptic disassembly. Thus, we propose that an ADBE-like mechanism is hijacked in this case to dismantle rather than recycle the presynaptic apparatus in remodeling GABA neurons. We are now using super resolution microscopy to monitor the dynamic structure of the actin cytoskeleton and presynaptic components in remodeling GABA neurons.

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Gene network analysis as a tool for studying pancreatic endocrine cell differentiation

Karrie D. Dudek, Anna Osipovich, Jacob Coeur, Jean-Philippe Cartailler, Emily Greenfest-Allen, Christian J. Stockert, Jr., and Mark A. Magnuson

Pancreatic endocrine cell identity is determined by a gene regulatory network (GRN) that is established as pre-endocrine cells delaminate from the pancreatic epithelium during mid-gestation. *Neurog3*, a helix-loop-helix transcription factor, plays a critical role in this process by regulating the expression of multiple downstream regulators, including *Insm1*, *NeuroD1*, *Pax6*, *Isl1* and *Rfx6*.

In order to gain a deeper understanding of the underlying gene regulatory logic responsible for pancreatic development, we generated RNASeq datasets from 11 different pancreatic cell populations that lie along the developmental lineage for mature β-cells, and used iterative whole genome correlation network analysis (iWGCNA) to construct a temporally-oriented gene correlation network (GCN). Our analysis, which spanned from E8.0 to P60, revealed a dramatic shift in gene expression as pre-pancreatic endocrine cells give rise to pancreatic islet cells and grouped both characterized and uncharacterized transcription factors (TFs) into 91 different modules, 14 of which were associated with endocrine cell development.

To further expand our knowledge of events that are occurring during the formation of pre-endocrine cells, we have extended our collection of datasets by performing RNASeq on FACS-purified endocrine progenitor cells that lack *NeuroD1, Pax6 and Insm1*, and then performing differential expression analysis. While our preliminary findings reveal a complex pattern of gene disruption, we find altered expression of over 150 zinc finger protein (ZFP)-containing TFs, including many proteins of the C_2H_2-type.

Using criteria parameters based on a gene's centrality measure within the developmental network, its regulation by *Neurog3* or *Neurod1,* protein structure, and conservation across species, we selected five ZFPs to explore the functional role of this understudied class of zinc finger proteins. We microinjected guide RNAs for each of the selected ZFPs into Cas9-expressing female pronuclei to generate global knockouts by non-homologous end joining. The progress we have made in analyzing these mice will be presented.

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Target-Based Screen to Identify Small Molecule Inhibitors of the Mitotic Kinesin Kif15

Megan Dumas, Geng-Yuan Chen, Nicole Kendrick, Josh Bauer, William Hancock, Alex Waterson, Gary Sulikowski, and Ryoma Ohi

The mitotic spindle is microtubule (MT)-based machine that segregates a replicated set of chromosomes during cell division. Many chemotherapeutics target the spindle by altering or disrupting microtubules, the polymer that forms the spindle. While these drugs are efficacious, microtubules are a major component of all cells and their disruption can have deleterious effects on cell types that rely on MTs for function, such as neurons. In addition to tubulin, MT-dependent motors that function during mitosis are logical targets for drug development. Eg5 (Kinesin-5) and Kif15 (Kinesin-12), in particular, is an attractive pair of motor proteins to pharmacologically target since they work in concert to drive centrosome separation and promote spindle bipolarity. Kinesin 5 inhibitors (K5Is) have been extensively studied since their discovery, and despite the initial excitement for K5Is due to their promising results in cell and mouse tumor models, they have largely failed in the clinic. Since Kif15 over expression has been shown to overcome K5I treatment in tissue culture cells, a potential explanation for K5I clinical failure may be due to the cell's ability to utilize a Kif15 dependent spindle assembly pathway. Recently, our laboratory discovered that the emergence of K5I resistance in tissue culture cells depends on the expression of Kif15. This result underscores the hypothesis that a combinatorial drug approach to target spindle assembly, by inhibiting both motors, will cripple rapidly dividing cancer cells. Therefore, we set out to perform a small molecule screen on a group of known kinase inhibitors, with the goal of identifying lead chemical scaffolds that inhibit Kif15. Using an *in-vitro* ATPase assay, the Published Kinase Inhibitor Set (distributed by GSK) was screened and two compounds that significantly inhibited Kif15's MT stimulated ATPase activity. The activities of both compounds were confirmed in a MT gliding assay as well as a second ATPase assay. Concentration response curves were performed in triplicate and IC50s were calculated for each. VU674 became our lead compound, exhibiting an IC50 of ~750nM in 3 different *in-vitro* assays. Furthermore, treatment with VU674 on K5I resistant cells (KIRCs), whose ability to form bipolar spindles relies on Kif15, results in nearly 100% monopolar spindles. Mechanistically, VU674 does not compete with ATP as expected, but instead interferes with Kif15's ability to bind MTs. While the exact nature of this inhibition remains unclear, it represents a novel function for this known kinase inhibitor. Structure Activity Relationship (SAR) analysis of VU674 is currently underway.

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F-actin is stabilized to form brush border microvilli

James (Bo) J. Faust and Matthew J. Tyska

The intestinal enterocyte undergoes a dramatic reorganization of the apical surface during differentiation as the cell transitions from the crypt to the villus. During the differentiation process filamentous actin (F-actin)-supported membrane protrusions known as microvilli are organized into a maximally packed highly ordered array of uniform length. Collectively, this ensemble of microvilli is referred to as the brush border, and acts to increase the cell surface area for maximum nutrient absorption and host defense. Previous studies in the laboratory have begun to dissect the molecular mechanisms responsible for nucleation of F-actin within microvilli. Despite our general understanding of the assembly process little is known about how F-actin length can be maintained to form brush border microvilli. To begin to address this question we analyzed the ability of F-actin to treadmill within brush border microvilli *in vivo* by applying cytochalasin or vehicle to the luminal surface of the intestine and measured length using structured illumination microscopy. At the villus, we observed no gross change in microvilli length *per se* for cytochalasin treated specimens compared to mock specimens indicating that F-actin within mature microvilli does not treadmill. At the base of the crypt we observed an ~50% reduction in microvilli length compared to mock specimens suggesting that, compared to the villus, F-actin in crypt microvilli are more dynamic. These data indicate that the transition from immature microvilli in the crypt to mature brush border microvilli is accompanied by stabilization of the underlying F-actin core bundle.

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Muscle specific stress fibers give rise to sarcomeres and are mechanistically distinct from stress fibers in non-muscle cells

Aidan M. Fenix, Nilay Taneja, Abigail C. Neininger, Mike R. Visetsouk, Benjamin R. Nixon, Annabelle E. Manalo, Jason R. Becker, Scott W. Crawley, David M. Bader, Matthew J. Tyska, Jennifer H. Gutzman, Dylan T. Burnette

The sarcomere is the fundamental contractile unit within cardiomyocytes driving heart muscle contraction. Dynamic and mechanistic information regulating sarcomere assembly is poorly understood. We sought to test the mechanisms regulating actin and myosin filament assembly during sarcomere formation. We developed an assay using human cardiomyocytes to test *de novo* sarcomere assembly. We report a population of muscle-specific stress fibers are essential sarcomere precursors. We show sarcomeric actin filaments arise directly from these muscle stress fibers. This requires formin-mediated but not Arp2/3-mediated actin polymerization and non-muscle myosin IIB but not non-muscle myosin IIA. Furthermore, we show short β cardiac myosin II filaments grow to form ~1.5 μm long filaments that "stitch" together to form the stack of filaments at the core of the sarcomere. Interestingly, these represent different mechanisms that have been reported during stress fiber assembly in non-muscle cells. Thus, we provide a model of cardiac sarcomere assembly based on distinct mechanisms of stress fiber regulation between non-muscle and muscle cells.

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Host Cells Resist *Helicobacter pylori* VacA Intoxication by Degrading VacA

Nora J. Foegeding, Krishnan Raghunathan, Timothy L. Cover, Melanie D. Ohi

Helicobacter pylori persistently colonizes the gastric mucosa of more than half of the world's population. Infection with *H. pylori* causes chronic gastric inflammation and is the leading cause of stomach ulcers and gastric cancer. An important *H. pylori* virulence factor is the pore-forming toxin known as vacuolating cytotoxin A (VacA). VacA enhances the ability of *H. pylori* to colonize the stomach and contributes to the pathogenesis of peptic ulcer disease and gastric cancer. VacA has been reported to trigger a wide range of cellular responses including cellular vacuolation, increased plasma membrane permeability, disruption of mitochondrial membrane permeability, and apoptosis. VacA activity is potentiated by the presence of weak bases, but the mechanistic basis for this phenomenon is not completely understood. Using both light microscopy techniques and western blot analysis, we assessed the trafficking and stability of intracellular VacA. During our investigation, we observed that VacA-treated cells recover from VacA-induced vacuolation. Furthermore, we found that VacA-induced cell death is dependent on the presence of supplemental ammonium chloride (NH_4Cl). It is proposed that VacA induces cell death by trafficking to mitochondria and forming a pore; therefore we first tested whether NH_4Cl is required for VacA to traffic to mitochondria. We determined that VacA colocalization with mitochondria was not altered by the presence or absence of NH_4Cl. As NH_4Cl is a weak base commonly used to inhibit lysosomal function and autophagy, we next tested that hypothesis that host cells are able to degrade VacA and that NH_4Cl inhibits VacA degradation. By assessing the intracellular levels of VacA, we show that host cells degrade VacA and that inhibiting lysosomal activity with NH_4Cl, chloroquine, or bafilomycin A1 inhibits VacA degradation. Additionally, we found that VacA colocalizes with lysosomes before colocalizing with autophagosomes. Finally, we determined that inhibiting autophagy either by use of the chemical inhibitor 3MA, by inhibition of ATG5 expression, or by inhibition of ATG16L1 expression does not inhibit VacA degradation. We propose that intracellular degradation of VacA is a defense mechanism that allows host cells to resist VacA-induced cell death, and weak bases like NH_4Cl enhance VacA activity by inhibiting its degradation.

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Targeting the MYC–WDR5 Nexus in Cancer

Audra M. Foshage, April M. Weissmiller, Lance R. Thomas, Shelly L. Lorey, William P. Tansey

Deregulation of MYC, an oncoprotein transcription factor, is one of the most prominent alterations in cancer and contributes to an estimated 100,000 cancer-related deaths in the U.S. every year. MYC induction is a critical event in the development and progression of cancer, as MYC uses transcriptional regulators and chromatin machinery to control expression of thousands of genes involved in cell cycle progression, cellular growth, metabolism, proliferation, differentiation, and genome integrity. Determining how MYC selects and regulates its target genes is necessary to understand the biology of MYC-driven tumorigenesis and may expose novel avenues for anti-cancer therapies.

MYC is known to heterodimerize with its obligate partner MAX to bind target genes. However, it is clear that the epigenetic environment of these sites is also key to MYC binding, with a particular bias towards genomic regions enriched in histone H3 lysine 4 trimethylation. Recently, the Tansey laboratory discovered that association of MYC with a majority of its target genes in the context of chromatin depends on interaction with WDR5, a core component of multiple chromatin regulatory complexes including those that catalyze H3K4me3. Based on these observations in HEK cells, we propose that target gene recognition by MYC is an avidity-based mechanism that involves two critical sets of interactions: one between MYC/MAX heterodimers and DNA, and another between MYC and chromatin-bound WDR5. We posit that MYC is recruited by WDR5 to the specific target genes required for the tumorigenic functions of MYC. ChIP-seq experiments in HEK cells indicate that at ~80% of MYC binding sites, WDR5 also co-localizes. Since MYC deregulation features in the majority of cancers, we want to know if this extensive overlap between MYC and WDR5 also occurs in cancer cells. Are there MYC–WDR5 sites that are common between cancer cell types? Sites that are different? Are MYC–WDR5 sites in cancer cells located in promoter or enhancer regions? To answer these questions, we compared MYC–WDR5 sites in multiple cancer contexts, including colorectal carcinoma and neuroblastoma. We determined that MYC and WDR5 do indeed bind in various cancer cells, and co-localize on chromatin. ChIP-seq experiments allowed us to identify all the MYC target genes that are also co-occupied by WDR5. Performed in various human and mouse cell lines, these studies indicate widespread overlap of MYC and WDR5 in cancer, and have identified a conserved set of MYC–WDR5 target genes, tied to the core tumorigenic potential of MYC.

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Dynamics of Golgi Organization in Proliferating, Motile Cells

Keyada Frye, Maria Fomicheva, Xiaodong Zhu, Lisa Gong, Elena Kolobova, James Goldering, Alexey Khodjakov, and Irina Kaverina

The mammalian Golgi complex is an organelle with several roles in the cell, including the processing and trafficking newly synthesized proteins. Additionally, the Golgi complex is a microtubule organizing center, with the ability to nucleate its own subset of microtubule tracks which facilitate post-mitotic Golgi formation and post-Golgi trafficking events. It has been shown that the highly dynamic Golgi membrane system undergoes extensive fragmentation in late G2/prophase. However, we lack an understanding of Golgi dynamics in interphase. While no fragmentation occurs during this long cell cycle stage, we have observed distinct changes in the Golgi complex's localization, specifically in relation to the centrosomes.

We seek to elucidate the mechanism whereby the Golgi complex dynamically associates and disassociates with the centrosomes during interphase. The current study presents whether microtubules, molecular motors, and/or the centrosomes themselves direct this dynamic localization of the Golgi. Through live cell spinning disk microcopy we were able to observe three categories of Golgi localization. In early G1, the Golgi is tightly compacted around the centrosome and we refer to this as C1 (compact stage 1). Starting in S-phase, we see the Golgi localizing around the equator of the nucleus, thus E-stage (equatorial stage). Finally, prior to mitosis, the Golgi is compact around the centrosomes yet again and we refer to this as C2 (compact stage 2). We quantified the association of the Golgi and centrosome by acquiring the XYZ coordinates for each Golgi pixel and center of homogenous mass of each centrosome, per cell. Next, we applied the distance formula to determine distance between each Golgi pixel and each centrosome. "*GC Distance"* was determined by averaging the distances between each Golgi pixel and its nearer centrosome (if two). Results show that when the Golgi is in compact stages, whether C1 or C2, the GC Distance is around 4um. The E-stage has the widest range of GC Distance, ranging from 4um to 10um.

Analysis of ~100 cells show that the E-stage of Golgi dynamics begins in S-phase. By depolymerizing microtubules through a combined ice and nocodazole treatment, we observed equatorial distribution is blocked. The specific microtubule motor facilitating this distribution is the plus end directed KIF5B. Data show there is a significant decrease ($p<0.03$) in equatorial distribution when KIF5B activity is blocked via a dominant negative approach. Centrosome depletion (Plk4 inhibitor) show that no Golgi stage is perturbed. Data show C1 influences cell velocity. In this stage, the cell moves faster as opposed to E and C2 stages. Finally, we observed that C2 compaction occurs directly before nuclear envelope breakdown.

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The progression from early- to late-born cell production in the developing retina is regulated by Lhx2

Alexandra Fuller, Patrick Gordon, and Edward Levine

Treatment of ocular disease with cell-replacement or reprogramming therapies necessitates a thorough understanding of the mechanisms governing retinal progenitor cell (RPC) proliferation and production of retinal cell-types. All 7 retinal cell-types are generated from RPCs. Although multipotent, RPCs do not generate all cell-types simultaneously. Rather, production of retinal cells is divided into two temporal groups, early-born and late-born, and how this temporal transition occurs is not well understood. Previous work from the Levine lab showed that Lhx2, a LIM homeodomain transcription factor, regulates the timing of ganglion cell (GC) production in mice. When Lhx2 is conditionally inactivated, new GCs continue to be produced beyond their normal production period; this suggests impaired temporal progression in Lhx2-inactivated retinas. However, it was unknown 1) whether temporal progression is delayed rather than prevented and 2) how production of other retinal cell-types is affected by absence of Lhx2. Using EdU birth-dating in Lhx2-inactivated mice, I quantified production of new retinal cells at and beyond the time when production shifts towards late-born cell-types. My studies reveal that Lhx2-inactivated retinas remain in an early-born production state, producing GCs, amacrine cells, and possibly cone photoreceptors, into postnatal development. Furthermore, production of rod photoreceptors and bipolar cells (late-born cell-types) was severely diminished. Ongoing efforts are underway to identify the mechanism by which Lhx2 promotes temporal progression of cell-type production in the embryonic retina.

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Mechanisms of EPS8 Function During Microvillar Growth

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Microvilli are ancient actin-based cellular protrusions that increase surface area for nutrient absorption and have been evolutionarily conserved from invertebrates to mammals. Despite the physiological and evolutionary importance of microvilli, little is known about the molecules that drive assembly of the supporting actin bundle against the apical plasma membrane. Electron microscopy studies ~50 years ago of intestinal brush border microvilli first revealed that the tip of the microvillus is occupied by an electron dense plaque known as the 'distal tip complex', which embeds the barbed ends of actin filaments. Because the barbed ends are the preferred sites of actin assembly, proteins that regulate actin dynamics typically target these ends. Thus, elucidation of distal tip complex components is critical to gain insight into the molecular basis of microvillar morphogenesis.

One candidate tip complex component is Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8), which localizes specifically to the distal tips of intestinal microvilli, and is able to bind actin, plasma membrane, and other signaling factors. In mouse intestinal tissue, EPS8 is present at the apical membrane of absorptive epithelial cells, along the full length of the crypt-villus. Additionally, EPS8 is localized to the distal tips of microvilli during the earliest stages of intestinal cell differentiation in cell culture models and in native intestinal tissue. Loss of EPS8 significantly decreases the length of microvilli, suggesting EPS8 plays a role in protrusion elongation. Thus, these studies seek to elucidate the mechanism EPS8 function in growing microvilli. Additionally, by taking advantage of the highly specific and persistent localization of EPS8 at the distal tips of intestinal microvilli, EPS8 will be used in a biotin proximity labeling assay (BioID2) to elucidate the distal tip complex proteome.

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Contributions of alterations in extracellular matrix composition to DR-relevant endothelial cell behaviors

Meredith Giblin, John Penn

An early structural abnormality of diabetic retinopathy (DR) is basement membrane (BM) thickening of the retinal microvasculature. Recent studies suggest that BM thickening is a product of increased extracellular matrix (ECM) deposition and contributes to pathogenic retinal cell behaviors. Yet, to date, studies regarding BM alterations and DR pathology remain inconclusive. The purpose of this study was to learn how diabetes-relevant stimuli affect expression of BM components and how these diabetes-induced changes affect human retinal microvascular endothelial cells' (HRMEC) expression of adhesion proteins involved in pathogenic leukostasis. HRMEC and human retinal pericytes (HRP) were treated with diabetes-relevant stimuli, including inflammatory cytokines (TNFα or IL1β, 10ng/mL) and high glucose conditions (25mM D-glucose). Concentrations and treatment times were systematically optimized and expression of two primary BM components, collagen IV (COL4) and fibronectin (FN), was measured by qRT-PCR. High glucose treatment of HRMEC or HRP produced no significant changes in COL4 or FN expression. In HRMEC, TNFα caused a 1.7-fold increase in COL4 ($p<0.01$) and a .5-fold decrease in FN ($p<0.01$). In HRP, TNFα caused a 2.7-fold ($p<0.01$) induction of COL4. IL-1β induced a 1.8-fold ($p<0.01$) induction of COL4 in both HRMEC and HRP. To study how changes in ECM deposited under diabetes-relevant conditions alter HRMEC behavior, HRMEC or HRP were treated with TNFα or IL-1β, respectively, for 48hrs before cultures were decellularized. Naïve HRMEC were then plated on the decellularized matrices and collected 16hrs later for qRT-PCR. TNFα-conditioned HRP ECM caused 349-, 6.5- and 3.3-fold inductions in SELE, ICAM and VCAM, respectively. IL-1β-conditioned HRMEC ECM caused 4.2 ($p<0.0001$), 2.2 ($p<0.0001$), and 1.6 ($p<0.0001$) -fold inductions in SELE, ICAM, and VCAM, respectively. In conclusion, cytokines were shown to be more potent inducers of COL4 expression than conditions designed to simulate hyperglycemia. Interestingly, TNFα caused contrasting expression changes in HRMEC indicating that ratios of BM constituents may be significantly altered in the diabetic retinal BM. HRP demonstrated higher levels of COL4 induction than HRMEC, arguing that HRMEC should not be the only focus in understanding BM thickening. Additionally, decellularization experiments suggest that diabetes-relevant alterations in ECM composition alone can alter adhesion molecule expression by HRMEC, indicating that BM thickening may drive other pathogenic behaviors in DR.

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Gle1A-mediated Stress Granule Assembly provides a Survival Advantage to Tumor Cells

Laura Glass and Susan R. Wente

Gle1 is a highly conserved, essential regulator of DEAD-box proteins that is required for mRNA export, translation and stress granule function. The *GLE1* gene is alternatively spliced in human cells to generate at least two isoforms (Gle1A and Gle1B), each with distinct functions and subcellular localization. Gle1 has been found to be upregulated after cellular transformation and is frequently upregulated in tumor cells compared to healthy tissue. "Evasion of apoptosis" is a hallmark of cancer and there is an emerging field of research into whether stress granule formation allows cells to evade apoptosis and survive. We have demonstrated that Gle1A specifically mediates and supports SG function, whereas Gle1B does not. These data led us to question whether overexpression of Gle1A modulates SG biology to provide a survival advantage to tumor cells during drug treatment. We discovered that when HeLa cells were treated with high doses of sodium arsenite (>100 μM), cells were able to form large stress granules that correlated with increased viability at 72 hours of treatment. In contrast, treatment with a lower dose of sodium arsenite, which did not induce SG formation, resulted in complete loss of viability. Moreover, presence of the structural SG component, G3BP is also necessary for this SG-mediated survival. Importantly, we further demonstrated that knockdown of Gle1 by siRNA diminished this protective effect at the high dose, and that Gle1A specifically rescued this survival defect. These data provide evidence that Gle1A-mediated stress granule formation in tumor cell lines offers a protective line of defense and a survival advantage in tumor cell populations subjected to chemotoxic drug treatment. We posit that Gle1A is required for evasion of apoptosis by tumor cells through its role in directing SG assembly and disassembly. Future studies aim to determine whether the mechanism of action for Gle1A in tumorigenic cells is consistent with its actions in response to general cellular stress. Combinatorial approaches targeting SG assembly alongside traditional chemotoxic agents may help to improve efficacy of anti-cancer treatment regimens.

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Determining the molecular details of the MYC–WDR5 interaction

Alissa duPuy Guarnaccia and William P. Tansey, Ph.D.

MYC is an oncoprotein that is overexpressed in the majority of malignancies and contributes to an estimated 70,000–100,000 cancer deaths in the United States every year. The broad pro-tumorigenic functions of MYC stem from its role as a sequence-specific transcriptional regulator, controlling the expression of thousands of genes linked to cell cycle control, growth, and metabolism. Key to understanding how MYC causes cancer, therefore, is understanding the mechanisms through which it selects its target genes. Our laboratory recently discovered that the chromatin regulatory protein WDR5 interacts directly with MYC and co-localizes with MYC at a majority of its target genes in human cells. Point mutations in MYC that disable interaction with WDR5 do not impact the ability of MYC to bind naked DNA but do prevent MYC from recognizing target genes in the context of chromatin and from driving tumorigenesis in mice. These studies led us to propose that the MYC–WDR5 interaction is a critical determinant in MYC target gene recognition. Two important questions are raised by these studies, however. How does WDR5 recognize and select its target genes? And what other functions, if any, does WDR5 play in regulating MYC target genes? To begin to answer these questions we used a proteomic approach to identify protein candidates that interact with wildtype MYC but not with the mutant MYC that is unable to bind WDR5. Initial validations highlighted one protein in particular: phosphoinositide-dependent kinase 1 (PDPK1), a protein central to the AKT pathway. PDPK1 is remarkable in that, in addition to being sensitive to mutant MYC, it is also sensitive to a point mutant of WDR5. These initial observations establish a firm understanding of the surfaces mediating interaction among these proteins and enable us to use targeted chemical and genetic manipulations to thoroughly characterize this new interaction. Such characterizations will enable us to dissect the functional role for this kinase with MYC and WDR5, and ultimately help define the potential for MYC–WDR5 inhibition as a therapeutic target for cancer.

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Investigating the functional role of the GAPVD1-CK1δ/ε interaction

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Human casein kinase 1 delta (CK1δ) and epsilon (CK1ε) are members of a conserved family of ubiquitously expressed serine/threonine kinases that regulate multiple processes including vesicular trafficking, circadian rhythm, and ribosome maturation. CK1δ/ε depletion results in a significant reduction of internalization of EGF and transferrin[1]. Inhibition of CK1δ/ε using two small molecule inhibitors (SR1277 and SR3029)[2] results in a 6-fold increase of mitotic cells exhibiting multipolar spindles, as well as 7-fold and 50-fold increases of mitotic cells with misaligned and unaligned chromosomes. These results suggest CK1δ/ε function to promote endocytosis, chromosome alignment, spindle polarity. We sought to investigate the molecular mechanism by which CK1δ/ε may modulate these processes by determining their interacting partners and substrates. As observed in fixed cells with indirect immunofluorescent microscopy, CK1δ and CK1ε endogenously tagged with a fluorescent, multifunctional affinity purification (MAP^2) tag localize to centrosomes, the nucleus and throughout the cytoplasm. MAP-tagged CK1δ and CK1ε from asynchronous and mitotic cell populations was used as bait to purify and identify associated proteins by mass spectrometry. GTPase-activating protein and VPS9 domain-containing protein 1 (GAPVD1) was consistently the most abundant interacting protein in the purifications of both enzymes. Unlike CK1δ/ε, GAPVD1 does not localize to the centrosome. However, CK1δ/ε and GAPVD1 co-localize with clathrin heavy chain, which is consistent with their roles in endocytosis. By conventional co-immunoprecipitation, we found that GAPVD1 and CK1δ/ε associate throughout the cell cycle and biochemical experiments showed that GAPVD1 interacts directly with the kinase domain of CK1δ/ε. GAPVD1 has a GAP domain at its N-terminus and a GEF domain at its C-terminus, which are separated by a predicted unstructured region. The GAP, but not GEF, domain is necessary for GAPVD1's association with CK1δ/ε. We also found that GAPVD1 is a substrate of CK1δ/ε *in vitro* and *in vivo*, with all of the phosphorylation sites located between the GAP and GEF domains. Our results suggest that GAPVD1 is a key interacting partner and substrate of CK1δ/ε and may potentially relate to CK1δ/ε's function in endocytosis.

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Ubiquitin Ser57 Phosphorylation as a Novel Component to the DNA Damage Response

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Ser57 is the most predominantly phosphorylated residue of ubiquitin (Ub) in yeast but the regulatory attributes of this modification are largely unknown. Here we identify the paralogous SKS1 and VHS1 kinases, which phosphorylate linear poly-Ub at Ser57 *in vitro*. Endogenous expression of the linear pentameric Ub (UBI4) is stress-inducible, suggesting regulatory implications of SKS1 and VHS1 in response to DNA-damaging agents and under oxidative stress. During H_2O_2 treatment cells exclusively expressing phosphoresistant Ub have growth phenotypes similar to Δ*SKS1*Δ*VHS1* cells which can be suppressed by the expression of wildtype or phosphomimetic Ub. In addition, hydroxyurea treatment, which causes replication fork stalling, decreases the growth rate of cells expressing Ub S57D, SKS1 or VHS1. Taken together, we propose that SKS1/VHS1-mediated phosphorylation of Ub Ser57 is a novel signaling component involved in checkpoint activation to ensure proper DNA damage recognition and response.

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Quantitation of 3D Structures in Pancreas

Brenda Jarvis, Jeff Duryea Jr, and Chris Wright

While it is increasingly clear that rodent and human islets and β cells have major structural and expression differences, new data from our groups and others indicate that "young" (0-5 years of age, or "juvenile") human islets are substantially different from adult human islets. New data in humans suggests that the juvenile period is an incredibly important developmental period of substantial islet plasticity, but our knowledge of the human pancreatic islet in this time period is quite limited. We know little about how human islets become vascularized and innervated, but both are essential for the normal β-cell microenvironment and function. These limitations result partly from difficulties in procuring human pancreas tissue, but also because standard immunofluorescent methods are limited in their 3D resolution. Using several types of advanced tissue-clearing protocols, which retain fine structures and allow imaging of large tissue blocks at relatively high resolution, we have imaged juvenile human pancreata with the intent of establishing 3D structural information on islets, neurons and vasculature.

We have developed a universally applicable strategy using a commercially available software for quantifying 3D structure, size and shape as well as distance between structures. With Imaris software, we can quantitate 3D volumes of endocrine cells within an islet, determine distances between neuronal bundles and vasculature in relation to islets, and measure the number of branch points and segment lengths of vasculature and neurons. We are defining the intra- and inter-islet vascular and neural networks and the 3D structure-function relationships at the cellular and organ-wide level for human juvenile pancreata. We examined the presence of multiple dispersed small endocrine clusters (often singlet cells or low numbers, particularly of insulin-positive cells) in juvenile human pancreas, which are lacking in adult human pancreas. We surmise that these clusters are important during early development, but lack of these cells during adulthood could result from migration to the nearest large islet cluster, cell-autonomous apoptosis or immune clearance during pancreas maturation.

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Quantitative proteomic analysis between exosomes and microvesicles reveals enrichment of adhesion proteins in exosomes

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Extracellular vesicles (EVs) are important mediators of cell-cell communication due to their cargo content of proteins, lipids and RNAs. We previously reported smaller EVs, such as exosomes, promote a variety of aggressive cancer cell traits, such as chemotaxis, cell motility and invasion. In contrast larger EVs, such as shed microvesicles (MVs), were not active in our systems. The goal of this study was to identify differences in the protein cargos of exosomes and MVs that may contribute to their different functional properties. We utilized isobaric tag for relative and absolute quantitation (iTRAQ)-liquid chromatography (LC) tandem mass spectrometry (MS) to perform a comprehensive comparison of protein cargos in exosomes and MVs obtained from the colorectal cancer line, DKs8. Statistically significant differences in proteins between these EV subtypes were identified by differential expression and gene set enrichment analysis methods. Our proteomic analysis showed that exosomes were enriched in proteins associated with cell-cell junctions, cell-matrix adhesion, exosome biogenesis machinery and various signaling pathways. In contrast, MVs were enriched in proteins associated with ribosome and RNA biogenesis and processing, and metabolism. Proteins of interest were validated by Western blot analysis of total cell lysates and EVs purified from DKs8 cells, as well as from HT1080 fibrosarcoma cells. Western blot analysis confirmed the presence of integrins, claudin 3, thrombospondin-1 and Ephrin receptors in exosomes. In addition, another highly abundant protein in the exosome preparation was arrestin-domain containing protein 1 (ARRDC1), which has been implicated in budding of ARRDC1-mediated microvesicles (ARMMs) from the plasma membrane. As a result of our EV isolation protocol, ARMMs are collected along with exosomes. Western blot analysis of the protein cargos carried by ARMMs versus exosomes did not identify protein cargos particularly enriched in ARMMs, thus suggesting that the ARMMs are a small subpopulation of the exosome population collected. Since we identified that exosomes were enriched in proteins associated with cell-matrix adhesion, we set out to determine whether exosomes promoted cell adhesion of DKs8 cells. We showed that the cell adhesion of DKs8 cells was enhanced with exosome coating compared to MV coating. In summary, iTRAQ is a useful method to identify protein differences in complex EV populations. Adhesion proteins appear to be particularly enriched in exosomes and may function to promote cell motility.

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Different expression pattern of monocarboxylate transporters in gastrointestinal epithelial cells

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The family of monocarboxylate transporters (MCTs) consists of 14 members that are essential for the transfer of energy precursors into cells that prefer short-chain fatty acids (SCFAs) or ketones. However, the expression pattern of each MCT member is largely unknown. Some gastrointestinal (GI) epithelial cells shuttle SCFAs from the lumen to the circulation, while other cells may uptake SCFAs from the circulation as an energy source. CD36 is another key protein for fatty acid absorption from the lumen. Depletion of CD36 alters expression levels of MCT2 and MCT4 in cardiomyocytes, which utilize fatty acids. The distribution of each MCT subtype and their interaction with CD36 has not been determined in GI epithelial cells. We investigated the expression of MCT1, MCT2, and MCT4 that co-transport SCFAs with protons by utilizing specific antibodies in GI mucosa from CD36 knockout (KO) and WT mice. Immunoreactivity and positive cell numbers were quantified by whole slide imaging and digital image analysis software. MCT1 was strongly expressed on the basolateral membranes of forestomach basal cells, gastric surface cells, and the majority of intestinal epithelial cells, including proliferating cells and enteroendocrine cells. No expression was observed in cells of Brunner's glands. The low affinity subtype MCT4 was localized in the first corpus gland epithelial cells and scattered parietal cells in the gastric surface area, suggesting that these cells may monitor the luminal concentration of SCFAs. Intestinal MCT4 showed a similar distribution to that of MCT1. In contrast, MCT2, which has the highest affinity for lactate, was strongly expressed on the basolateral membrane of secretory cells, including parietal and chief cells of the stomach, Brunner's gland cells, and Paneth cells of the intestine. The distinct MCT2 expression suggests that GI secretory cells require more SCFAs as an energy source and MCT2 may contribute to intracellular pH homeostasis. CD36 KO mice showed similar expression patterns of each MCT with lower expression of MCT1 and MCT2, but not MCT4, compared to WT. While differences in substrates between MCT1 and MCT2 are not fully characterized, their reciprocal cellular distribution in the same epithelium suggests that each positive cell type may have different fuel preferences. Malignant cells from a wide range of tumor types highly express MCTs and MCT inhibition is proposed as an anti-cancer therapy. The identification of cell types that express distinct MCT subtypes will help to elucidate the pathogenesis of epithelial cell malignancy and to develop novel therapies.

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Microvillar sensation of shear stress induces autophagic flux in the intestinal epithelium

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The intestinal epithelial cells lining the lumen of the neonatal gut are exposed to the flow of fluid from a liquid diet. Mechanosensors, such as the primary cilia of the kidney epithelium or stereocilia of the inner hair cell of the ear, have been identified to respond to shear forces. However, whether intestinal epithelial cells can respond to fluid shear stress is unknown.

To study the effect of shear on intestinal epithelial cells, we applied fluid shear stress on the intestinal epithelial cell line, Caco-2 BBE, through the use of a microfluidics design. Interestingly, we observed that exposure to fluid shear stress causes the formation of large vacuoles within intestinal epithelial cells when they are organized as monolayers. These observations raised two questions: first, what structural elements allow intestinal epithelial cells to transduce shear stress, and second, what mechanisms support the formation of large vacuoles that can exceed 80% of cell volume. As a result, we found that structurally, intestinal epithelial microvilli play a role in sensing extracellular shear stress, since shear-induced vacuole formation was significantly diminished when the intestinal microvilli were experimentally reduced. In addition, we identified that autophagic flux, and not endocytosis, to be the major pathway connecting mechanical sensation to vacuole formation. Loss-of-function studies by pharmaceutical and genetic inhibition of autophagy pathway components support our observations that shear stress-induced vacuole formation is mediated by the autophagic machinery.

In conclusion, our results revealed a novel link between the intestinal microvilli, the macroscopic transport of fluids across cells, and the autophagy pathway in organized epithelial monolayers. Our study contributes to the understanding of how physical stress affects cellular response in both intestinal physiological and pathological contexts.

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Rab11-FIP1B Locates at the Apical Junction and is Involved in Regulating Tight Junction Composition

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Previously we have shown that pSer227-Rab11-FIP2 and Rab11-FIP1C were involved in cell polarity and apical junctional composition. In the current work we now demonstrate that pSer234-Rab11-FIP1B is also involved in the regulation of tight junction composition. Interestingly both Rab11-FIP1B on serine 234 and Rab11-FIP2 on serine 227 are phosphorylated by the same kinase, MARK2 (Par1b/EMK1) but at different spatial and temporal times. By immunofluorescence pS234-Rab11-FIP1 localized to both apical vesicles and to the apical lateral membrane. The serine at amino acid 234 is shared by at least two Rab11-FIP1 splice variants, B and C. Western blotting for pSer234-Rab11-FIP1 indicated that in Caco-2BBE and murine intestine the predominate phosphorylated Rab11-FIP1 splice variant was Rab11-FIP1B. When murine intestine was homogenized in hypotonic buffer, separated by differential centrifugation and on a Optiprep gradient the fractions that contained pS234-Rab11-FIP1B also contained both ZO-2 and ZO-3 but not calreticulin or NaK-ATPase. Staining the intestine from a Rab11-FIP1 knockout mouse indicated that ZO-1 was no longer observed at the tight junction while Occludin and ZO-2 remained. This work places a subpopulation of pSer234-Rab11-FIP1Bat the apical lateral membrane between the tight and adherens junctions and that this localization of pSer234-Rab11-FIP1B plays an important role in the localization or stabilization of ZO-1 at the tight junction.

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Ppz phosphatases regulate endocytosis by dephosphorylation of ubiquitin ligase adaptors in yeast

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Regulation of plasma membrane (PM) protein abundance by selective turnover is critical for cellular physiology – particularly for adaptation to stress or a changing microenvironment. Such selection requires sorting of specific PM proteins into endocytic vesicles, a process that relies on coordination of multiple signaling cues including phosphorylation and ubiquitylation. Here we report that loss of Ppz phosphatases results in significant endocytic trafficking defects – with both catalytic activity and PM localization required for Ppz endocytic function. Surprisingly, Ppz phosphatases were found to regulate phosphorylation of Rsp5 adaptors, including Thr93 phosphorylation of Art1. Phenotypic analysis of phosphomimetic (Thr93Asp) and phosphorylation defective (Thr93Ala) variants indicates that Thr93 phosphorylation/dephosphorylation toggles Art1 activity. Our results reveal complex phosphoregulation of Art1 by multiple kinases and phosphatases, demonstrating how ART proteins function as signal processors that translate multiple upstream signals into specific ubiquitylation outcomes at the plasma membrane.

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Notch-independent progenitor maintenance reveals a novel pre-neurogenic state in mouse RPCs

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In the developing retina, there exists a heterogeneous population of retinal progenitor cells, RPCs, that must be maintained from the early optic cup stage (~embryonic day 10) throughout neurogenesis and into the postnatal mature retina (after ~post-natal day 7). Past studies in the eye and other developing tissue systems amongst many different model organisms have shown that the canonical Notch pathway is responsible for maintaining a population of progenitors during active differentiation. While the function of the Notch pathway is very well established in a tissue that is actively differentiating it remains unknown if Notch functions in the same manor prior to differentiation. To investigate the role of active Notch signaling prior to differentiation in the retina (neurogenesis) we inhibited active Notch signaling during and before neurogenesis in the retina. We show evidence that Notch signaling is not required for progenitor maintenance prior to neurogenesis, and we use this distinction to begin characterizing a novel progenitor state in RPCs. We have observed that inhibiting Notch signaling during neurogenesis results in increased cellular differentiation when retinal sections are antibody stained for cell differentiation markers. However, when Notch is inhibited prior to neurogenesis the results show no difference in cell differentiation markers. RNA sequencing will be used to find candidate factors with expression profiles tracking with the change in response to Notch inhibition. In conclusion, our study aims to characterize a novel pre-neurogenic state of RPCs. The finding of a novel progenitor state may become important for future studies with a therapeutic focus on regeneration or cell replacement.

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Polyphosphate synthesis mediates life span extension in *S. cerevisiae* mutants with increased levels of phosphate transport

Christopher L. Lord and Susan R. Wente

Phosphates play critical roles in several metabolic pathways and signaling events, and mouse research has shown they can also influence the aging process; however, the precise cellular functions regulated by phosphates that modulate life span are currently undefined. In order to mechanistically define such functions, we measured the replicative life spans of several *Saccharomyces cerevisiae* mutants that inhibit distinct aspects of phosphate metabolism. *S. cerevisiae* cells divide asymmetrically, and replicative life span (RLS) is defined as the number of daughter cells a single mother produces prior to senescence or death. Importantly, many factors that influence RLS also modulate life span in higher eukaryotes. While most mutations had little or no significant effect on RLS, *spl2*Δ cells displayed life spans ~25% greater than wild type cells. Spl2 is a ~17 kD protein that promotes degradation of the plasma membrane phosphate transporters Pho87 and Pho90. We therefore tested whether *spl2*Δ cells have elevated levels of orthophosphate or inorganic polyphosphate using live cell ^{31}P NMR. While cytoplasmic orthophosphate levels were unaffected in *spl2*Δ cells, these mutants displayed significantly increased amounts of inorganic polyphosphate, which likely is synthesized to store excess orthophosphate in the vacuoles of *S. cerevisiae* cells. Polyphosphates also appear to play important roles in protein folding and signaling events, but in large part it is unclear exactly how this occurs in yeast and mammalian cells. Using a *vtc4*Δ *spl2*Δ double mutant, we showed that life span extension in *spl2*Δ cells is completely dependent on the polyphosphate polymerase subunit Vtc4. Although phosphate levels are unaltered in long-lived *los1*Δ cells, genetic experiments show Spl2 and Los1 regulate longevity through a common downstream signaling event. Importantly, these experiments reveal a novel role for polyphosphates in longevity and provide mechanistic insights into how increasing phosphate transport extends yeast life span. Current experiments are aimed at testing whether gene expression changes mediate the effects of polyphosphate on RLS and determining the threshold of polyphosphate production that is sufficient to increase longevity.

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Distinguishing the role of ERC1 isoforms in membrane trafficking during craniofacial and neuronal development

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Membrane trafficking is one of the most essential cellular functions, however, mutations impairing proper membrane trafficking result in changes to cell shape, ultimately affecting tissue integrity. These cellular faults can result in developmental defects, affecting both craniofacial morphology and behavior. Clinical reports have identified both craniofacial and abnormal behavior phenotypes associated with distal chromosome 12 deletions. ERC1 (ELKS, Rab6IP2, CAST), a regulator of membrane trafficking, is found within the smallest region of overlap between deleted chromosome 12 regions. However, a role for ERC1 in regulating craniofacial and neuronal development has not been easy to determine, particularly due to multiple alternatively spliced ERC1 isoforms. ERC1 isoforms are reported to have differential expression, and therefore, could have separate cellular function. Unlike other vertebrate models, zebrafish have paralogous *erc1* genes, *erc1a* and *erc1b*, both of which are homologous to separate human ERC1 isoforms. Utilizing either a forward genetics screen or CRISPR/Cas9 to target zebrafish *erc1* paralogs identified differential effects on dorsal or ventral cartilage formation, causing craniofacial dysmorphology in zebrafish embryos. The aims of this project are to a) identify zebrafish *erc1* paralog effect on chondrocyte maturation and neuronal development, and b) define involvement of zebrafish *erc1* paralogs in Rab-mediated membrane trafficking during development. This project uses in vivo vertebrate animal modeling and imaging techniques to map the functional contribution of human ERC1 isoforms to craniofacial and neuronal development. Investigating the molecular function of separate ERC1 isoforms in membrane trafficking during development will help explain a mechanistic link between craniofacial dysmorphology and neurological defect comorbidity occurring in patients with distal chromosome 12 deletions.

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Determining functions of the disordered central region of the F-BAR protein Cdc15

MariaSanta Mangione, Nathan A. McDonald, Kathleen L. Gould

F-BAR proteins link membranes to actin cytoskeleton remodeling and participate in endocytosis, phagocytosis, cell migration, and cytokinesis. F-BAR proteins have a crescent-shaped, membrane-binding F-BAR domain that is commonly coupled to additional adaptor domains (e.g. SH3, μHD, RhoGAPs), often via a disordered protein region. The purpose of this study is to determine mechanistically how disordered regions contribute to overall F-BAR protein function by studying the F-BAR protein Cdc15 in the model organism *Schizosaccharomyces pombe*. *cdc15* is essential for cytokinesis, which is the last step in the cell cycle. Cytokinesis is accomplished using an actin-based contractile ring (CR) that assembles at the equator of *S. pombe* cells and eventually constricts to separate two daughter cells. Domain analysis revealed that Cdc15's N-terminal F-BAR domain is strictly essential; it binds membranes, mediates oligomerization, and recruits directly the formin Cdc12 to the division site. The F-BAR domain is linked by a disordered central region to a C-terminal SH3 domain. The SH3 domain scaffolds a network of proteins that participate in cytokinesis, but the SH3 domain is not essential.

The goal of this study is to determine whether—and if yes, then how—the central disordered region contributes to Cdc15's essential function. We found that truncating Cdc15 so that only the F-BAR domain is expressed (*cdc15ΔC)* is lethal. Furthermore, deleting the central region *(cdc15Δcentral)* is also lethal. A series of deletion mutants revealed that cells tolerate loss of at most one third of the central region. However, *cdc15* central region deletion mutants have defects in cytokinesis, including increased constriction length and asymmetric deposition of septum material. These defects are due to loss of CR integrity and we are currently testing multiple hypotheses to determine what aspect of the CR is perturbed in *cdc15* deletion mutants. Our mutants provide an in vivo system to test theoretical models of CR constriction. Furthermore, the results of this work will expose any unknown activities of the disordered central region, refining our model for Cdc15 function and potentially identifying a novel function of F-BAR proteins generally.

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Revealing the CUL9-APC/C connection during human cortical differentiation

Ortolano, N.A., Kline, L. A., Chalkley, M., Gama, V.

The cullin-ring ligase (CRL) family of E3 ubiquitin ligases play a critical role in cell cycle regulation and development. However, cullin-9 (CUL9) has proven to be a unique CRL with an elusive function. CRLs generally form large complexes that ubiquitinate a set of specific substrates. CUL9 has not been shown to form large complexes and has only two identified substrates. My data suggest a role for CUL9 in the modulation of cell cycle in human pluripotent stem cells (hPSCs) and human forebrain neural precursor cells (hNPCs). Through an unbiased mass spectrometric analysis, I determined that CUL9 interacts with several subunits of the well-characterized cell cycle regulator, the anaphase promoting complex/cyclosome (APC/C) in hPSCs and hNPCs. CUL9 protein levels are induced during reprogramming of fibroblasts into hPSCs and increase even further during cortical glutamatergic differentiation. Interestingly, knockdown of CUL9 protein levels by lentiviral transduction in hPSCs caused abnormal cortical glutamatergic differentiation. During the neural induction phase of cortical differentiation, neural rosette formation was delayed. Neural rosettes in CUL9 depleted cells were poorly formed and much smaller in size than those in control cells. We are currently developing a method, in collaboration with the Nikon Center of Excellence, to quantify and characterize neural rosette morphology using various parameters (e.g. size, cell intercalation, lumen formation and expression of polarity and neuronal markers). We hypothesize that the CUL9-APC/C interaction may be critical for proper neural rosette formation and neurogenesis, and thus efficient cortical glutamatergic differentiation. Additionally, we will determine the underlying molecular mechanism linking CUL9 and neural cell fate. We will assess the affects of CUL9 deficiency on APC/C activity and cell cycle progression using Fluorescent Ubiquitination Cell Cycle Indicator (FUCCI). We anticipate that elucidating the function of the CUL9-APC/C interaction will provide key insight into the underlying mechanisms linking cell cycle control, differentiation, and neurodevelopment.

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Non-random gamma-TuNA dependent spatial patter of microtubule nucleation at the Golgi

Anna A.W.M. Sanders, Kevin Chang, Xiaodong Zhu, Roslin J. Thoppil, William R. Holmes, Irina Kaverina

Non-centrosomal microtubule (MT) nucleation at the Golgi generates MT network asymmetry in motile vertebrate cells. Investigating Golgi-derived MT (GDMT) distribution, we find that MT asymmetry arises from non-random nucleation sites at the Golgi (hotspots). Using computational simulations, we have tested two plausible mechanistic models of GDMT nucleation leading to this phenotype. In the "Cooperativity" model, formation of a single GDMT promotes further nucleation at the same site. In the "Heterogeneous Golgi" model, MT nucleation is dramatically upregulated at discrete and sparse locations within the Golgi. While the distribution of GDMT nucleation sites is equally consistent with both models, GDMT nucleation timing and length distribution allows us to cautiously favor the Cooperativity model. Investigating the molecular mechanism underlying hotspot formation, we have found that hotspots are significantly smaller than Golgi subdomains positive for either scaffolding protein AKAP450, which is thought to recruit GDMT nucleation factors, γ-TuRC-mediated nucleation activator (γ-TuNA) domain-containing protein MMG8, or MT stabilizers CLASPs. We have further probed potential roles of known GDMT-promoting molecules, including γ-TuNA domain-containing proteins and CLASPs. While both γ-TuNA inhibition and lack of CLASPs resulted in drastically decreased GDMT nucleation, computational modeling revealed only γ-TuNA inhibition suppressed hotspot formation. We conclude that clustered GDMT nucleation is a result of γ-TuNA-dependent local activation of γ-TuRC at the Golgi. In light of the Cooperativity model, such activation should be stimulated by positive feedback by initial GDMT formation.

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PI(4,5)P_2-mediated ring anchoring resists perpendicular forces to promote medial cytokinesis

Chloe E. Snider, Alaina H. Willet, Jun-Song Chen, HannahSofia T. Brown, Göker Arpağ, Marija Zanic, and Kathleen L. Gould

Many eukaryotic cells divide by assembling and constricting an actin- and myosin- based contractile ring (CR) that is physically coupled to the plasma membrane (PM). It is not well understood how the attachment of the proteinaceous CR and the lipid-based PM is mediated. In *Schizosaccharomyces pombe* cells lacking *efr3*, which encodes a PM scaffold for a conserved phosphatidylinositol-4-kinase, the CR forms in the cell center but subsequently slides away from its original position during anaphase in a type V myosin-dependent manner. We find that CR anchoring defects are common to PIP kinase mutants that reduce PM PI(4,5)P_2 levels, while increasing PI(4,5)P_2 levels does not affect medial cytokinesis. Interestingly, the concentration of multiple membrane-binding proteins at the PM/CR is reduced in *efr3Δ*. This suggests that an ensemble of PI(4,5)P_2-sensitive proteins may coordinate to mediate CR-PM attachment. Our work indicates that PI(4,5)P_2 is important to stabilize the central position of the CR and resist myosin V-based forces to promote the fidelity of cell division.

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An iPSC-Derived Neuronal Model to Determine the Pathophysiology of Alternating Hemiplegia of Childhood

Snow JP, Westlake G, Grier M, Ess KC

Alternating Hemiplegia of Childhood (AHC) is a rare genetic neurodevelopmental disease caused by heterozygous missense mutations in the *ATP1A3* gene, encoding the neuronal specific α3 subunit of the sodium-potassium adenosine triphosphatase (Na,K-ATPase) pump. This protein complex is crucial for many cellular functions including setting the resting membrane potential and regulating ion homeostasis. AHC patients display unique symptoms beginning in early childhood, including episodes of weakness or paralysis often triggered by stress, abnormal eye movements, seizures, painful dystonia, developmental delay, and intellectual disability. These alternating hemiplegic episodes last for minutes to hours and symptoms can often be terminated by sleep. It is known that the majority of AHC patients possess one of three mutations in the *ATP1A3* gene, resulting in D801N, E815K, or G947R mutants of the α3 Na,K-ATPase subunit. However, mechanisms underlying symptoms in patients remain poorly understood and there are no empirically proven treatments for AHC. We have generated iPSCs from patients with the three most common mutations in AHC, and will focus our efforts here on the most phenotypically severe *ATP1A3* mutation, E815K. Patient-specific iPSCs have been differentiated into mixed cortical and GABAergic neurons to test our hypothesis that E815K mutant α3 protein decreases Na,K-ATPase function in a dominant-negative manner. We further hypothesize that mutant α3 protein perturbs normal neurodevelopment and results in neuronal depolarization that is exacerbated during cellular stress. Initial studies have focused on characterizing Na,K-ATPase subunit expression in developing cortical neurons. Results indicate that iPSC-derived neurons have increased levels of α3 protein and decreased levels of the α1 subunit compared to iPSCs. Expression of the α3 subunit during neuronal differentiation has similar temporal dynamics to control cells. Fluorescent indicators of sodium and calcium concentration in patient-specific iPSC-derived cortical neurons are being used to investigate ion homeostasis in the presence of the E815K *ATP1A3* mutation. Current studies involve multielectrode array electrophysiological analyses, along with testing for lineage-specific consequences of mutant α3 expression in iPSC-derived GABAergic neurons using newly published differentiation protocols. We are increasing the potential impact of our findings and probing the hypothesis of a dominant negative disease mechanism by utilizing CRISPR/Cas9 techniques to create isogenic controls by correcting *ATP1A3* mutations in patient lines, as well as through creation of heterozygous and homozygous *ATP1A3* knockout lines.

This research was generously supported by the Alternating Hemiplegia of Childhood Foundation as well as the Association Française de l'Hémiplégie Alternante.

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Impact of tip-enriched adhesion on the morphology and dynamics of actin-based protrusions

Meredith L. Weck, Scott W. Crawley, and Matthew J. Tyska

During intestinal epithelial differentiation, apical microvillar packing and organization are driven by adhesion complexes formed between two protocadherins, CDHR2 and CDHR5, that localize to the distal tips of microvilli, where they drive physical interactions between neighboring protrusions. CDHR2 is also capable of weak homophilic interactions. We recently reported that the actin-based motor myosin-7b (Myo7b) promotes the accumulation of the intermicrovillar adhesion complex (IMAC) components at microvillar tips, which is essential for the proper function of the complex. Additionally, two scaffolding proteins, USH1C and ANKS4B, play critical roles in complex formation and function. However, many questions still remain about how Myo7b, a monomeric motor, is capable of localizing these proteins and why there are so many seemingly redundant interactions. To address these questions, we developed an in-cell reconstitution assay using filopodial protrusions and their myosin transporter, myosin-10 (Myo10). The use of filopodia provides better spatial and temporal resolution, and heterologous expression allows us to control which IMAC components are present and able to interact. To allow us to take advantage of filopodia, we generated a chimera containing the motor domain of Myo10 and the cargo-binding tail domain of Myo7b, which localizes to the distal tips of filopodia. IMAC components that can interact with Myo7b will become enriched at filopodial tips when coexpressed with the chimeric motor. Using this assay, we found that the chimeric motor alone can target CDHR2 and CDHR5 to the distal tips of filopodia. Tip enrichment of CDHR2 results in increased filopodial number and stability. Additionally, CDHR2 forms homophilic interactions between adjacent filopodia, resulting in interfilopodial adhesion and tipi-like clusters of dorsal filopodia, which resemble the clusters of microvilli found on differentiating epithelial cells. The chimeric motor can also transport both USH1C and ANKS4B individually to filopodial tips. Future studies will focus on determining how the adaptors promote the transport efficiency, stability, and lifetime of the IMAC.

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The Role of Self-Association in Gle1 Activity

Aaron C. Mason and Susan R. Wente

Gle1 is a conserved multidomain protein that functions in multiple aspects of gene expression including nuclear mRNA export, translation, and the stress response. Mutations in GLE1 are causal for multiple forms of arthrogryposis, including lethal congenital contracture syndrome-1 (LCCS1). The LCCS-1 gle1-Finmajor mutation disrupts the amino-terminal coiled-coil domain of Gle1, resulting in abrogated nucleocytoplasmic shuttling, reduced mRNA export, and perturbed self-association in vitro. In this study, we expand efforts to understand how Gle1 self-association is mediated and its contribution to each of Gle1's critical cellular functions.

Gle1 has been shown to form at least a homodimer in living cells and higher ordered homomultimers in vitro. To further define which sub-domains of the N-terminus and coiled-coil domain contribute to self-association, we purified a series of bacterially expressed recombinant fragments of Gle1 and assessed their apparent molecular weight by SEC. This analysis uncovered two regions in the N-terminus that differentially mediated oligomerization. One region was the coiled-coil domain that comprised of amino acids 152-360. Using the predictive software COILS, a canonical coiled-coil heptad repeat was identified that is required for coiled-coil domains to self-associate. Mutational analysis of the predicted core hydrophobic residues within the repeat demonstrated that the coiled-coil association could be disrupted. To specifically assess the functional role of Gle1's coiled-coil interactions for mRNA export, we used a knockdown:add-back system in HeLa cells to introduce mutations that disrupt the coiled-coil oligomerization. Indirect immunofluorescence for poly(A)+ RNA revealed nuclear mRNA accumulation indicative of defective mRNA export when the coiled-coil based oligomerization of Gle1 was disrupted. We next sought to determine the orientation of the coiled-coil interaction. We utilized the biochemical method termed ACCORD to discriminate between parallel and antiparallel orientation. This method uses a dimeric fusion protein attached to the coiled-coil domain, which allows detection between a dimer (parallel) and a tetramer (antiparallel) by SEC-MALS. Our results show that the Gle1 coiled-coil domain was associated in a parallel orientation.

The second region identified in mediating Gle1 oligomerization N-terminally flanks the coiled-coil domain in an area predicted to have low structural complexity. Using AGGRESCAN, software that predicts aggregation-prone segments derived from in vivo experimental measurements, a 10 amino acid stretch was predicted to have the highest probability of aggregation. Deleting this aggregation-prone motif reduced the fraction of aggregated protein and increased the monomeric fraction. These studies demonstrate that Gle1 oligomerization is promoted through at least two discrete mechanisms.

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Role of Exosomal Endoglin in Filopodia and Tumor Cell Motility

Caitlin McAtee, Nan Hyung Hong, Daisuke Hoshino, Anthony Maldonado, Andries Zijlstra, Alissa Weaver

Exosomes are small secreted vesicles that carry a variety of cargoes and have been shown to promote tumor cell motility and metastasis. Cell motility is influenced by dynamic formation and stability of filopodia: actin-rich protrusions that extend from the leading edge and control directional movement. Furthermore, filopodia regulators such as fascin are upregulated in multiple epithelial cancers and can also promote invasive phenotypes. However, how filopodia are induced and controlled is poorly understood. Recently, our lab has shown that exosomes promote adhesion formation and control tumor cell motility. In our current work, we have identified exosomes as key regulators of filopodia formation and stability. We have also preliminarily found that the TGF-β coreceptor endoglin is a unique exosome cargo that influences filopodia formation and stability. Knockdown of endoglin reduces filopodia in melanoma cells, and this defect can be rescued by exosomes from control cells, but not exosomes from endoglin-KD cells. These data are relevant to cancer as endoglin expression is altered in many cancers; however, its function in cancer cells is not known. In addition, endoglin is highly expressed by endothelial cells, is the disease gene for hereditary hemorrhagic telangiectasia, and may influence angiogenesis. Thus, it may be important to tumor angiogenesis, either in an autocrine or paracrine manner.

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Role of a novel Senataxin-associated nuclease in DNA repair

Heather J. McCartney, Alex Andrews, Tim M. Errington, and Ian G. Macara

Yeast two-hybrid analysis has identified a novel, uncharacterized Senataxin-interacting protein with 5' exonuclease activity, and homology to the FEN1 nuclease domain. Senataxin is an RNA/DNA helicase that functions in resolving R loop structures which occur as a result of normal replication and transcription but can also persist resulting in genomic instability. We found that SAN1 (Senataxin-Associated Nuclease 1) is unable to cut dsDNA or bubbles, but is active against 5' overhangs and against ssDNAs. SAN1 cleaves 3 or 8 nt fragments from the 5' end of ssDNA and utilizes magnesium as a cofactor. As the definitive 5' nuclease responsible for unhooking ICLs has yet to be identified we speculate that SAN1 might function in excising crosslinked nucleotides that result from interstrand crosslink (ICL) damage. We generated a SAN1 -/- HeLa cell line through CRISPR/Cas gene editing and found that SAN1 -/- cells are sensitized to ICL agents mitomycin-c and cisplatin. Interestingly, colony survival assays have shown that SAN1 does not appear to be epistatic of FANCD2, a key component of the classical pathway for resolving ICLs, the Fanconi Anemia pathway. Additionally, although the nuclease domain of SAN1 is homologous to the FEN1 family of structure-specific nucleases, SAN1 also possesses a unique and conserved C terminus of unknown function that has been found to interact with the nuclease domain of SAN1. Our goal is to understand the relationship between the structure and nuclease activity of SAN1 in DNA repair and how SAN1 relates to other known ICL-repair pathways. Understanding the components that regulate genome stability is crucial in understanding how cancer occurs and what protein targets will be the most advantageous to pursue in developing potential therapeutics. This study may also provide a better understanding of a novel factor in resistance to ICL-inducing chemotherapeutics such as cisplatin and MMC which are still widely used in the treatment of breast and ovarian cancers.

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Use of a Phenotypic Screen to Identify Small Molecules that Selectively Enhance Repair in Certain Models of Acute Kidney Injury

Anna Menshikh, Lauren Scarfe, Rachel Delgado, Charlene Finney, Mark deCaestecker

Repeated episodes of acute kidney injury (AKI) increase the risk of developing chronic kidney disease (CKD), a progressive loss of kidney function over time, which may lead to end-stage renal disease requiring life-long dialysis or a kidney transplant. A therapy that enhances recovery following an episode of AKI may reduce the risk of CKD progression, however no such treatment currently exists. Importantly, the therapy would need to be effective when treatment is delayed, as many patients do not present clinical symptoms of AKI until hours or days after the initiating event.

A high-content phenotypic screen in zebrafish embryos has identified a new histone deacetylase inhibitor, 4-(phenylthio)butanoic acid (PTBA), which enhanced proliferation of renal progenitor cells in both zebrafish embryos and in a model of gentamicin-induced AKI in zebrafish larvae. Subsequently, the PTBA analog UPHD186 was identified. UPHD186 acts as a PTBA prodrug and has favorable pharmacokinetics in vivo compared with alternative analogs. Recently, PTBA analogs have been shown to accelerate recovery and reduce postinjury fibrosis in several mouse models of AKI, including ischemia reperfusion injury, unilateral ureteric obstruction, and aristolochic acid nephropathy. Importantly, beneficial results were observed even when treatment was delayed up to 4 days after the onset of AKI.

Repeated administration of cisplatin to mice models the clinical setting whereby some cancer patients receiving cisplatin chemotherapy develop subclinical AKI that may progress to CKD. Mice were given 7.5mg/kg cisplatin once a week for 4 weeks, and were treated with either vehicle, one dose of UPHD186, or three doses of UPHD186, starting one day after cisplatin administration each week. Kidney function was monitored weekly by blood urea nitrogen (BUN), and glomerular filtration rate (GFR) was measured by transcutaneous assessment of FITC-sinistrin clearance at day 28. Mice were sacrificed at day 28 and renal fibrosis was assessed histologically by Picro-Sirius Red staining and by qRT-PCR of renal Col1α1 and LoxL2 mRNA expression.

Cisplatin-treated mice progressively lost body weight over the 28-day experiment, exhibited increasingly elevated BUN levels each week, and significantly decreased GFR and increased renal fibrosis on day 28, indicating that the repeated dosing cisplatin regime successfully induced CKD. However, none of the parameters measured showed any statistically significant difference between UPHD186- and vehicle-treated mice.

Unlike previous studies in zebrafish and mouse models of kidney injury, UPHD186 did not improve kidney function or reduce postinjury fibrosis in a mouse model of repeat dosing cisplatin nephropathy.

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CD44v9-xCT system linked to cellular plasticity in the stomach

Anne R. Meyer, Amy Engevik, Eunyoung Choi, James R. Goldenring

Many differentiated epithelial cell types are able to reprogram in response to tissue damage. While reprogramming represents an important physiological response to injury, the regulation of cellular plasticity is not well understood. Damage to the gastric epithelium initiates reprogramming and proliferation of digestive enzyme-secreting chief cells into a metaplastic cell lineage known as spasmolytic polypeptide-expressing metaplasia or SPEM. Here, we investigated the role of the CD44v9-xCT cystine-glutamate antiporter system in the plasticity of chief cells. CD44v9-xCT regulates cystine uptake, necessary for glutathione synthesis and ROS detoxification. We utilized sulfasalazine, an inhibitor of xCT-mediated cystine transport, with established metaplastic cell lines and mouse models of acute gastric damage in order to determine the role of this transporter system in chief cell plasticity. We revealed that CD44v9-xCT is required for cystine uptake, defense against ROS, the complete transition of chief cells into metaplasia, and metaplasia proliferation. Treatment with metabolites of sulfasalazine, known anti-inflammatory agents, did not prevent the development of metaplasia. This data suggests that sulfasalazine is a useful drug to target metaplasia in the stomach and may represent an important tool that can be used to study chief cell reprogramming.

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Identifying new components of the Intermicrovillar Adhesion Complex (IMAC)

E. Angelo Morales, Matthew J. Tyska

Intestinal epithelial cells give rise to actin-based protrusions called microvilli, which are collectively known as brush border and are crucial for nutrient absorption and host defense against microbes. Microvilli are tightly packed and highly organized structures connected with each other by adhesion molecules termed cadherins. Cadherins are transmembrane proteins that connect to actin bundles via myosin-7b and two other cytoplasmic proteins at the distal tips of microvilli. Together, they form the Intermicrovillar Adhesion Complex (IMAC), which is important for brush border assembly and normal function of the intestinal epithelial cells. Recent evidence has shown that myosin-7b promotes the enrichment of IMAC cargoes to the microvillar tips. Myosin-7b harbors a motor domain that binds to actin, a tail domain that interacts with cargoes, and a neck region that links both domains. The tail domain of myosin-7b harbors two MyTH4-FERM domains and an intervening Src Homology 3 (SH3) domain. Whereas the interacting partners of the MyTH4-FERM domains have been characterized, the SH3 domain has no known interacting partners. In the attempt to identify new interacting partners of the SH3 domain of myosin-7b, we conducted a native pulldown assay coupled with mass spectrometry. Briefly, we expressed the His-tagged SH3 domain of myosin-7b in *E. coli*, we then purified the SH3 domain using Ni-NTA resin beads and subsequently cross-linked to NHS magnetic beads. Native brush border lysates were incubated with the SH3-coupled magnetic beads, eluted and analyzed by mass spectrometry. Out of 140 proteins pulled down, we identified four potential candidates: Caprice, Esp8L3, Epcam and Protein 4.1. Caprice and Eps8L3 have preferred localization to the brush border of intestinal epithelial cells. Interestingly, Caprice was recently identified as a novel F-actin bundling protein and showed colocalization with myosin-7a in stereocilia (microvilli-like structures in hair cells). Similarly, Eps8L3 also shows preferred localization to the brush border and belong to Eps8 family that display actin bundling and capping functions. Although Epcam and Protein 4.1 localization is not restricted to the brush border, they are known as adaptor proteins at the interface of the membrane and the actin cytoskeleton. Given that myosin-7b is an actin-based motor, these proteins represent potential interacting partners of the SH3 domain that could provide insights on how myosin-7b is regulated and/or anchored to the tips of microvilli to support the IMAC assembly. Further validation, including biochemical and tip targeting assays, will be conducted to determine if they are true interacting partners.

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Clasp Regulates Actin Stress Fiber Organization and Mechanotransduction

Shwetha Narasimhan, Nadia Efimova, Tatyana Svitkina, Irina Kaverina

Study of the interplay between microtubule-actin networks provides a fuller understanding of cellular physiology. Microtubules are dynamic polymers made of tubulin. They have a distinct polarity of plus and minus ends and facilitate processes such as intracellular trafficking, cell polarization and chromosome segregation. Actin stress fibres (SFs) form contractile networks that generate forces essential for processes such as migration, morphogenesis and remodeling of the extracellular matrix. SFs in motile cells are classified into different subtypes such as dorsal stress fibres, ventral stress fibres and transverse arcs. The regulatory mechanisms defining complex organization and dynamics of SFs are an ongoing field of study. To this end, my experiments show that SFs are altered in cells lacking microtubule-associated proteins CLASPs.

CLASPs are plus tip binding proteins that stabilize microtubules, rescue them from catastrophe and mediate interactions between microtubules and the cell cortex. In CLASP-depleted cells, actin bundles are disorganized, with ventral stress fibres being severely affected. CLASP depleted cells also have loss of actin bundling, leading to a model where CLASPs regulate actin bundling to form stress fibers. Furthermore, silicon contractility assays show an abnormal, spiral pattern of contraction in CLASP-depleted cells compared to the wild type, implying a vital role of CLASPs in facilitating directional force transduction to the environment. Thus, my project investigates a novel mechanism of microtubule-actin crosstalk in the organization of actin stress fibres, providing new information on how microtubule-associated proteins can govern actin organization and mechanotransduction.

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Focal adhesion kinase regulates early steps of myofibrillogenesis in cardiomyocytes

Abigail C. Neininger, Nilay Taneja, Matthew R. Bersi, W. David Merryman, Dylan T. Burnette

Forces generated by myofibrils within cardiomyocytes must be balanced by adhesion to the substrate and to other cardiomyocytes for proper heart function. Loss of this force balance results in cardiomyopathies that ultimately cause heart failure. How this force balance is first established during the assembly of myofibrils is poorly understood. Using human induced pluripotent stem cell derived cardiomyocytes, we show coupling of focal adhesions to myofibrils during early steps of de novo myofibrillogenesis is essential for myofibril maturation. We also establish a key role for Focal adhesion kinase (FAK), a known regulator of adhesion dynamics in non-muscle cells, in regulating focal adhesion dynamics in cardiomyocytes. Specifically, FAK inhibition increased the stability of vinculin in focal adhesions, allowing greater substrate coupling of assembling myofibrils. Furthermore, this coupling is critical for regulating myofibril tension and viscosity. Taken together, our findings uncover a fundamental mechanism regulating the maturation of myofibrils in human cardiomyocytes.

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N-acetyl glucosamine kinase is required for anteroposterior patterning in *Xenopus laevis* embryos

Leif Neitzel, Christopher Cselenyi, CheyAnne Youngblood, Alya Zouaoui, Ethan Lee

Wnt ligands play a critical role in embryonic development and misregulation of the Wnt pathway results in numerous human diseases. The use of *Xenopus* embryos as an *in vivo* system for studying Wnt signaling is supported by an exceedingly large body of work that serves as a clear precedent to support the physiological relevance of the data. Using a novel overexpression screen developed by our lab, we identified kinases that modulate axis formation in *Xenopus laevis* embryos upon their overexpression.

One kinase, N-acetyl glucosamine kinase (NAGK), exhibited the strongest and most penetrant phenotype and we decided to pursue its further characterization. Injection of NAGK mRNA or recombinant protein robustly inhibits anterior trunk and head structure formation. Conversely, Morpholino mediated knockdown of NAGK promotes expansion of anterior structures. In *Danio rerio* (zebrafish), NAGK overexpression inhibits eye formation while inhibition of NAGK results in cyclopia. These data are consistent with NAGK acting as a positive regulator of canonical Wnt signaling in *Xenopus* and zebrafish. Experiments are ongoing to determine whether NAGK regulates Wnt target gene transcription and the level at which NAGK acts on the Wnt pathway.

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The USP46 deubiquitinase complex promotes Wnt signaling by blocking receptor turnover

Victoria Ng, Amanda Hansen, and Ethan Lee

The Wnt signaling pathway is critical for human development and its misregulation leads to a variety of human diseases. In the latter case, the most notable is evidenced by mutational activation of the pathway in over 90% of non-hereditary colorectal cancer. Controlling the abundance of Wnt receptors (LRP5/6 and Frizzleds) has been shown to be important in maintaining pathway homeostasis. It has been previously shown that the E3 ligases, RNF43/ZNRF3, ubiquitinate Wnt receptors to promote their degradation. The secreted Wnt agonist, R-spondin (RSPO), promotes receptor abundance and potentiates Wnt signaling by inhibiting the activities of RNF43/ZNRF3. The evidence for the importance of controlling receptor homeostasis is demonstrated by the fact that deletion of both *RNF43* and *ZNRF3* in mice results in intestinal adenoma formation and that *RSPO* gene fusions occur in 10% of colorectal cancers. In a forward genetics screen we identified WDR20 and UAF1 as positive regulators of Wnt signaling. WDR20 and UAF1 only have modest effects on Wnt activation when overexpressed. However, in association with USP46, we show that the USP46/WDR20/UAF1 complex ("USP46 complex") greatly potentiates Wnt signaling. In addition, we demonstrate that 1) the USP46 complex itself is stabilized by Wnt signaling, 2) overexpression of the USP46 complex decreases LRP6 ubiquitination, and 3) the USP46 complex is recruited to LRP6 in the presence of Wnt. Finally, we show that overexpression of the USP46 complex with a constitutively active form of LRP6 lacking the extracellular region (TM-LRP6ICD) is sufficient to increase the steady-state levels of both the USP46 complex and TM-LRP6ICD. These findings suggest that Wnt signaling stabilizes the USP46 complex and promotes its association and deubiquitination of LRP6. It remains unclear how the USP46 complex is recruited to LRP6 and what structural features of LRP6 are necessary for its binding to the USP46 complex. Our studies represent a unique opportunity to delineate the important role of deubiquitination in regulating Wnt receptor homeostasis and have great potential to inform the development of novel therapeutics targeting Wnt-driven diseases.

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Calcium signaling dynamics in the early response to epithelial wounds

James O'Connor, Erica Shannon, M. Shane Hutson, Andrea Page-McCaw

Epithelial tissue is an important structure in all complex organisms, lining all surfaces that come in contact with the environment and protecting the inside of the organism from the outside world. Normally, these epithelial cells are stationary, non-invasive, non-proliferative, and polarized along the apical-basal axis. However, upon sensing a wound, these cells can become migratory, invasive, proliferative, and polarized along the front-rear axis. This state change serves as a mechanism by which these cells can rearrange in order to heal wounds and restore epithelial structure and function. One fundamental question is how these cells can detect and interpret signals from a wound in order to properly respond to the epithelial breach.

The earliest detectable signal in wounds is a dramatic increase in cytosolic calcium that spreads from the wound margin, which is conserved among model organisms and humans. This signal has been shown to trigger a healing response around the wound margin, as well as in cells distant from the wound. Through a collaborative effort, we have used complex genetic tools coupled with quantitative analysis to create a wound model in the *Drosophila* pupal notum, an epithelial monolayer on the dorsal side of *Drosophila* pupae. We created mosaic-like flies that express genetic changes in one part of the pupal notum, while maintaining an internal control in the neighboring section of epithelium. By wounding on the border of gene expression in this tissue using pulsed laser ablation, we can create reproducible epithelial wounds, and monitor the dynamics of the calcium signal that is released in real time using a genetically encoded GCaMP.

We have shown that an initial influx of calcium is due to plasma membrane micro-tears at the wound site, allowing extracellular calcium to flood into the cells. This calcium then diffuses from the wound through gap junction mediated diffusion. Here we will present data showing that a second calcium expansion is mediated by a G-protein signal transduction pathway that releases calcium stored in the endoplasmic reticulum. Our goal is to develop an integrated model of how wounded cells signal their neighbors and control their epithelial state change in order to properly respond to damage.

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Role of exosomes in the development of dendritic filopodia and spines

Mikin Patel, Mingjian Shi, Caitlin McAtee, Daisuke Hoshino, Andrew McKenzie, Donna Webb, Alissa Weaver

Dendritic spines are actin-rich structures at the postsynaptic sites of most excitatory synapses in the central nervous system (CNS). They are highly important structures for the cognitive functions of learning and memory. Abnormalities in dendritic spines are observed in many neurological disorders such as Alzheimer's, schizophrenia and autism spectrum disorders. Several live imaging studies have shown that long, thin, actin-rich protrusions called dendritic filopodia are precursors of dendritic spines in hippocampal and cortical neurons. So far, many different intracellular factors that regulate filopodia formation have been identified. However, extracellular mechanisms of filopodia formation are largely unknown. In recent years, there has been growing interest in the field of exosome research due to accumulating evidence indicating their role in cellular communication. Exosomes are small extracellular vesicles that are released from the cell upon fusion of a multi-vesicular body (MVB) to the plasma membrane. They are enriched in proteins, lipids and various nucleic acids. Due to their unique composition, exosomes have been shown to regulate behavior of recipient cells in an autocrine or paracrine manner. Although progress has been made in understanding functions of exosomes in various physiological and pathological processes of the CNS, their role in neuronal development remains unexplored. Previous studies from several different cell types have shown that Hrs, an ESCRT-0 protein, is a critical regulator of exosome biogenesis. While Rab27b, a small Rab GTPase, is involved in MVB transport to the plasma membrane for exosome release. Here, we show that actin-rich filopodia and spines are sites of exosome secretion using MVB marker GFP-Rab27b. Furthermore, overexpression of Rab27b leads to increase in the number of filopodia and spines while knockdown of Hrs or Rab27b significantly reduces filopodia and spine density. Also, we demonstrate that defects of Hrs or Rab27b knockdown neurons in filopodia density are rescued by the addition of isolated neuronal exosomes to the cultures. Collectively, these results indicate that exosomes promote the development of dendritic filopodia and spines.

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Understanding the MYC-HCF-1 interaction

Tessa M. Popay and William P. Tansey

The oncoprotein c-MYC is over-expressed in the majority of cancers. Despite extensive studies into the function of c-MYC, much of this has been limited to the N-terminal transactivation domain and the C-terminal DNA binding domain. Within the understudied central portion of c-MYC are three highly conserved MYC boxes; MbIIIa, MbIIIb, and MbIV. We recently identified and validated MbIV as interacting with the abundant transcriptional co-regulator host cell factor (HCF)-1. HCF-1 is an essential component of many chromatin-associated complexes, and is necessary for progression through the cell cycle. Mutations in c-MYC that disrupt its interaction HCF-1 limit the growth of MYC-driven tumorigenesis. The goal of my research is to understand the molecular processes that contribute to this phenotype, specifically at the level of chromatin and transcriptional regulation. To achieve this, I am combining simple genetic mutants with the CRISPR/Cas9 system and powerful genomic approaches, including chromatin immunoprecipitation (ChIP) and precision nuclear run-on (PRO) sequencing. From this, I have found that almost 40% of MYC binding sites on DNA are also occupied by HCF-1, and established how disrupting the MYC-HCF-1 interaction affects this overlap. These data suggest an important functional relationship between c-MYC and HCF-1.

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IRTKS elongates brush border microvilli using EPS8-dependent and independent mechanisms

Meagan M. Postema, Nathan E. Grega Larson, Matthew J. Tyska

The intestinal brush border lines the apical surface of enterocytes and is composed of thousands of actin-supported protrusions called microvilli, which extend into the intestinal lumen. Microvilli function to increase intestinal surface area and a properly formed brush border is critical for maintaining intestinal homeostasis. However, the mechanisms underlying the formation of microvillar protrusions remain unclear. Here we provide evidence that the I-BAR domain containing protein, insulin receptor tyrosine kinase substrate (IRTKS), is essential for normal microvillar growth and elongation. IRTKS targets to the tips of actively growing microvilli, elongates microvilli when overexpressed, and is required for microvilli to achieve their normal length during BB formation. Our experiments reveal that the SH3 domain of IRTKS binds to the actin capping and bundling protein EPS8, and the two proteins colocalize at the distal tips of microvilli. Similar to IRTKS, knockdown of EPS8 impairs microvillar elongation. Interestingly, when IRTKS is knocked down, EPS8 levels at microvillar tips are reduced. This latter finding suggests that IRTKS promotes tip enrichment of EPS8 and provides at least one explanation for the short microvillar phenotype upon IRTKS knockdown. We also found that an IRTKS construct lacking its C-terminal WH2 domain generates microvilli that are significantly shorter; however, the length of these protrusions can be rescued to WT levels when EPS8 is simultaneously overexpressed. Together these results suggest that IRTKS elongates microvilli in two ways: directly using its WH2 domain, and indirectly using its SH3 domain to promote the tip localization EPS8, which in turn promotes microvillar elongation using a separate mechanism. Thus, an IRTKS/EPS8 complex targets to the tips of microvilli where it controls the length of these protrusions during brush border formation.

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MCL-1 maintains stem cell pluripotency through its regulation of mitochondrial dynamics and metabolism

Megan Rasmussen, Alejandra Romero-Morales, Leigh A. Kline, Jeffrey Rathmell, Kathryn Beckermann, Vivian Gama

Mitochondrial dynamics, which are maintained by the balance of mitochondrial fission and fusion, affect not only cellular metabolic profiles, but also proliferation and apoptosis. Preserving this balance is essential to maintain mitochondrial genome integrity, efficient ATP generation, and control ROS levels. Emerging studies show that the mitochondrial network in stem cells is shifted to promote a highly fragmented state. However, the mechanisms by which mitochondrial dynamics are maintained and regulated in pluripotent stem cells remain unknown. Data from our laboratory demonstrate that Myeloid Cell Leukemia-1 (MCL-1), an anti-apoptotic protein belonging to the BCL-2 family, is a fundamental regulator of mitochondrial dynamics in human pluripotent stem cells. My studies demonstrate that MCL-1 not only inhibits cell death, but also has additional roles in the regulation of stem cell fate through the modulation of mitochondrial fragmentation and metabolism. MCL-1 is induced upon reprogramming, and MCL-1 inhibition or knockdown induces dramatic changes to the mitochondrial network, as well as the loss of the key pluripotency transcription factors, NANOG and OCT-4. Aside from localizing at the outer mitochondrial membrane, as with other BCL-2 family members, MCL-1 is unique in that it also resides at the mitochondrial matrix in pluripotent stem cells. Mechanistically, my studies show that MCL-1 interacts with DRP-1 and OPA1, two GTPases responsible for remodeling of the mitochondrial network. We were able to disrupt these interactions using a recently published small molecule inhibitor of MCL-1, which specifically binds with high affinity to MCL-1's BH3-binding groove. To further decipher this mechanism for MCL-1 regulation of DRP-1 and OPA1, we will perform structure-function analysis coupled with high resolution imaging to examine the effects of disrupting these protein interactions. Our experimental system is ideal for elucidating how MCL-1 can regulate both cell death and mitochondrial dynamics in stem cells. In addition, my preliminary data indicate that MCL-1 may also support stem cell metabolism through its interaction with OPA1 at the matrix. Upon treatment with the MCL-1 inhibitor, pluripotent stem cells showed reduced levels of basal respiration, ATP production, and respiration capacity by Seahorse analysis. These findings suggest that MCL-1 mediates a potential link between mitochondrial dynamics and metabolism, and uncovers an unexpected, non-apoptotic function for MCL-1 in stem cell fate. Future studies using cellular reprogramming will examine the requirement of this non-canonical function of MCL-1 for cell fate conversion.

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The role of MCL-1 in early human brain development

Alejandra Romero-Morales, Megan Rasmussen, Ela Contreras Panta, Shannon Faley, Leon Bellan, Ethan Lippmann and Vivian Gama

Myeloid cell leukemia-1 (MCL-1) is an anti-apoptotic protein that prevents mitochondrial outer membrane permeabilization after cell death stimuli. Studies from our laboratory showed that MCL-1 is essential in human pluripotent stem cells (hPSCs) not only for the regulation of cell death, but also for the maintenance of pluripotency. We are interested in uncovering the function of MCL-1 as pluripotent stem cells undergo neuronal differentiation. Previous studies from our laboratory indicate that when MCL-1 is deleted in neural progenitor cells (NPCs) of the mouse central nervous system together with BAX/BAK -key pro-apoptotic proteins- their brains still exhibit signs of dysfunction (*e.g.* loss of cellular architecture and increased gliosis). My studies demonstrate that the levels of MCL-1 are reduced but maintained as the cells commit to a neural progenitor fate and mature into neurons. We found MCL-1 to be localized at the outer mitochondrial membrane and at the matrix in hPSCs, thus, we are currently investigating whether both forms of MCL-1 are reduced during neural differentiation. Interestingly, when MCL-1 was downregulated in human NPCs, using RNAi, the neural markers PAX6 and NESTIN were significantly decreased without any signs of cell death, suggesting that MCL-1 is required to maintain the identity of neural progenitors, independently of its anti-apoptotic function. We are currently investigating whether this effect is due to MCL-1's non-apoptotic function in regulating mitochondrial dynamics or metabolism. To gain a deeper understanding of the physiological effects of MCL-1 during neural differentiation and maturation in the context of the tissue microenvironment, we are developing, in collaboration with our colleagues at the School of Engineering, a 3D brain organoid system in which vascularization will be incorporated. With this approach, we seek to model the behavior of different neuronal populations and to elucidate the function of MCL-1 during early human brain development. My overarching goal is to uncover the function of core mitochondrial proteins in early stages of differentiation and to reveal potential new targets in mitochondrial-related human diseases.

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DYRK2 enhances Wnt signaling activation through GSK3b directed phosphorylation of LRP6

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Protein kinases (e.g. GSK3 and CK1) have been shown to control multiple steps in the Wnt pathway. To identify other kinases that could regulate the Wnt signaling, we performed an expression cloning developmental screening in Xenopus embryos using a kinase specific library. One candidate identified was the dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2). We found that DYRK2 overexpression in Xenopus embryos promoted axis duplication whereas DYRK2 morphant embryos were ventralization. Consistent with our Xenopus results, human cells overexpressing DYRK2 potentiate Wnt3a signaling as evidenced by elevated phospho-LRP6, elevated cytoplasmic b-catenin, and enhanced TOPflash reporter signal. Furthermore, knockdown of DYRK2 in human cells inhibited Wnt reporter activation and prevented Wnt3a-induced LRP6 phosphorylation. In order to determine the mechanism of DYRK2 action, we found that DYRK2 and GSK3 co-immunoprecipitated from cells, and that DYRK2 enhances GSK3-dependent LRP6 phosphorylation in vitro. Taken together, our results suggest a model where DYRK2 cooperates with GSK3 to activate and enhance LRP6 phosphorylation and Wnt pathway activity.

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Bid maintains mitochondrial cristae structure and function in mice and protects against human cardiac disease in an integrative genomics study

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Mitochondria are structurally dynamic double membrane organelles that harbor inner membrane invaginations known as cristae. Cristae harbor the electron transport chain among other proteins and can rearrange depending on metabolic demand or during intrinsic apoptosis to mobilize cytochrome c, facilitating its release. While maintenance of cristae structure is essential for mitochondrial function, only recently are some of the proteins involved in this process beginning to be understood.

We find that in the absence of the pro-apoptotic Bcl-2 protein, Bid, Myeloid progenitor cells (MPC) have severe mitochondrial defects, with decreased numbers of cristae as well as decreased respiration. Mitochondrial structure and function can be rescued with the re-introduction of non-apoptotic forms of Bid, BH3-mutated and caspase-8 cleavage mutated (D59A) Bid. Furthermore, we find that cardiomyocytes from *Bid-/-* mice have decreased complex I (CI) supported respiration and ATP production, as well as decreased CI supercomplex and ATP synthase dimer enzymatic activity. Interestingly, upon acute stress, *Bid-/-* mice display left ventricular dysfunction, as well as decreased ejection fraction and fractional shortening.

To determine if our findings have implications for human disease, we used a gene-based approach applied to a large-scale biobank (BioVU) called PrediXcan. We observed decreased BID gene expression significantly correlates with myocardial infarction (MI). We also find that this result is highly significant for BID among other BH3-only proteins. Moreover, we have validated our PrediXcan findings in two independent studies; the CARDIoGRAMplusC4D GWAS as well as the UK Biobank.

In combination with PrediXcan, we have also used BioVU for exome analysis of BID and find that carrier status for the coding SNP M148T, located in Bid's membrane binding domain (Helix 6), associates with diagnosis of MI. Helix 6 has been previously identified as important for cBid dependent cristae reorganization. While Bid mutated in its apoptosis-promoting (BH3) domain restores mitochondrial respiration, it does not when combined with M148T. Furthermore, BH3M148T-double mutant does not restore cristae numbers.

In sum, we have identified a novel role for Bid in the regulation of mitochondrial cristae with implications for MI. To our knowledge, we are the first to incorporate multiple large scale-human genetics studies with findings in cell lines and a mouse model. This multi-scale translational approach may serve as a model approach for elucidating the genetic basis of complex human disease traits.

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HNSCC exosomes drive tumor angiogenesis via ephrin reverse signaling

Shinya Sato and Alissa M. Weaver

Exosomes are small extracellular vesicles that are secreted upon fusion of multivesicular endosomes (MVE) with the plasma membrane and carry bioactive protein and RNA cargoes. Increasing numbers of studies have identified key roles for exosomes in driving aggressive tumor behaviors, including angiogenesis. However, the mechanisms are unclear. Our goal is to identify the role of head and neck squamous cell carcinoma (HNSCC) exosomes in tumor angiogenesis.

In an orthotopic mouse model of HNSCC, microvessel density of primary tumors correlated with exosome secretion rates of original HNSCC lines. In vitro, conditioned medium (CM) and purified exosomes but not exosome-depleted CM from HNSCC cells drove tube formation of Human Umbilical Vein Endothelial Cells (HUVECs) and human lymphatic endothelial cells. Inhibition of nucleic acid cargo activity by psoralen cross-linking or inhibition of miRNA processing by knockdown of Dicer had no effect on endothelial tube formation, suggesting that transfer of RNAs is not responsible for HNSCC-induced angiogenesis. Proteomics analysis of HNSCC exosomes revealed multiple potential angiogenic proteins, including EphB2 and EphB4. The addition of purified HNSCC exosomes to HUVECs induced reverse ephrin-B signaling in endothelial cells, as assessed by Western blot analysis. To test whether reverse ephrin-B signaling might account for exosome-induced angiogenesis, we pre-incubated purified exosomes with Fc-ephrin-B2 to block the interaction between exosomal EphB2 and ephrin-B2 on endothelial cells. We found that low concentrations of this reagent had little effect on endothelial tube formation in the absence of exosomes but blocked the pro-angiogenic effect of the exosomes.

We find that tumor-derived exosomes can induce reverse ephrin-B signaling and angiogenesis. This mechanism may be important in the HNSCC microenvironment.

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Microvesicles Contribute to Matrix Remodeling and Cancer Heterogeneity

Samantha C. Schwager, Lauren A. Hapach, Francois Bordeleau, Marc A. Antonyak, Richard A. Cerione, Cynthia A. Reinhart-King

As cancer cells metastasize from a primary tumor, the stromal microenvironment plays a crucial role in the determination of metastatic potential. This feedback is bidirectional as the microenvironment is often manipulated by cancer cells through both direct and indirect mechanisms. Recent studies have shown that extracellular vesicles secreted by cancer cells, specifically exosomes and microvesicles, communicate with other cell types, including epithelial and stromal cells, to alter the local tumor microenvironment as well as the pre metastatic niche. Microvesicles contain a wide variety of cargo, including ECM components, growth factor receptors, cytoskeletal proteins, and signaling molecules, but the effects of these components on exposed cells and subsequently the microenvironment are just beginning to be uncovered. Here, we show that microvesicles isolated from breast carcinoma cells alter the morphology of both breast epithelial cells and stromal fibroblasts. Additionally, we found that breast cancer-derived microvesicles increased breast epithelial cell-mediated collagen bundling and contractility in 3D collagen gels. As cancer cells within the primary tumor exhibit heterogeneity in migratory capability, we sorted breast carcinoma cells based on migration phenotype into ‘invasive’ and ‘noninvasive’ subpopulations using a transwell migration assay. Microvesicles released from these migratory subpopulations were then analyzed based on microvesicle concentration, size, and protein content. The phenotypic changes caused by cancer cell-derived microvesicles and the differences in subpopulation-derived microvesicles may play a role in the development of the pre metastatic niche.

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Colorectal Cancer Cell-of-origin and Cancer Stem Cell Dynamics

Cherie R. Scurrah, Alan J. Simmons, Wei Li, Yu-Ping Yang, Eunyoung Choi, Robert J. Coffey, James R. Goldenring, Ken S. Lau

Colorectal cancer (CRC) is the third leading cause of cancer mortality in the United States; a consequence of therapeutic resistance, tumor recurrence, and metastasis due to the heterogeneous nature of colon tumors. Tumor cellular heterogeneity is suggested to be a consequence of stem-like activities of cancer stem cells (CSCs), but the relationship of CSCs with the tumor cell-of-origin, the cell that acquires the first driver mutations, remains to be defined. We hypothesize that the tumor cell-of-origin is directly related to the CSCs present in tumors that consequently arise. To test this hypothesis, plasticity and tumorigenesis of stem cells (Lrig1-CreERT2) and non-stem cell (Mist1-CreERT2) were compared and analyzed through the use of lineage mapping and single cell RNA sequencing. We observed that although Mist1 non-stem cells do not express stem cell characteristics, they can serve as tumor cells-of-origin and these tumors have different characteristics than Lrig1 stem cells driven tumors. This suggests that the origin of tumorigenesis influences tumor CSCs thus aids in the understanding of tumor heterogeneity and the possible development of novel therapies targeting such heterogeneity.

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Nucleocytoplasmic shuttling of Gle1 contributes to R-loop resolution

Manisha Sharma and Susan R. Wente

Gle1 is a master regulator of RNA-dependent DEAD box ATPase proteins (Dbps) involved in the remodeling of messenger (m)RNA-protein complexes (mRNPs) during nuclear export, translation and stress granule biology. The mechanism of action during mRNA export has been well defined in budding yeast, where Gle1 bound to inositol hexakisphosphate (IP_6) at the cytoplasmic NPC face stimulates Dbp5 (human DDX19) to activate mRNP remodeling. This alters the composition of proteins bound to an mRNP, enabling its directional release to the cytosol with the proper protein complement to direct its fate. In HeLa cells, a two-fold nuclear accumulation of poly(A) mRNA is observed when nucleocytoplasmic shuttling of Gle1 is perturbed by uptake of a unique 39 amino acid peptide comprising the Gle1 shuttling domain (SD). To determine the composition of the mRNA pool sequestered in the nucleus, HeLa cells were treated with Gle1-SD or scrambled peptide, and nuclear RNA was isolated for RNA-seq analysis of the poly(A)+RNA library. Differential analyses comparing Gle1-SD peptide versus scrambled peptide treatments revealed that 56 protein coding mRNAs were differentially accumulated in the nucleus with greater than or equal to a 4 fold change. To validate the dataset, nuclear accumulation of nine transcripts identified in the RNA-seq analysis was confirmed by RT-qPCR analysis. In-cell analysis of nascent transcript levels using click-IT chemistry further revealed that treatment with Gle1-SD peptide activates transcription of these target genes. Bioinformatic analyses of accumulated transcripts revealed a striking enrichment of R-loop prone transcripts with 58% of the mRNAs possessing R-loop propensity in their 5'UTR or gene coding region, and 22% containing R-loop prone sequences in their 3'UTR. R-loops are DNA:RNA hybrids that form during transcription at sites with high GC skew. Indirect immunofluorescence confirmed an increase in nuclear R-loop signal intensity upon Gle1-SD peptide treatment. Additionally, immunoprecipitation of R-loops using DNA:RNA structure specific antibody (DRIP) revealed an increase in R-loop signal at certain target transcripts after Gle1-SD peptide treatment. These results provide evidence that Gle1 is involved in resolving R-loops, potentially at the site of transcription termination. Senataxin is a known RNA helicase that resolves R-loops during transcription termination and its distribution is altered when Gle1 is silenced. We hypothesize that Gle1 may be modulating the ATPase activity of senataxin to resolve R-loops formed during transcription termination. Future studies seek to determine the mechanistic underpinnings of Gle1's role in modulating senataxin and R-loop biology.

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miR-148a regulates chief cell plasticity in the stomach

Yoojin Sohn, Takahiro Shimizu, Eunyoung Choi and James R. Goldenring

Metaplasia is defined as a conversion of one mature cell type to another mature cell type, taking place when the cells are exposed to a stimulus or stress. This abnormal cell growth can be problematic, for it has capability to develop and progress into cancer. In normal stomach, gastric chief cell are terminally differentiated cells located at the base of gastric gland, and they are responsible for secreting digestive enzymes. In the event of acid-secreting parietal cell loss in stomach, chief cells transdifferentiate into mucous cell metaplasia, designated as spasmolytic polypeptide-expressing metaplasia (SPEM). SPEM is considered a precursor of intestinal-type gastric adenocarcinoma. In this study, we used microRNA sequencing to examine the role of miRNA gene regulation in chief cell transdifferentiation and SPEM development. Normal chief cells isolated from mouse stomach and cell lines established by isolating chief cell and SPEM cell population from mice (ImChief and ImSPEM cells) were used in miRNA sequencing, and a number of miRNAs that were downregulated in SPEM were identified. From the range of miRNA, miR-148a showed the strongest expression in normal chief cells and was significantly downregulated during the process of SPEM development. *In situ* hybridization using miR-148a probe confirmed that miR-148a expression decreases as SPEM develops in mouse stomach. *In vitro* suppression of miR-148a in chief cells induced upregulation of SPEM cell marker, CD44 variant 9, along with DNA methyltransferase, Dnmt1, which is a validated target of miR-148a. Immunofluorescence staining analysis showed that Dnmt1 was upregulated in SPEM cells as well as in chief cells before the emergence of SPEM in drug-induced SPEM mouse model. These findings suggest that a miR-148a-Dnmt1 regulatory network has important roles for the initiation process of chief cell transdifferentiation into SPEM cells.

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Dynamics and Regulation of Microtubule Minus Ends

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Dynamic organization of microtubule minus ends is essential for the formation and maintenance of acentrosomal microtubule arrays, such as those found in epithelial cells, neurons, or cells undergoing meiosis. In vitro, both microtubule ends switch between phases of assembly and disassembly, behavior known as microtubule dynamic instability. However, while the minus ends have characteristically slower growth rates, the frequency at which they transition from the growing to the shrinking state (known as 'catastrophe frequency') is similar to that observed at the plus ends. The mechanisms underlying this difference between the two ends are not known. Here, we use an in vitro reconstitution approach with total internal reflection fluorescence (TIRF) microscopy to determine the parameters characterizing minus-end dynamics. Interestingly, we find that the microtubule catastrophe rates are not defined by the size of the protective GTP-cap at either end. Rather, we show that kinetic on and off rates of tubulin are the primary determinants of the differences between plus and minus end dynamics. Additionally, we find that the minus-end directed kinesin HSET/KIFC1 is a regulator of microtubule minus-end dynamics, which specifically suppresses minus end catastrophe, without changing the minus end growth rate, further potentiating the differences between the two microtubule ends.

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Netrin/UNC-6 triggers actin assembly and non-muscle myosin activity to drive dendrite retraction in a self-avoidance response

Lakshmi Sundararajan, Cody Smith, Bryan Millis, Matthew Tyska, David Miller III

Dendritic arbors are defined by the combined effects of dendrite outgrowth versus contact-dependent self-avoidance but the downstream pathways that balance these opposing mechanisms are unknown. We have previously shown that the diffusible cue UNC-6/Netrin functions as a short-range signal with its receptors UNC-40/DCC and UNC-5 to trigger dendrite retraction in the self-avoidance response for PVD neurons in *C. elegans*. Here we report that the actin-polymerizing proteins UNC-34/Ena/VASP and WSP-1/WASP function downstream of UNC-5 and that dendrites show a burst of distal F-actin assembly upon mutual contact. The paradoxical idea that actin polymerization results in shorter rather than longer dendrites may be explained by our finding that non-muscle myosin II, NMY-1, is necessary for retraction and could therefore mediate this effect by accelerating retrograde flow. Our results also show that dendrite length is defined by the antagonistic effects on the actin cytoskeleton of retraction mediated by UNC-6/Netrin versus outgrowth promoted by the DMA-1 receptor. Thus, our findings suggest that the dendrite length depends on distinct modes of actin assembly for growth versus retraction.

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Precise tuning of cortical contractility regulates mechanical equilibrium during cytokinesis

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The cell cortex comprises a thin network of actin filaments underneath the plasma membrane. Contractile force generation by myosin II (MII) in the cortex drives shape changes during cell division, cell migration and tissue morphogenesis. During the cytokinetic (C)- phase of cell division, large contractile forces and cortical remodeling events are required at the equatorial cortical network to drive furrow ingression. Outward pressures created by furrow ingression must be balanced by MII contractility at the polar cortical network. The roles for the two MII isoforms, MIIA and MIIB, in the establishment of a mechanical equilibrium between these two cortical networks are not understood. We found MIIA depletion resulted in slower cleavage furrow ingression, and loss of both MIIA and MIIB filaments in the furrow, with no changes at the polar cortex. MIIB depletion, in turn, resulted in intense blebbing at the polar cortex, with no changes in furrow ingression. This suggested an imbalance of mechanical forces at the polar cortex could drive blebbing. We therefore developed a coarse-grained mathematical description of cortical behavior, incorporating experimentally measured kinetic parameters to predict pressure and tension at the cortex. Modeling the cortex based on active gel theory surprisingly revealed no significant differences in outward pressure and cortical tension in the two knockdown conditions. Instead, our model predicted differences in the duty ratios of MII isoforms and total contractility must account for the observed differences in cortical stability in the two knockdown conditions. We verified these predictions using both MII chimeras and biophysical assays. We further found Rho kinase and Myosin Light Chain Kinase regulated the recruitment and turnover of MII isoforms, respectively. Our results suggest in the unperturbed state, competition between MIIA and MIIB regulates cortical turnover, while competition between MLCK and ROCK regulates turnover of MII itself. Depletion of either isoform leads to compensation at the polar cortex, driving the cell to extremes of contractility, with MIIA and MIIB depletion leading to hypo- and hyper-contractility, respectively, leading to cytokinetic failure. Therefore, our data support a model where an intermediate level of contractility leads to efficient cytokinesis, allowing MIIA driven furrow ingression balanced by MIIB driven polar tension generation.

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Increased Extracellular Matrix Stiffness Enhances Biglycan Expression in Endothelial Cells

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The extracellular matrix is a dynamic environment with important structural and regulatory roles for cells/tumors. The tumor microenvironment is significantly stiffer than normal tissue and extracellular matrix stiffness can regulate gene expression. To investigate the effects of stiffening on endothelial cells, RNA-seq was performed on endothelial cells seeded on compliant and stiff matrices. Numerous transcriptional differences were found including a robust increase in biglycan on stiff substrates which was confirmed via qPCR. Biglycan, a small proteoglycan of the extracellular matrix has recently been implicated in tumor progression. High biglycan expression is a characteristic of the tumor microenvironment, and specifically tumor endothelial cells. Endothelial cell secreted biglycan has been shown to promote angiogenesis in an autocrine manner and enhance cancer cell metastasis in a paracrine manner. However, the tumor microenvironment factor responsible for upregulating biglycan expression in endothelial cells remains unclear. To further investigate our RNA-seq data, we utilized non-enzymatic glycation of collagen to generate 3D collagen matrices of varying stiffnesses. HUVECs cultured in stiffer collagen matrices displayed higher biglycan mRNA and protein expression relative to softer matrices. Mechanotransduction of substrate stiffness is known to be mediated by cellular contractility. Thus, to examine the role of contractility in biglycan expression, we treated cells seeded in stiffer collagen matrices with the ROCK inhibitor Y-27632 for 24 hours and observed decreased biglycan mRNA transcript levels and a similar trend in protein levels compared to untreated controls. To confirm the relevance of these findings in an *in vivo* setting, we utilized the MMTV-PyMT transgenic mouse model to obtain soft and stiff tumors by treating the mice with either **β-aminopropionitrile** or control, respectively. Immunohistochemistry revealed endothelial specific upregulation of biglycan in the stiffer tumors relative to the softer tumors. These results suggest that the rigidity of the extracellular matrix regulates the expression of biglycan in endothelial cells and this may be dependent upon cellular contractility.

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C. elegans Neuronal Gene Expression Map & Network

Seth R. Taylor, Rebecca McWhirter, Abby Poff, David M. Miller III

Differential gene expression defines the architecture of the nervous system by encoding the morphology, connectivity and function of individual neuron types. With the goal of delineating the genetic blueprint for an entire nervous system, we are using the model organism, *C. elegans*, to map gene expression at single neuron resolution. The CeNGEN (*C. elegans* Neuronal Gene Expression Map and Network) project is supported by NINDS and involves collaborating laboratories at Vanderbilt, Yale and Columbia. The adult *C. elegans* nervous system is comprised of 302 neurons, which have been categorized into 118 classes. We are using RNA sequencing to define the transcriptome of each of the 118 neuronal classes. For this purpose, we use Fluorescent Activated Cell Sorting (FACS) to isolate individual neuron classes labeled with unique combinations of fluorescent reporters. Our lab has previously used this approach to profile multiple neuron classes (AVK, NSM, PVD, RIS, VA, VD, DD) at various stages of development. We are currently optimizing our methodology for FACS isolation of neurons from adults. Bulk RNA sequencing will reveal differential expression of protein-coding genes, including alternative splicing, as well as expression of microRNAs across an entire nervous system. Single-cell sequencing may detect previously indistinguishable subclasses of neurons. We will first profile the transcriptomes of the motor neuron circuit to understand the genetic regulation of its development and connectivity. CeNGEN promises to complement the existing maps of *C. elegans* genome, cell lineage and connectivity with transcriptome profiles at single-cell resolution.

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Clathrin Independendent Endocytosis at plasma membrane is regulated by microtubules.

Ajit Tiwari, Nico Fricke and Anne Kenworthy

Endocytosis- a process by which micronutrient and other molecules are internalized within the cell, initiates at the plasma membrane. The endocytic process also is intricately involved with turnover of membrane components. Clathrin-mediated endocytosis (CME) mechanisms have been widely studied and very elaborately delineated. In addition to CME, clathrin independent endocytosis (CIE) that is not dependent on clathrin, has been proposed to be one of the major and important endocytic pathway. CIE has been shown to be indispensable for transport of variety of endogenous cargoes and is also hijacked by viruses and bacteria to gain entry into the host cell. Recent studies have shown that a variety of CIE cargoes including cholera toxin and Shiga toxin utilize Endophilin A2 (a N-bar domain protein) to gain entry into the cell by a mechanism described as Fast Endophilin Mediated Endocytosis (FEME). The FEME pathway has been proposed to occur specifically at the leading edge and regulates cell surface level of G-coupled receptors and receptor tyrosine kinases. Endophilin A2 has been shown to be recruited to tubular FEME carriers in response to bacterial toxin binding or activation receptor tyrosine kinases by growth factors. However, compared to CME, the mechanism and machinery driving CIE and FEME pathways is poorly understood. Our studies have shown that microtubules, dynein, and dynactin help bend the plasma membrane to form tubular CIE carriers. In ongoing current studies, we have investigated whether microtubules contribute to the biogenesis of endophilin positive structure that are ultimately required for the CIE cargoes to gain entry into the cells via the FEME pathway. We show that the recruitment of Endophilin A2 at the leading edge in response to various growth factor is dependent on intact microtubules. Our data suggest that microtubules play a role in the formation of FEME carriers at the leading edge. Our ongoing study is focused towards identification of protein complexes that enable microtubule plus end to contact the plasma membrane at the sites of FEME.

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Elucidating the Gene Signature Driving Multipotency in Mammary Epithelial Cells

Erica Tross, Armelle Le Guelte, Ph.D. Ian Macara, Ph.D.

The mammary ductal tree is believed to develop from multipotent stem cells which may play important roles in the elaboration of the tree at puberty and in alveolar formation during pregnancy. In search for the stem cell population that maintains the mammary gland a study identified a population of cells expressing a splice variant of the SH2 domain-containing 5'-inositol phosphatase (s-SHIP). Using transplantation assays, the study claimed that this cell population was multipotent giving rise to myoepithelial and luminal cells. Lineage tracing done by my lab showed that *in vivo* these cells were not multipotent, but instead are unipotent basal progenitors. When cultivated *in vitro*, s-SHIP+ cells converted into multipotent stem cells. We also have found that cultivating differentiated myoepithelial cells isolated from the mammary gland results in dedifferentiation and an onset of stem cell properties. My goal is to take advantage of the ability to control the cell state of s-SHIP-expressing cells and myoepithelial cells to study cell plasticity and self-renewal. My lab has shown that depletion of s-SHIP+ cells results in improper mammary gland development. Using High-Throughput Next Generation Sequencing, I have generated the gene expression profile for s-SHIP+ cells and have also identified the transcription factors most likely to regulate the genes that define the s-SHIP+ cells population using iRegulon.I will use this data to determine the factors that are important for s-SHIP+ cells to switch cell states from unipotent progenitors to multipotent stem cells, driving normal mammary gland development. Transplantation assays will be used to test the factors critical in regulating cell state.

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Hal5 kinase activity regulates Art1-dependent endocytosis in *Saccharomyces cerevisiae*

Tumolo JM, MacGurn JA

Regulation of endocytic trafficking is critical to human health and disease. Endocytic trafficking and signal transduction networks regulate each other in response to changing environmental conditions to achieve a variety of biological outcomes such as endocytic downregulation, plasma membrane remodeling, and protein degradation. The AMPK/Snf1-related family of kinases in yeast is known to regulate nutrient uptake and metabolism, but many of these kinases remain poorly characterized in their regulation of membrane trafficking. Hal4 and Hal5 are members of this kinase family thought to function redundantly in regulation nutrient transporter stability. The stage of membrane trafficking impacted by these kinases, their mechanism of action, and their individual contributions in regulation are unknown. My data suggests that Hal5 kinase activity regulates art1-dependent endocytosis of Mup1, and that Hal4 and Hal5 may have similar but different mechanisms of regulation in the cell.

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Rab6a activation by Ric1-Rgp1 GEF complex drives collagen secretion in skeletal tissues

Gokhan Unlu, Kinsey Qi, Amy R. Rushing, Eric Gamazon, David B. Melville, Fowzan S. Alkuraya, Nancy J. Cox, Ela W. Knapik

Fibrillar collagens are main components of extracellular matrix in animal tissues. Molecular machinery mediating efficient secretion of fibrillar procollagens during development and tissue homeostasis remain largely unknown. By phenotype-driven forward genetic screen and positional cloning of variants, we identified Ric1 as a key component of Rab6a activating GEF complex that is required for collagen secretion to ECM and basement membrane in zebrafish chondrocytes and notochord sheath cells. Genetic depletion of Ric1-Rgp1 GEF blocks secretion of procollagen-II, in a cargo-specific manner. Constitutive activation of Rab6a bypasses requirement for Ric1-Rgp1 complex, and is sufficient to restore collagen secretion in *ric1*$^{-/-}$ mutant zebrafish. Genetic mutations of *ric1* cause skeletal and cranio-dental malformations, chondrocyte dysfunction and shortened body length. Patients with pediatric cataract and microcephaly carry a R1265P variant in *RIC1* gene, which fails to rescue zebrafish *ric1* mutation and collagen secretion unlike human WT gene suggesting highly evolutionarily conserved biological function. Furthermore, transcriptome-wide association study (TWAS) by PrediXcan in the Vanderbilt University DNA biobank (BioVU) revealed highly significant associations with skeletal and dental defects in patients with predicted reduced expression of *RIC1*. We show, for the first time, requirement for Rab6a activation by Ric1-Rgp1 GEF complex in fibrillar procollagen secretion that is essential for development and homeostasis of multiple tissues.

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Sphingolipids modulate the coordination of growth and cell wall formation in yeast

Marcin P. Woś and Kathleen L. Gould

Lipids containing a sphingosine backbone take part in multiple intracellular and intercellular signaling pathways. Disruption of signaling pathways mediated by sphingolipids are the origin of very severe neurodegenerative and metabolic disorders in humans. Through our study of a cytokinesis mutant, we have found that in the yeast, *Schizosaccharomyces pombe,* defects in sphingolipid metabolism uncouple growth from cell wall synthesis and cell division. Coupling cell wall construction with cell growth is a universal challenge for all walled organisms such as yeast, plants, and bacteria.

Css1 is an integral plasma membrane protein with phospholipase C and neutral sphingomyelinase activity. It cleaves the polar head group of sphingolipids, leaving ceramide. In strains conditionally mutant for Css1, cells cease growth and division and accumulate massive amounts of cell wall material at previous sites of growth. This phenotype can be partially suppressed by mutations in any one of three enzymes that produce cell wall constituents ags1, bgs1, bgs4, indicating that sphingolipid metabolites do not affect cell wall synthesis per se.

The sphingolipid biosynthesis pathway in *S. pombe* is very poorly understood. However, drugs that inhibit conserved enzymes acting early in sphingolipid biosynthesis and mutation of other enzymes acting early in the pathway suppress the *css1-3* temperature-sensitive phenotype. These results are helping us to pinpoint the precise metabolite(s) whose increase uncouples growth from wall formation. We are constructing new gene mutations to help in this endeavor as well as employing mass spectrometry to identify metabolic intermediates.

Detailed investigation of sphingolipid synthesis will help us better understand this important pathway, signaling that lies behind it as well as its coupling with homeostatic cell growth.

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Identification of Crumbs family protein functions in MDCK cells using CRISPR gene editing

Chih-Chao Yang and Ian Macara

Apical-basal cell polarity is essential for normal epithelial cell function. Disruption of apical-basal polarity is associated with various diseases, including tumor formation. Crumbs (Crb) family proteins (Crb1, Crb2 and Crb3) are a group of apically localized proteins that play a pivotal role in apical membrane specification during epithelial cell polarity establishment. Crb family proteins also regulate tight junction formation and ciliogenesis. However, the mechanisms by which Crb family proteins contribute to cell junction formation and ciliogenesis in mammalian cells are poorly characterized. To understand their roles in epithelial cells, we knocked out Crbs in MDCK cells using CRISPR/Cas9 technology. As expected, a significant fraction of the Crb3 knockout cells displayed defects in tight junction formation and a multi-lumen phenotype in 3D cyst formation assay, suggesting the cell polarity is disrupted in Crb3 knockout cells. Surprisingly, however, ~30% of the knockout cells still have intact tight junctions. These data implied that although Crb3 is important, it is not the only factor governing tight junction formation. We also observed a substantial loss of primary cilia in Crb3 knockout cells, confirming that Crb3 is important for ciliogenesis. Overexpression of the ERLI but not the CLPI splicing variant of Crb3 can reverse the defects in tight junction, lumen formation and ciliogenesis in the Crb3 knockout cells. We suspected that the partial tight junction defect might be due to redundancy with other members of the family, Crb1 and Crb2. However, CRISPR-mediated knockout of Crb2 had no detectable phenotype. We also attempted to generate Crb1 knockout MDCK cells, but failed to recover any homozygous deletions. Therefore, we generated small hairpin RNAs to knockdown Crb1 and found that Crb1 depletion had no effect on tight junctions but instead suppressed cell growth. This effect is a consequence of increased cell-autonomous apoptosis, mediated by JNK activation. Ectopic expression of human Crb1 recued the cell viability in Crb1 knockdown MDCK cells. Together, our data suggest that Crb family proteins not only regulate the tight junction, cell polarity establishment and ciliogenesis, but also mediate cell homeostasis.

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Sin3A is required for postnatal pancreatic β-cell function and survival

Xiaodun Yang, Yanwen Xu, Ruiying Hu, Guoqiang Gu

Sin3A (SWI-independent-3A) is a transcriptional co-regulator that together with transcription factors (TFs) recruits chromatin-modifying enzymes to regulate target gene transcription. In pancreatic β cells, Sin3A co-purifies with MafA that is essential for β cell function. In neuronal cells, its close paralog Sin3B directly interacts with Myt factors (myelin transcription factors) that are required for β-cell maturation and survival. These premises led to our hypothesis that Sin3A is involved in β-cell production and functional maintenance. In wild type mice, Sin3A is expressed in all pancreatic cells at embryonic and early postnatal days. Its expression was then gradually enriched in endocrine pancreatic cells after 3 weeks of age. To examine the roles of Sin3A in β-cell differentiation, function, and survival, we used Cre/LoxP system to knock out *Sin3A* in pancreatic endocrine progenitors (*Sin3A F/-; Ngn3-Cre*) in mouse models. Loss of *Sin3A* resulted in elevated blood glucose levels in mice 2 weeks after birth, which reached overt diabetic levels 4 weeks after birth (492.78 ± 57.39 mg/dl, mean ± SD). Consistent with this *in vivo* phenotype, loss of *Sin3A* impaired glucose-stimulated insulin secretion and KCl-stimulated insulin secretion from P7 mutant islets. Loss of *Sin3A* induced Myt1 cytoplasmic translocation in P14 islet β cells, while the expression and nuclear localization of Pdx1 or Nkx6.1 was not affected. The expression of MafA, MafB, Urocotrin III (Ucn3), and Glut2 will be examined. Furthermore, loss of *Sin3A* had no effect on β-cell proliferation but resulted in β-cell death. Thus, *Sin3A* mutant mice had significantly reduced β-cell mass on P7 and P14 (P7: 45% decrease; P14: 69% decrease), although their β-cell cycling status remains unchanged over control β cells. RNA-seq and ChIP-seq will be performed to examine gene expression and Sin3A direct targets, respectively. Our data indicate that Sin3A is not required for islet-cell differentiation during embryogenesis but is required for β-cell function and survival after birth.

Transcriptional Profiling of the Ductus Arteriosus: Identification of Candidate Genes from Rodent Microarrays and Human RNA Sequencing

Michael T. Yarboro, Matthew D. Durbin, Jennifer L. Herington, Elaine L. Shelton, Stanley D. Poole, Naoko Brown, Jason Z. Stoller, Ronald I. Clyman, Jeff Reese

The ductus arteriosus (DA) is an essential vascular shunt which connects the pulmonary artery and the aorta, allowing oxygenated blood to bypass the developing lungs *in utero*. After birth, closure of the DA is required for transition to neonatal life. Often, this process is disrupted, leading to persistent patency of the DA (PDA). Normal postnatal closure is supported by alterations in signaling and structural composition of the DA which distinguish it from its surrounding vessels. These changes must have a transcriptional basis. Previous studies have examined the DA using microarrays in several animal models, but conflicting experimental design and criteria have led to ambiguity in the literature. Further, the human DA has yet to be explored using current transcriptomic techniques. We hypothesized that: 1) a discrete set of genes distinguish the DA from other vascular beds, 2) evolve with advancing gestation, and 3) overlap of key DA-specific regulatory genes exists between species. We identified nine microarray studies of the DA in three species (mouse, rat, sheep). Data from four rodent studies met pre-specified criteria (data quality, gestational stage, vessel type) for further analysis. Meta-analysis of raw data from term gestation rodent microarrays revealed 276 differentially expressed genes which were consistently increased (n=87) or decreased (n=189) in the DA compared to aorta, in at least 3 of the 4 studies. RNAseq of preterm human tissue revealed differentially expressed genes which consistently distinguished the DA from the ascending aorta. As expected, overlap between rodent microarrays and human RNAseq was limited, but successfully identified previously known or suspected regulators of DA function, including *ABCC9*, *PDE1C*, *PTGER4*, and *TFAP2b*. Comparing functional annotation of gene sets from rodent microarray and human RNAseq identified key pathways likely involved in preparing the DA for muscular constriction and subsequent closure. Of the top 30 UniProt Keyword terms from rodent microarray and human RNAseq, 16 were found in common, including 'Calcium', 'Cell adhesion', 'collagen', 'extracellular matrix', and 'metalloprotease' genes, which align with pathways known to be important for DA constriction and remodeling. A better understanding of the DA transcriptome is essential to guiding future research, both to provide targeted treatments in the clinic and new information on the basic mechanisms of DA function.

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Programmed Necrosis, a key role of Development for Myelodysplastic Syndromes

Jing Zou, Qiong Shi, Heidi Chen, Ridas Juskevicius, and Sandra S. Zinkel

Background**:** Myelodysplastic syndrome (MDS) is a bone marrow failure disorder characterized by persistent cytopenias and evidence of morphologic dysplasia in the bone marrow. Lower-grade MDS (bone marrow blasts <5%) is particularly challenging to diagnose, as the morphologic dysplasia may be subtle and many patients lack characteristic cytogenetic abnormalities, thus biomarkers of early disease are needed. Excessive hematopoietic cell death has been demonstrated in MDS patients' bone marrow and is thought to play an important role in the pathogenesis of the disease. Besides apoptosis, necroptosis has been identified as a new pathway of programmed cell death, which is driven by Rip kinases and is characterized by premature rupture of the plasma membrane, resulting in an immune response. Necroptosis has been shown to play a role in inflammatory diseases as well as host response to infection, and tumors, and necroptotic cell death has important implications for the bone marrow microenvironment. However the role of necrosis in human MDS has not been extensively investigated. In this study, we evaluate the cell death status both apoptosis and necrosis in MDS.

Methods: To assess cell death status in normal bone marrow as compared to MDS and de novo AML, we evaluated a total of 28 de novo MDS cases, 23 normal control cases and 5 AML patients. Formalin fixed paraffin embedded bone marrow core biopsies were immunostained with the apoptosis marker (cleaved caspase 3) and necroptosis markers (Rip1, pMLKL) and visualized by immunofluorescence. Whole slide images were obtained using Aperio Versa 200 (Leica Microsystems). A pipeline for statistical analysis developed to evaluate the immunofluorescence of stained and scanned images was performance on CellProfiler image analysis software. For each slide over 10,000 cells were analyzed.

Results: We demonstrated RIPK1 and pMLKL but not Caspase 3 showed a similar increasing expression pattern in MDS relative to normal controls or de novo AML. Furthermore RIP1 expression increased in low-grade morphology and low IPSS-R score cases. RIP1 positive cells were highly correlated with CD71 but not CD34 staining, showing that erythroid progenitor cells are the predominant cell that displays elevated RipK1 expression in MDS.

Conclusions: In summary, our findings reveal that the necroptosis cell death pathway is upregulated in early MDS relative to normal controls and warrants further study to define its role in the pathogenesis of MDS and as a potential biomarker for the diagnosis of low-grade MDS.

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Investigating the role of the GTP hydrolysis rate in regulation of microtubule stability

Veronica Farmer, Göker Arpag, Anika Rahman, and Marija Zanic

Dynamic instability of microtubules, the switching between phases of microtubule growth and shrinkage, enables the microtubule network to remodel throughout the cell cycle. A growing microtubule end incorporates GTP-bound beta-tubulin subunits, which are subsequently hydrolyzed, resulting in a microtubule lattice consisting of GDP-bound beta-tubulin. The growing end maintains a cap of GTP-tubulin, thought to protect the microtubule against transitions from growth to shrinkage (known as 'microtubule catastrophe'). Recent *in vitro* studies found that microtubule tip-tracking protein EB1 is sensitive to the nucleotide state of tubulin in the microtubule lattice, and can therefore be used as a marker for the stabilizing nucleotide cap ('GTP cap'). Fluorescent EB1 reveals that EB1 localization resembles a 'comet' shape, decorating the end of a growing microtubule, consistent with the expected shape of the GTP cap. The size of the GTP cap is defined by microtubule growth and GTP hydrolysis rates, and thus modulation of either of these parameters has the potential of producing a more or less stable microtubule. Indeed, increasing microtubule growth rate, as achieved by increasing tubulin concentration *in vitro,* results in a linear increase of EB1 comet lengths. At the same time, increase in tubulin concentration leads to a moderate suppression of microtubule catastrophe. Here, we investigate the GTP hydrolysis rate over a range of microtubule growth rates to determine if the hydrolysis rate is impacted by the growth rate. We aim to perturb the rate of GTP hydrolysis, using microtubule associated proteins and a number of agents that have been implicated in modulating the GTP hydrolysis rate of tubulin, including glycerol, magnesium, and phosphate analogues. We use an *in vitro* biochemical reconstitution assay with total internal reflection fluorescence (TIRF) microscopy to investigate the effect of these perturbations on both EB1 comet size, as well as the corresponding rates of microtubule catastrophe. Our goal is to elucidate how the properties of the GTP cap influence microtubule stability.

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Establishing a model of BM damage and analyzing its repair

Angela Howard, Gautam Bhave, and Andrea Page-McCaw

The basement membrane is a sheet-like extracellular matrix that wraps around muscle fibers and underlies epithelia. Although the basement membrane is often considered to be static, there are indications that the BM is a dynamic structure in vivo, as it can grow, shrink, and repair. We have developed a system to analyze basement membrane repair in adult animals, using an adult gut injury model in Drosophila. The gut has a well-defined architecture of epithelial cells (enterocytes) residing on top of a basement membrane sheet, and the gut tube is wrapped in visceral muscles also surrounded by basement membrane.

To injure the basement membrane of the gut, flies are fed Dextran Sodium Sulfate (DSS); DSS administration has been previously used as a model for ulcerative colitis in mice. In Drosophila, DSS induces morphological changes consistent with basement membrane damage. Using fluorescently tagged DSS, we determined that DSS becomes lodged in the gut BM. Both electron and structured-illumination microscopy indicate that the BM thickens after DSS feeding. The stiffness of the BM is decreased upon DSS damage, as assessed by a stress/strain analysis. Moreover, there are clear morphological changes to the muscles that indicate the weakening of the basement membrane. Importantly, the basement membrane is repaired within 48 hours after removal of the DSS irritant. Interestingly, inhibiting or knocking down a collagen-IV crosslinking enzyme, peroxidasin, mimics the tissue changes seen in response to DSS. In addition, peroxidasin and laminin are required for the repair of basement membrane upon damage with DSS. Peroxidasin transcription levels are increased as a result of damage. We are investigating whether the requirement for peroxidasin indicates a structural change in BM during repair.

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The Integrated Biological Systems Training in Oncology (IBSTO) program prepares pre-doctoral students and postdoctoral fellows for careers in cancer research with comprehensive training in basic and translational research. The IBSTO program takes place in an active Medical Center environment with state-of-the-art facilities, experienced preceptors in an interactive education and research environment with extensive resources and institutional commitment. The IBSTO program provides our productive trainees unique mentoring, useful academic skills, interactions with faculty and other trainees, exposure to current cancer research discoveries and exposure to clinical cancer treatment and translational research.

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The Vanderbilt University Program in Developmental Biology offers a stimulating and supportive environment for researchers to study pattern formation, cellular differentiation and morphogenesis during the developmental process.

Membership in the Developmental Biology Program is currently over 200 researchers, postdoctoral fellows, graduate students, technicians and support personal across the School of Medicine and Arts & Sciences campuses. Program members utilize many different approaches in their attempt to answer developmental questions based on their particular field of study. This mixing pot of scientific disciplines has given our members the freedom to adopt an interspecies approach to their research.

As our members work to answer challenging developmental questions they are encouraged to participate in weekly activities that are sponsored by the Program.

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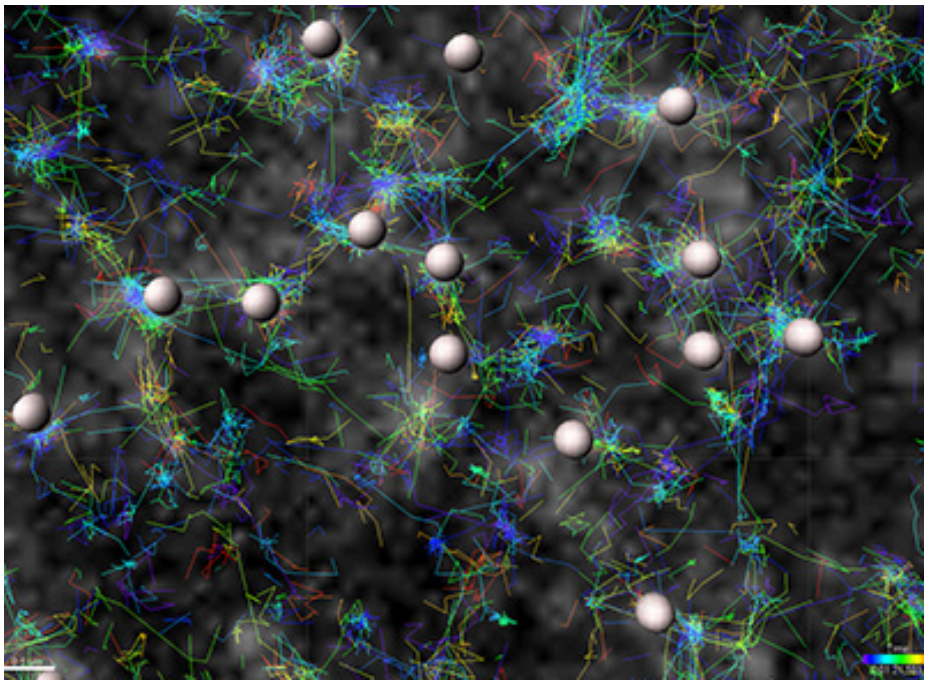
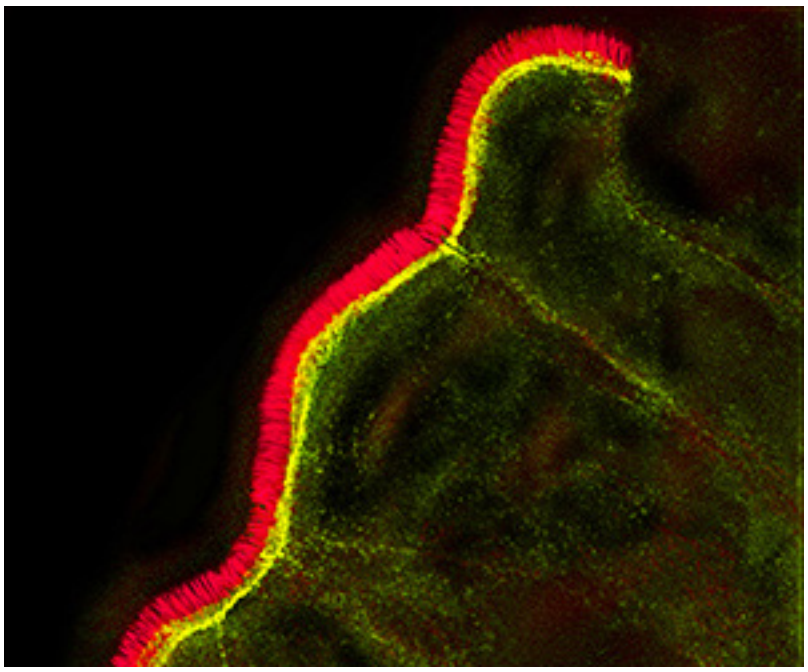
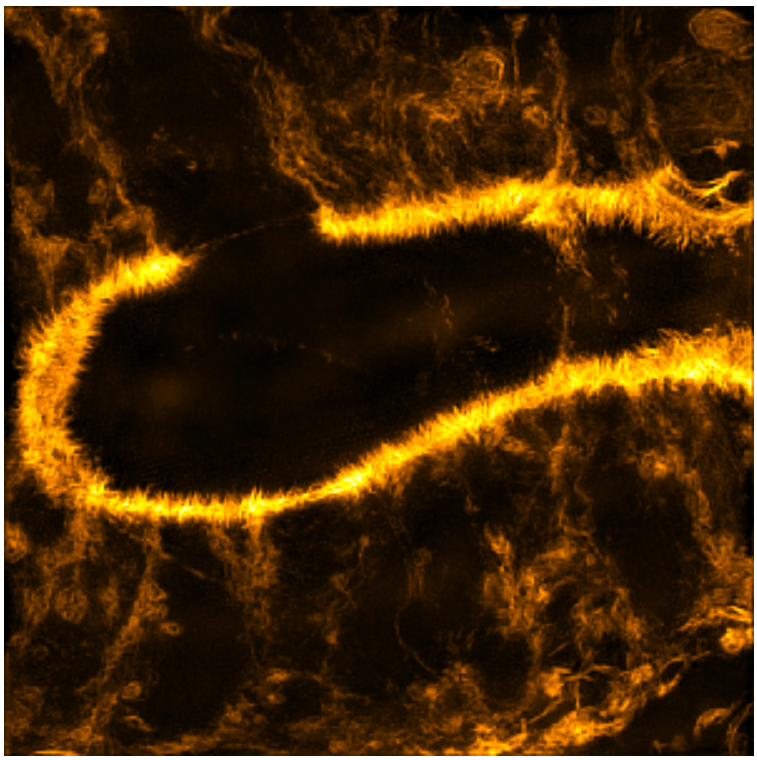
Additional Images



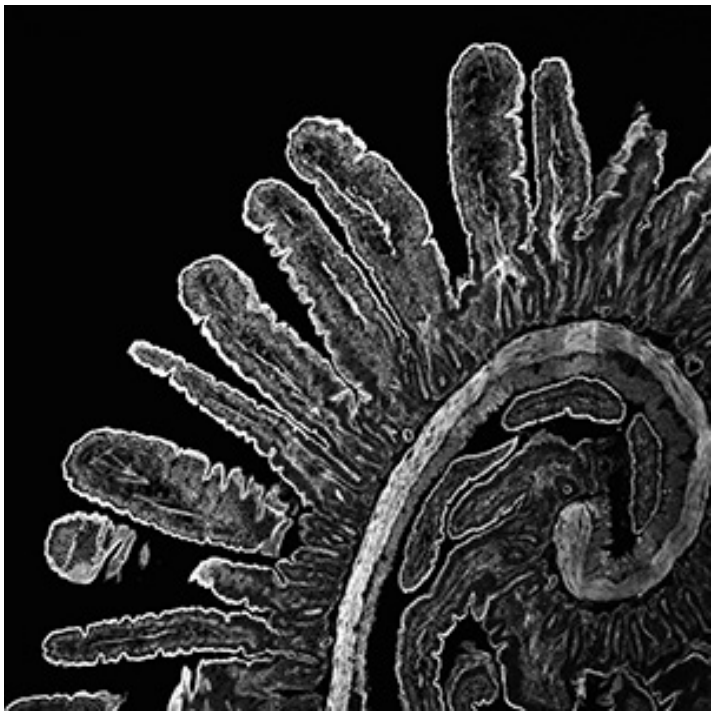
Mukhtar Ahmed. 2D particle tracking of endogenous exocyst subunit molecules imaged using high-speed TIRF microscopy. Colored lines indicate particle trajectory over a period of 1 min 21 sec, blue being at the beginning of the movie and red towards the end.



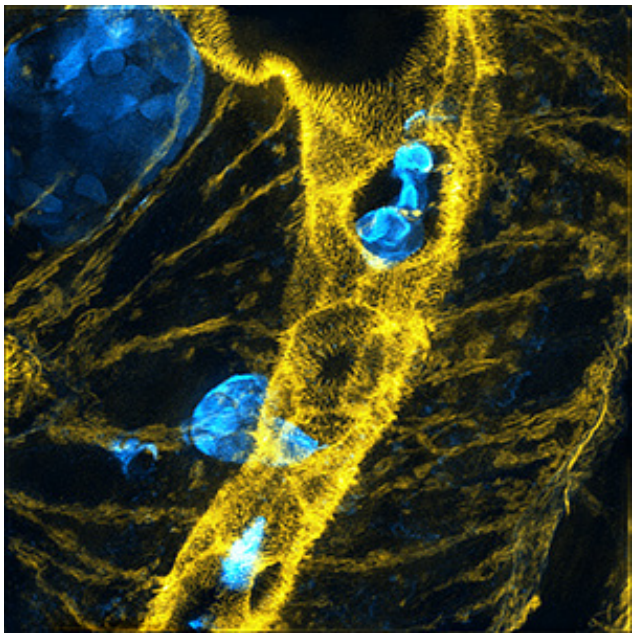
Colbie Chinowsky. Actin in red, non-muscle myosin IIC (NMII-C) in green. Image of murine jejunum from an animal expressing endogenous GFP-NMII-C and stained with Phalloidin 568 to visual microvilli at the apical cell surface, taken using structured illumination microscopy.



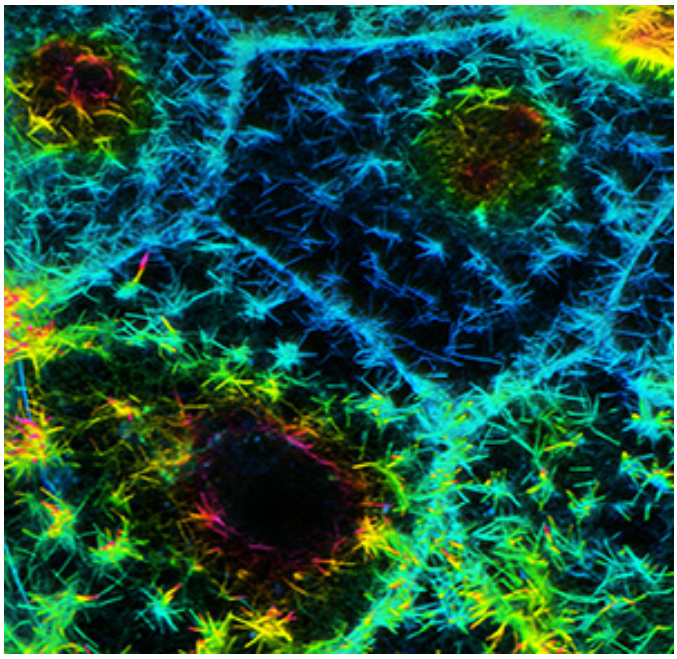
Bo Faust. The devil is in the details: Structured illumination micrograph of a CK666-treated tissue section. Shown in orange is the actin-cytoskeleton. Note the abundance of microvilli at the apex of these cells in the crypt.



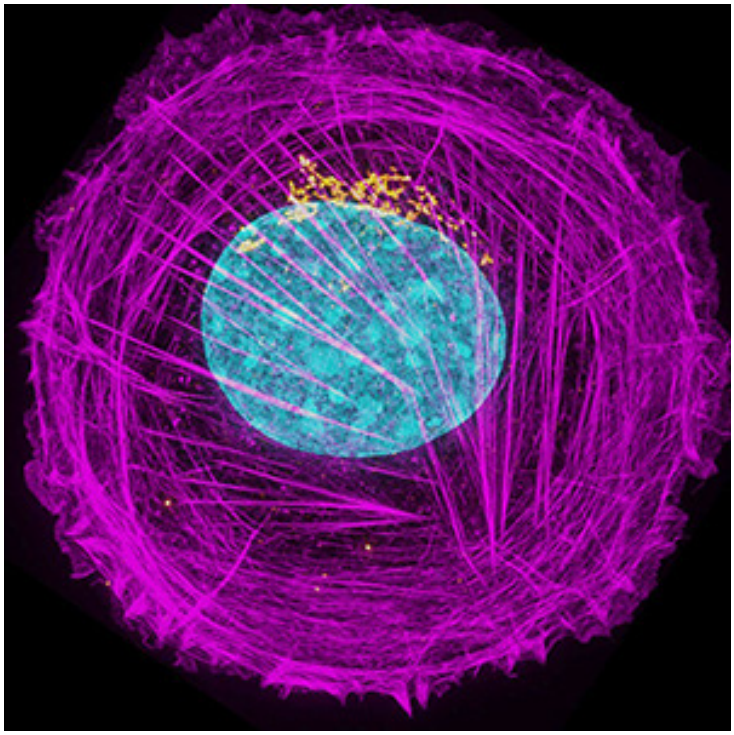
Bo Faust. Thrown into folds: Shown is a laser scanning confocal micrograph of a section from the jejunum. Villi, which appear in the image as finger-like projections, are an anatomic feature of the intestine that increases the surface area.



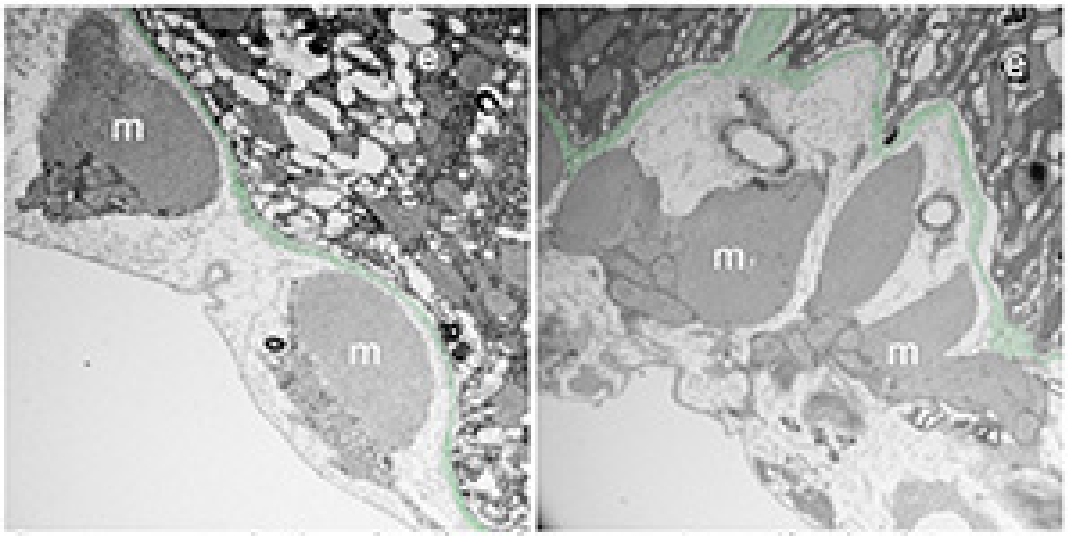
Bo Faust. Goblets at the gate: Structured illumination micrograph of a tissue section that shows the boundary between the crypt and villus domains. Goblet cells are identified by mucus (blue) surrounded by actin (yellow).



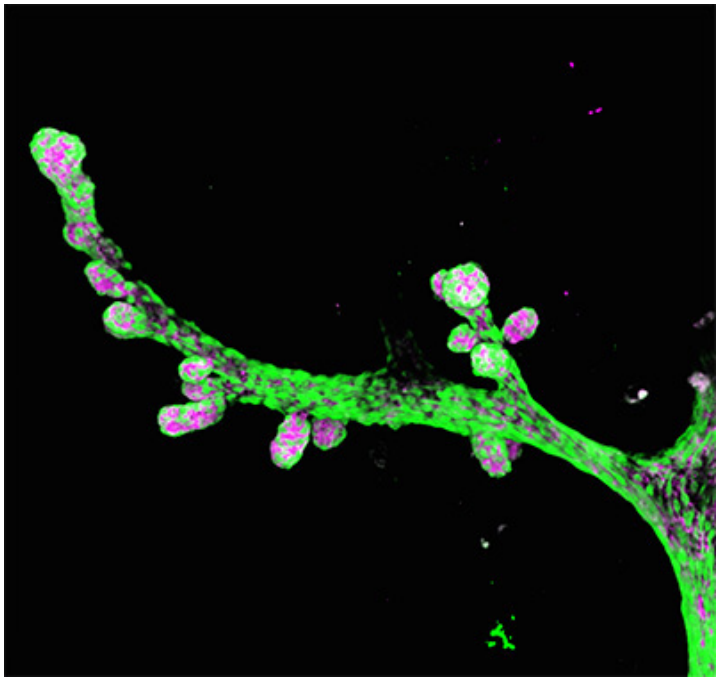
Bo Faust. Brush border rosettes: Laser scanning confocal micrograph of a cultured epithelium. The image shows a maximum intensity projection of F-actin where specimen depth is color-coded.



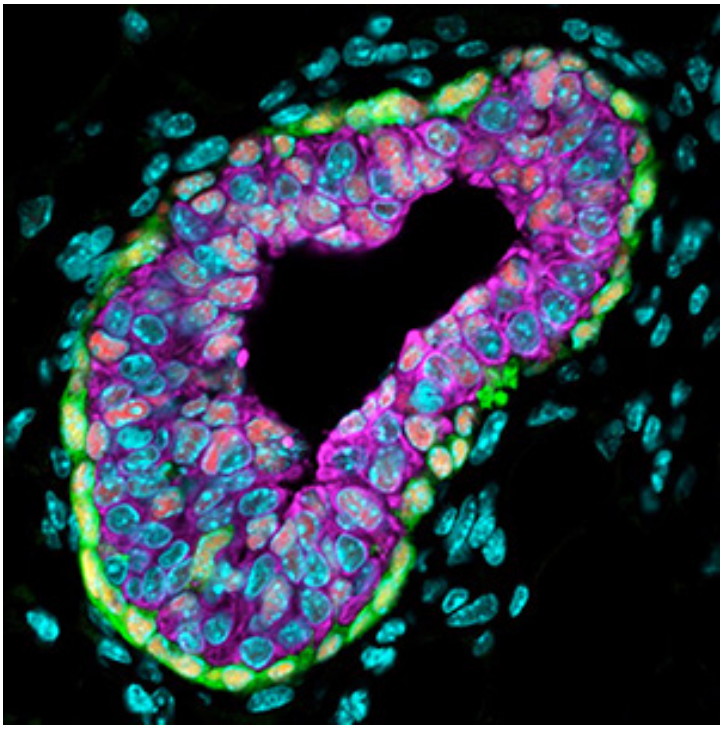
Aidan Fenix. Put a Golgi Cap on It: Cell polarization is crucial for homeostasis and processes such as cell migration and vesicle transport. This mouse embryonic fibroblast (MEF) displays a highly polarized golgi (yellow) adjacent to a nucleus (blue) with tightly packed chromatin. These structures are maintained in the cytoplasm of the cell whose structure is maintained by the actin cytoskeleton (magenta).



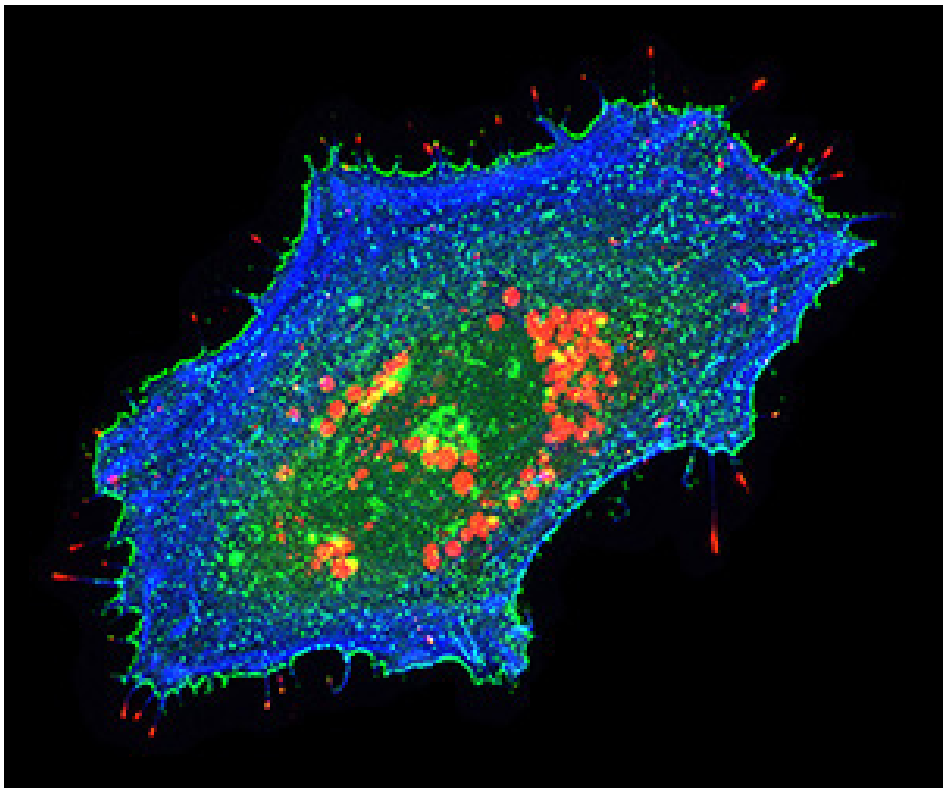
Angela Howard. Electron micrographs show the effect of dextran-sodium-sulfate (DSS) damage on the Drosophila gut tube (e, enterocytes) and adjacent tissue architecture (basement membrane (BM) is green pseudo-colored; m, muscle cells). Compared to the control (left panel), DSS treatment greatly disrupts the BM, causing swelling and massive distortion along the gut tube. Because BM is a foundation for the arrangement of underlying muscle cells, they also become mispositioned and malfunctional. Each panel is ~ 6 microns square.



Armelle Leguelte. 3D epithelial structure of mouse mammary ductal tree during morphogenesis. The tissue was cleared using SeeDB protocol and stained for Keratin 8 (luminal cells in purple) and Keratin 14 (Myoepithelial cells in green.

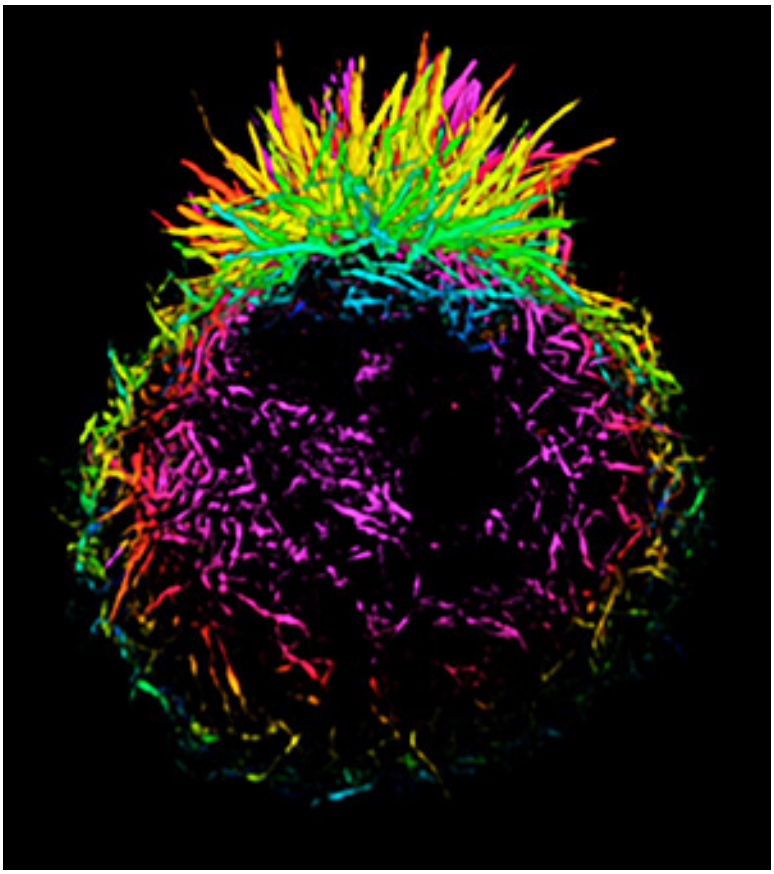


Armelle Leguelte. Section of a mouse epithelial Terminal End Bud (TEB) from a s-Ship-GFP transgenic mouse during puberty . GFP+ cells are represented in green, luminal cells in purple and Dapi in cyan. Sox9 (red) expression is uniform in GFP+ cell population but is heterogenous in luminal cells.

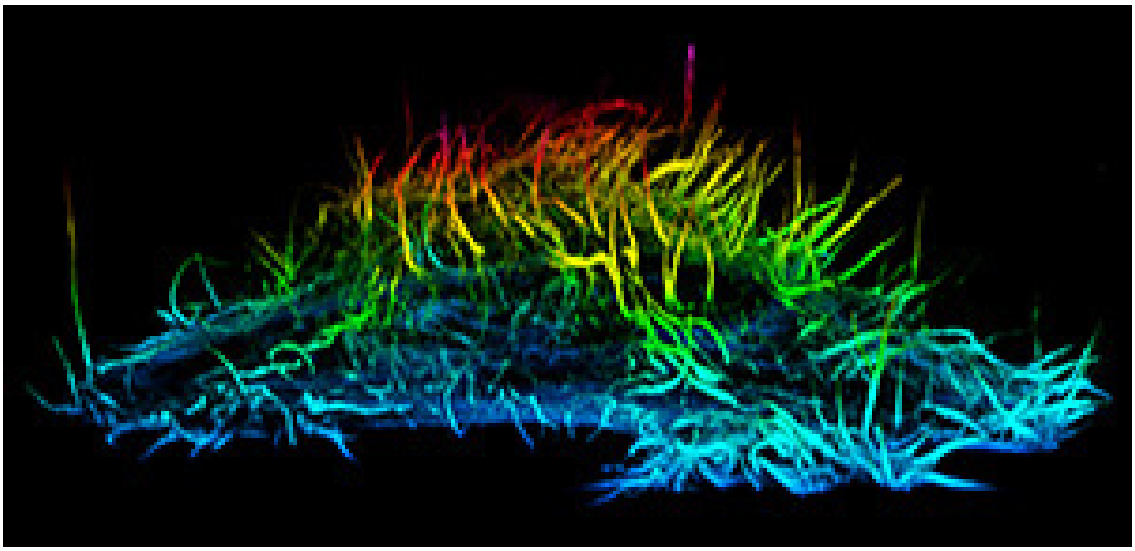


Angelo Morales. LSCM deconvolved image of a HeLa cell stained for F-actin (blue) that is overexpressing myosin-10/7b chimeric motor proteins (red) and Eps8L3 (green), a protein of unknown function that is positioned at the cell edge and filopodial tips.

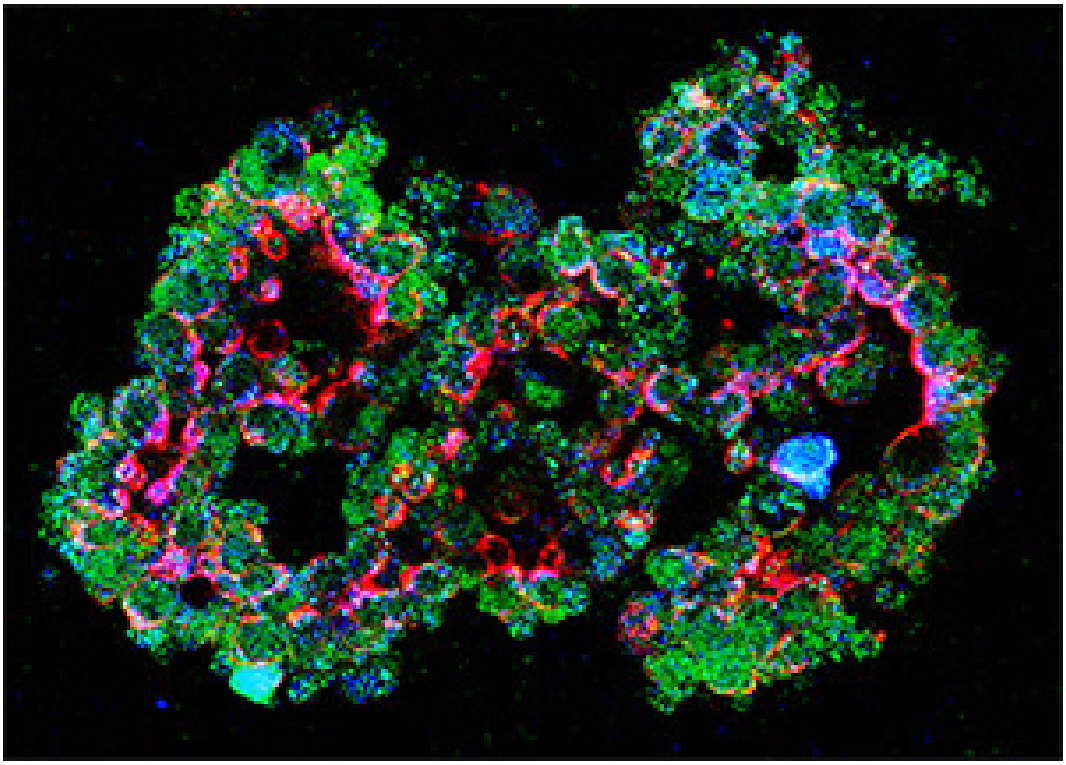
Meagan Postema. Scanning electron micrograph of an Ls174T-W4 cultured cell showing microvillar protrusions.



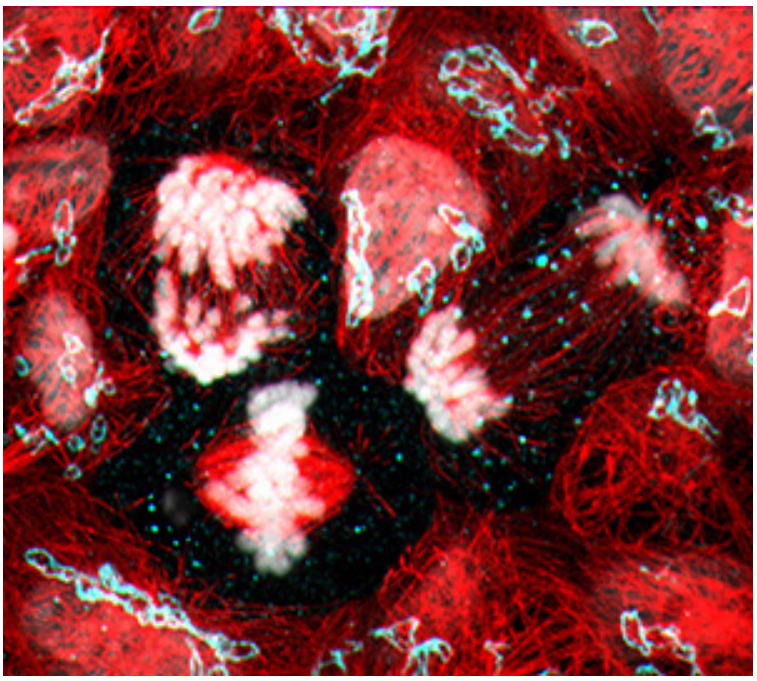
Meagan Postema. A structured illumination microscopy (SIM) image of microvilli protruding from am Ls174T-W4 epithelial cell. The cell is color coded for Z-depth (Magenta=low, Blue=high).



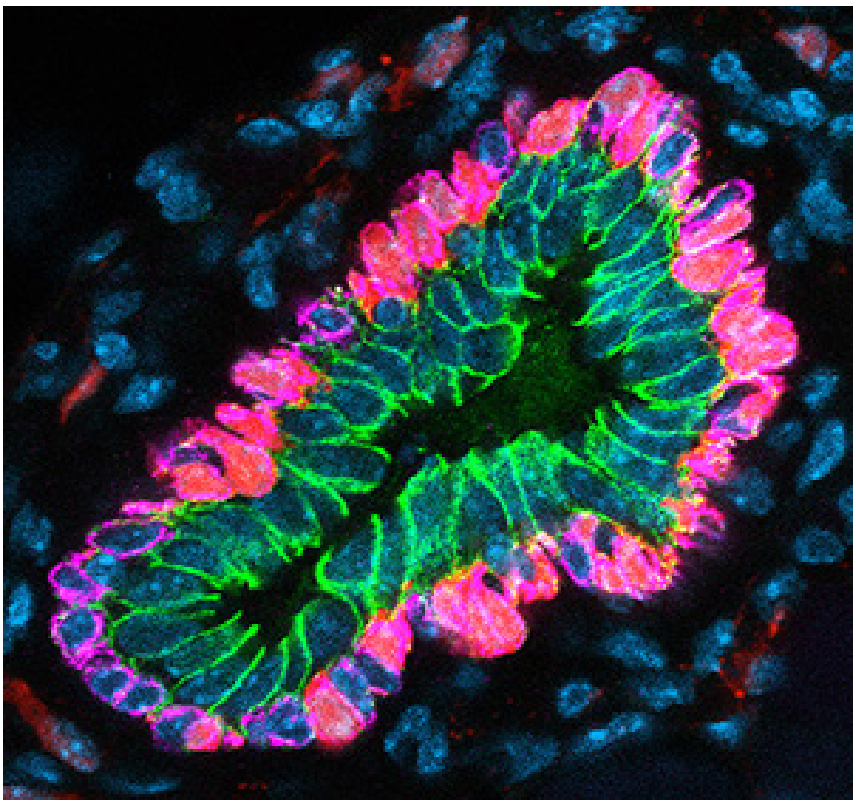
Meagan Postema. Filopodial protrusions formed from dual overexpression of the I-BAR domain containing protein IRTKS and the actin binding protein EPS8 in a B16F1 cultured cell. Image is color coded for Z depth (Blue=low, Magenta=high).



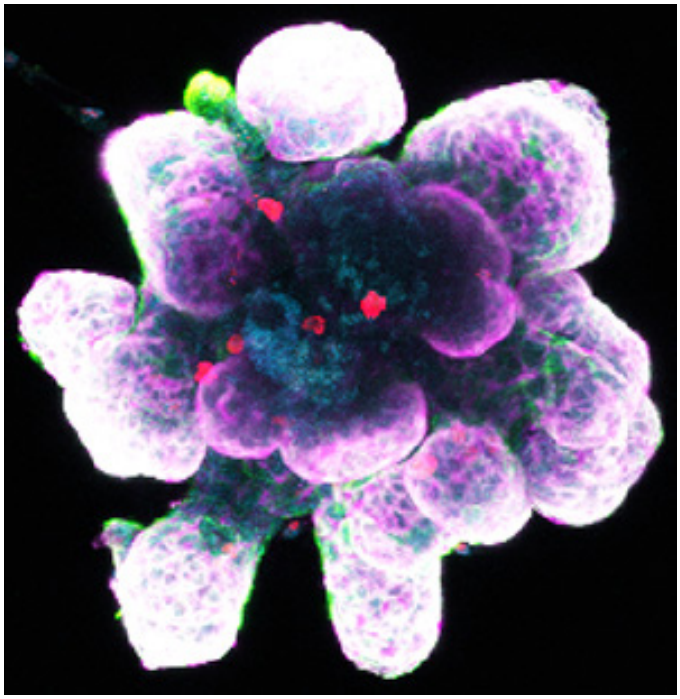
Kenyi Saito-Diaz. Mouse-derived enteroid fixed and stained for actin (red), b-catenin (blue), and USP47 (green).



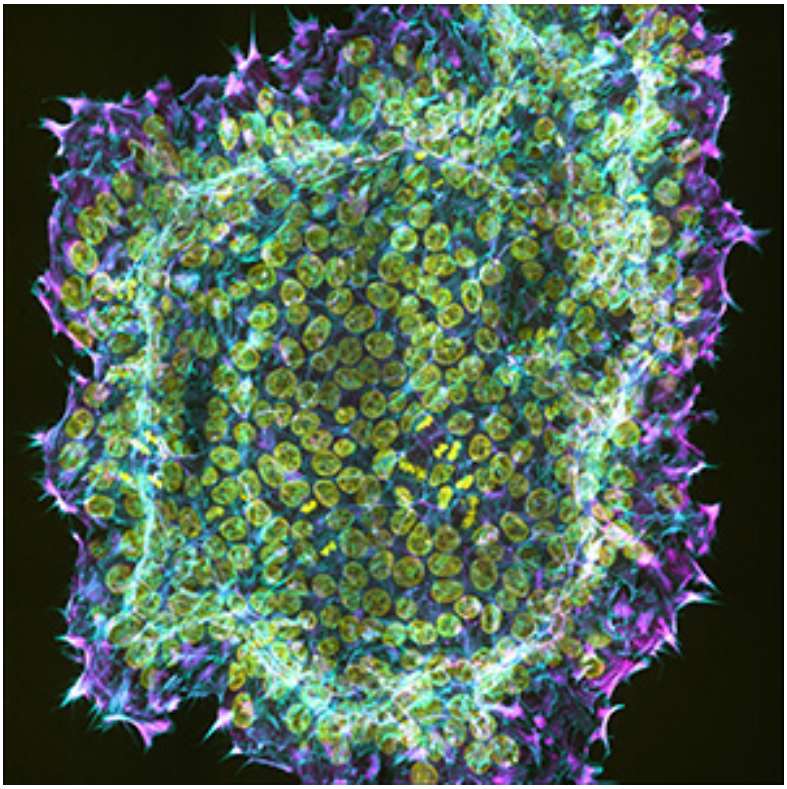
Anneke Sanders. Human iPSCs at three different stages of mitosis: early and late anaphase (top left and right cells) and metaphase (bottom cell). Red; microtubules, white; DNA, cyan, Golgi, acquired on laser scanning confocal microscope.



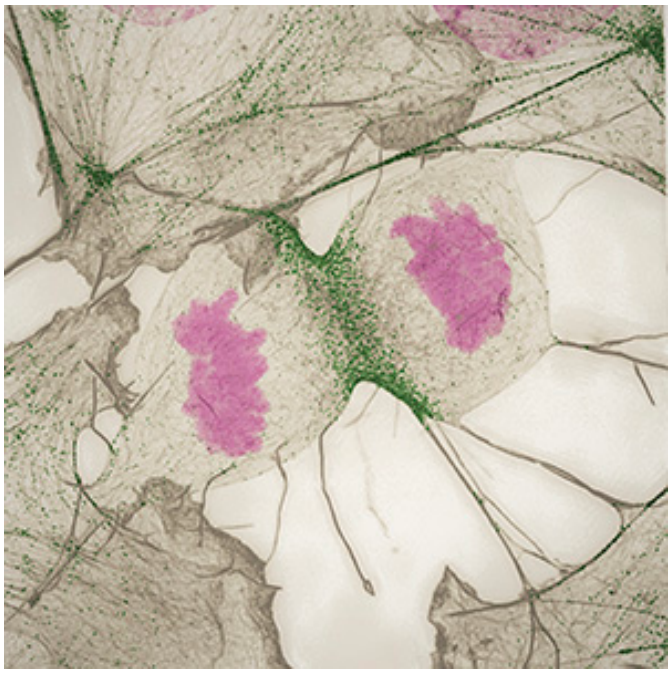
Lindsey Seldin. Cryosection of mouse mammary gland immunostained for myoepithelial cells (purple), luminal cells (green) and nuclei (blue). Lineage-tracing of myoepithelial cells throughout mammary morphogenesis reveals their lineage-restricted unipotency (red).



Lindsey Seldin. Mouse mammary gland organoid cultured in Matrigel and immunostained for myoepithelial cells (green), luminal epithelial cells (purple) and a proliferation marker (red). Maximum intensity projection of a 3D confocal image stack.



Nilay Taneja. A colony of human embryonic stem cells "fenced" by actin (cyan) and myosin II (magenta). Nuclei are shown in yellow. This "fence" is required for stem cells in the center of the colony to maintain their identity. Method of acquisition- Large image stitching, confocal microscopy.



Nilay Taneja. Super resolution image of the actin cytoskeleton of a dividing fibroblast. This intricate network of filaments allows a cell to change shape, allowing it to divide or move around. DNA is visualized in magenta and the contractile ring is in green.