

A fluorescence microscopy image showing a dense population of cells. The nuclei are stained blue, the cytoplasm and some organelles are green, and there are prominent red structures, possibly representing specific proteins or organelles. The cells are interconnected, forming a complex network.

Department of Cell & Developmental Biology  
Fifteenth Annual Retreat  
May 19, 2017

Nelson Andrews Leadership Lodge

CO-SPONSORS

INTEGRATED BIOLOGICAL SYSTEMS TRAINING IN ONCOLOGY & THE PROGRAM IN DEVELOPMENTAL BIOLOGY

**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 Matt Tyska**  
Alex Andrews (Macara), Andrea Cuentas-Condori (Miller), Zac Elmore (Gould)

**9:40 - 10:55 am Poster Session I (ODD NUMBERS)**  
**Breakout Session I (Students/Post Docs Only) Moderated by Karrie Dudek**  
Christine Jones (Gould), Elizabeth Lawrence (Zanic), Megan Merolla (Gama),  
Meagan Postema (Tyska), Lindsey Seldin (Macara)

**11:00 - 11:45 am Second Session Talks - Moderated by Rebecca Ihrle**  
Kung-Hsien Ho (Gu), Amy Engevik (Goldenring), Meredith Weck (Tyska)

**11:45 am-12:30 pm CATERED HOT LUNCH AND POSTER SESSION II SETUP**

**12:30 - 2:30 pm ACTIVITIES AND FREE TIME**

**2:30 - 3:15 pm Third Session Talks - Moderated by Dylan Burnette**  
Chuck Herring (Lau), Amrita Pathak (Fuhrmann), Erica Shannon (Page-McCaw)

**3:20 - 4:35 pm Poster Session II (EVEN NUMBERS)**  
**Breakout Session II (Students/Post Docs Only) Moderated by Angela Howard**  
Goker Arpag (Zanic), Amrita Banerjee (Lau), Keyada Frye (Kaverina),  
Alissa Guarnaccia (Tansey), Nathaniel Hepowit (MacGurn)

**4:40 - 5:25 pm Fourth Session Talks - Moderated by David Miller**  
Bhuminder Singh (Coffey), Nilay Taneja (Burnette), Stephen Norris (R. Ohi)

**5:25 - 5:30 pm Award Presentations by Andrea Page-McCaw**

**5:30 - 8:30 RECEPTION**



**Erica Tross**

**2017 Retreat Cover Art Winner**

## Erica's Research Project Description

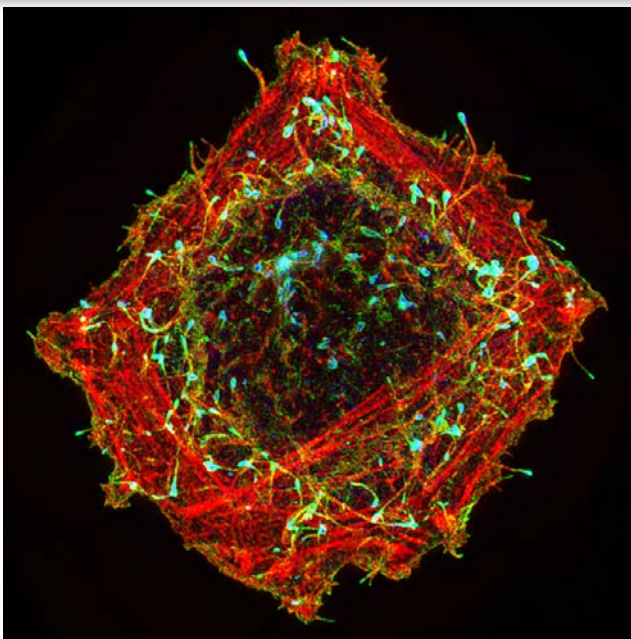
Erica is working on the molecular mechanisms that control stem cell behavior in mammary glands. She is using RNAseq and other techniques to identify the gene regulatory network that determine whether progenitor cells remain unipotent or become multipotent. This network is likely important in the development of breast cancer. The image she submitted shows the remarkable phenotypic heterogeneity of a triple negative breast cancer cell line.

## Front Cover Art Caption

Triple Negative Breast Cancer Cells Blue: Nucleus Green: Cytokeratin 5 Red: Alpha Smooth Muscle Actin Magenta: Cytokeratin 8 Photo Credit: Erica M. Tross

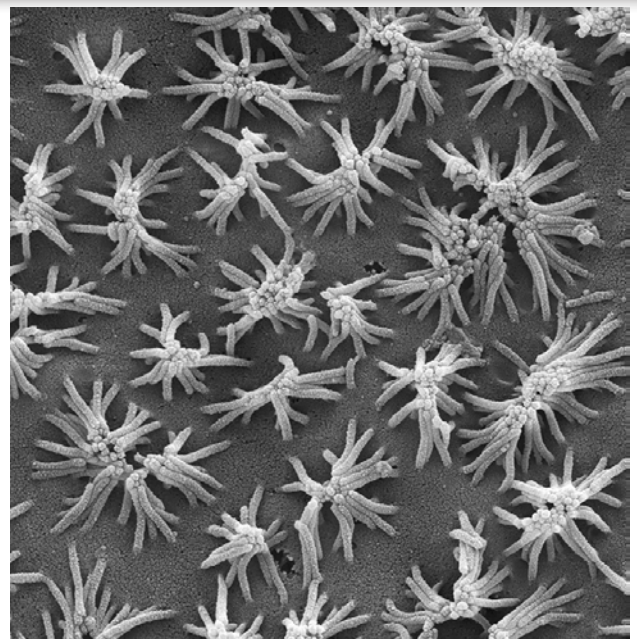
## 2nd Place - Meredith Weck

SIM image of HeLa cells overexpressing CDHR2 (green), an adhesion protein within the intermicrovillar adhesion complex, and a chimeric motor protein consisting of the motor domain of myosin-10 and the cargo-binding tail domain of myosin-7b (blue), stained for F-actin (red).



## 3rd Place - Meredith Weck

Scanning electron micrograph of clustering microvilli on the surface of differentiating CACO-2BBE intestinal epithelial cells.



\* Other image submissions may be found at the end of this book.



**First Place Movie Winner  
Meagan Postema**

## **First Place Movie Description**

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. EGFP-EPS8 (green) and mCherry-IRTKS (magenta).

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



[Click Here To View Films](#)

\* Click on the film roll to view all movie submissions.

# Oral Presentations - Nelson Andrews Leadership Lodge

## First Session – 8:50 – 9: 35 Moderated by Matt Tyska

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

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

### **Branched actin promotes synapse disassembly in remodeling GABAergic neurons**

*Andrea Cuentas-Condori, Tyne Miller-Fleming and David M Miller*

### **Spindle pole localization of CK1 is essential for its role in mitotic checkpoint signaling**

*Zachary C. Elmore, Rodrigo X. Guillen, and Kathleen L. Gould*

## Second Session – 11:00 – 11:45 Moderated by Rebecca Ihrle

### **Tau is a key regulator of glucose-dependent MT destabilization in pancreatic beta cells**

*Kung-Hsien Ho, Guoqiang Gu, and Irina Kaverina*

### **Deficits in enterocyte apical transporters associated with loss of myosin Vb**

*Amy C. Engevik, Victoria G. Weis, Byron C. Knowles, Cameron Schlegel, Nadia Ameen, Hermann Koepsell, Nicholas C. Zachos, Mark Donowitz, and James R. Goldenring*

### **In-cell reconstitution of intermicrovillar adhesion complex transport**

*Meredith L. Weck, Colbie R. Chinowsky, Scott W. Crawley, and Matthew J. Tyska*

## Third Session – 2:30- 3:15 Moderated by Dylan Burnette

### **Computational mapping of single-cell data reveals alternative tuft cell origin in the gut**

*Charles A. Herring, Amrita Banerjee, Eliot T. McKinley, Alan J. Simmons, Joseph T. Roland, Jeffrey L. Franklin, Michael J. Gerdes, Robert J. Coffey, Ken S. Lau*

### **Analysis of cell-cell interaction during optic fissure closure in the developing mouse eye**

*Amrita Pathak, Katrina S. Hofstetter, Jonathon Kuntz, Dylan T. Burnette, Sabine Fuhrmann*

### **Multiple mechanisms drive calcium dynamics around laser-induced epithelial wounds**

*Erica Shannon, Aaron Stevens, Wes Edrington, Yunhua Zhao, M. Shane Hutson, Andrea Page-McCaw*

## Fourth Session – 4:40 – 5:25 Moderated by David Miller

### **A 3D culture system identifies a new mode of cetuximab resistance**

*Bhuminder Singh, Cunxi Lia, Ramona Graves-Deal, Gregory Daniel Ayers, Mary Kay Washington, Timothy J. Yeatman, Oliver G. McDonald, Qi Liu, and Robert J. Coffey*

### **Precise control of cortical contractility maintains mechanical equilibrium during cell division**

*Nilay Taneja, Aidan M. Fenix, Matthew Bersi, Caleb Snider, James Cooper, Vivian Gama, David Merryman and Dylan T. Burnette*

### **Microtubule minus-end aster organization is driven by processive HSET-tubulin clusters**

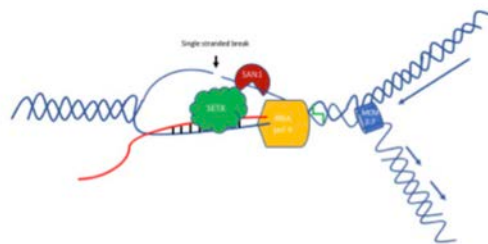
*Stephen R. Norris, Claire E. Strothman, Marija Zanic and Ryoma Ohi*

# 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<sup>-/-</sup> 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<sup>-/-</sup> 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. Future work is aimed at determining the specific step of ICL repair that SAN1 participates in, how and when during repair SAN1 is recruited to ICL sites, and which other DNA repair proteins SAN1 functions with.

SAN1 participates in interstrand cross-link repair by resecting single stranded DNA formed at R loops, at sites where transcriptional complexes have stalled from encountering an ICL.



# **Branched actin promotes synapse disassembly in remodeling GABAergic neurons**

Andrea Cuentas-Condori, Tyne Miller-Fleming and David M Miller

Actin is dynamically reorganized to sculpt postsynaptic structures during circuit refinement in the developing brain. Less is understood, however, about the role of actin in presynaptic remodeling. Here we report that branched actin promotes the disassembly of the presynaptic apparatus.

We use a developmentally regulated example of circuit remodeling in *Caenorhabditis elegans*. Dorsal D (DD) GABAergic motor neurons relocate their presynaptic structures from ventral to dorsal sites during early larval development.

We have previously proposed that the DEG/ENaC cation channel, UNC-8, drives disassembly of ventral GABAergic synapses by elevating intracellular calcium and neuronal activity. We have now shown that the Arp2/3 complex, the branched actin nucleator, is also required for synapse elimination in this circuit. This idea is consistent with our finding that two known activators of the Arp2/3 complex, the Wave Regulatory Complex and the F-BAR protein TOCA-1 are also necessary and function in a common pathway. A proximal role in disassembly is suggested by the co-localization of both Arp2/3 and TOCA-1 with the presynaptic terminals of remodeling DD neurons. We have established that TOCA-1 functions upstream of UNC-8 and in a common pathway with the calcium-activated phosphatase, TAX-6/Calcineurin. Calcineurin functions as a key regulator of bulk endocytosis, which is the dominant endocytic mechanism for recycling synaptic vesicle components in highly active neurons. Thus, we propose that a similar mechanism involving TOCA-1/Arp2/3-dependent actin branching triggers removal of the presynaptic apparatus in remodeling GABAergic neurons.

# Spindle pole localization of CK1 is essential for its role in mitotic checkpoint signaling

Zachary C. Elmore, Rodrigo X. Guillen, and Kathleen L. Gould

Members of the casein kinase 1 (CK1) family are widely distributed in the cell and function in multiple processes. In the fission yeast *Schizosaccharomyces pombe*, Hhp1/2 are the soluble CK1 family members, and one of their functions is to inhibit the septation initiation network (SIN) during a mitotic checkpoint arrest. Hhp1/2 phospho-prime the SIN scaffold protein Sid4 for ubiquitination by the E3 ubiquitin ligase Dma1, which results in a delay to SIN activation and cell division. The SIN is assembled at spindle pole bodies (SPBs), and though Hhp1/2 also localize to SPBs, the mechanism(s) of their recruitment is not understood, nor is it known if their SPB localization is required to inhibit the SIN. Here, we establish that both Hhp1 and Hhp2 localize to SPBs throughout the cell cycle, as well as to the nucleus, cell tips, and the site of cell division. We find that amino acids at the base of the catalytic domain of Hhp1/2 mediate SPB interaction; mutation of these residues selectively disrupts Hhp1/2 association with the core SPB protein Ppc89, Sid4 phosphorylation, Sid4 ubiquitination by Dma1, and the prevention of cytokinesis upon spindle stress. We are testing whether analogous residues in human CK1 $\delta/\epsilon$  facilitate their centrosomal location. Taken together, this study is likely to define a conserved molecular mechanism used by CK1 enzymes to target to a specific cellular locale.

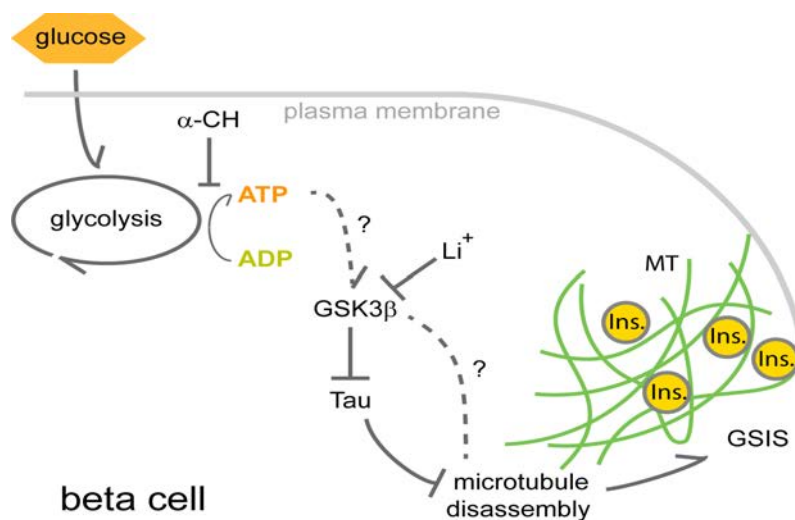


# Tau is a key regulator of glucose-dependent MT destabilization in pancreatic beta cells

Kung-Hsien Ho, Guoqiang Gu, and Irina Kaverina

The homeostasis of blood glucose depends on glucose-induced insulin secretion (GSIS) mediated by pancreatic beta cells. Impairment of GSIS results in prolonged high blood glucose, leading to diabetes. To maintain a high capacity and fast responsiveness to blood glucose surge after a meal, beta cells store thousands of insulin vesicles in the cytoplasm. Our lab has previously demonstrated that microtubule (MT) negatively regulates both phases I and II of GSIS. In resting conditions, MTs actively withdraw cytoplasmic insulin vesicles away from the plasma membrane to restrict the readily releasable pool; upon high glucose stimulation, MTs are destabilized to facilitate GSIS. By using the photoconversion assay, which directly measures MT disassembly, I found that MTs are destabilized within 5-8 minutes after the high glucose stimulation, followed by a brief stabilization (back to the basal stability) and then a prolonged period of low stability. This glucose-induced MT disassembly depends on glycolysis and matches the temporal profiling of GSIS, suggesting that the fine control of MT dynamics is involved in regulating both phases I and II of GSIS. Interestingly, my preliminary data suggest that the neuronal MT-associated protein (MAP) Tau is involved in this process. Tau binds to and stabilizes MTs, whereas the binding is inhibited by phosphorylation of Tau. I found that high glucose induces Tau hyperphosphorylation and dissociation from MTs in beta cells, whereas inhibition of the major Tau kinase, GSK3 $\beta$ , abolishes this response. These results suggest that Tau could be a critical MAP regulating glucose-dependent MT dynamics in beta cells to fine-tune GSIS and glucose homeostasis.

Tau regulates MT dynamics in  $\beta$  cells responding to glucose metabolism.



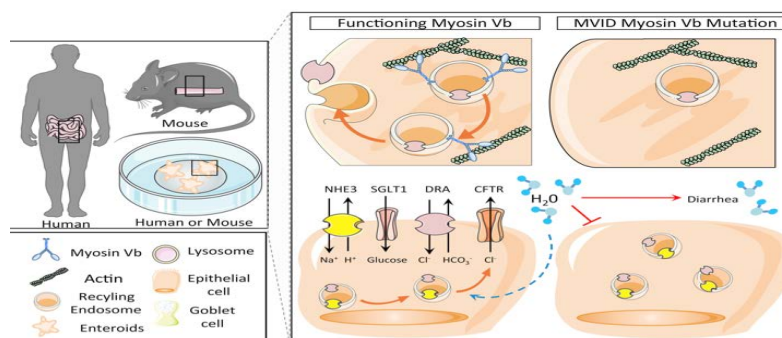
# Deficits in Enterocyte Apical Transporters Associated with Loss of Myosin Vb

Amy C. Engevik, Victoria G. Weis, Byron C. Knowles, Cameron Schlegel, Nadia Ameen, Hermann Koepsell, Nicholas C. Zachos, Mark Donowitz, and James R. Goldenring

**Background:** Microvillus Inclusion Disease (MVID) is a rare form of congenital diarrhea resulting from inactivating mutations in Myosin Vb (MYO5B). Treatment options for patients with MVID are extremely limited and carry a high risk of complications and mortality. Thus, despite the rarity of MVID new treatment strategies are necessary to improve patient outcome. Currently, the mechanism driving MVID diarrhea is unknown. We hypothesize that loss of MYO5B results in aberrant expression of key apical enterocyte membrane transporters that promote the absorption of nutrients and water.

**Methods:** Our lab has generated a mouse model of MVID with a germline deletion of MYO5B (MYO5B KO) to elucidate the mechanism by which loss of MYO5B results in untreatable diarrhea. Duodenum tissue was collected from neonatal MYO5B KO mice and heterozygous or wildtype littermates (control). Immunostaining and quantitative real time PCR (qPCR) was performed to determine the localization of apical transporters and the relative gene expression of transporters respectively in intestinal tissue of MYO5B KO and control mice. Results: Phalloidin conjugates identified apical F-actin in the intestinal brush border of both MYO5B KO and control mice. Similar to MVID patients, numerous F-actin positive inclusions were observed in MYO5B KO enterocytes. To assess the localization of key transporters known to regulate water absorption, we performed immunofluorescence for the sodium hydrogen exchanger isoform 3 (NHE3), cystic fibrosis transmembrane regulator (CFTR), sodium glucose co-transporter (SGLT1) and downregulated in adenoma (DRA). We also assessed NHE regulating factor 1 (NHERF1), a component known to anchor ion transporters in the plasma membrane. Control mice showed apical localization of NHE3, CFTR, SGLT1, DRA and NHERF1. In contrast MYO5B KO mice exhibited NHE3 and CFTR in F-actin positive inclusions. Likewise, SGLT1 had decreased apical expression in MYO5B KO mice and was present in intracellular inclusions. MYO5B KO mice also had decreased apical expression of DRA and alkaline phosphatase 1. qPCR showed decreased expression of NHE3, DRA and GLUT2 and increased expression of SGLT1 in the intestinal tissue of MYO5B KO mice compared to control mice. Enteroids generated from MYO5B KO mice recapitulated *in vivo* findings with the presence of F-actin positive inclusions and subapical expression of SGLT1 and CFTR. Staining of human intestinal tissue from an individual with MVID also showed decreased apical expression of NHE3. Conclusions: Our data suggest that loss of MYO5B results in altered expression of transporters known to regulate water and nutrient absorption. These data implicate intestinal ion transporter deficits as a likely explanation for the pathogenesis of MVID.

Loss of MYO5B alters expression of key apical transporters.

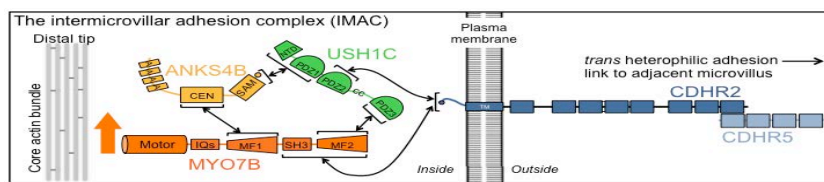


# In-cell reconstitution of intermicrovillar adhesion complex transport

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

The intestinal brush border (BB) serves as the sole site of nutrient absorption within the body and also acts as an important barrier against luminal pathogens. The BB is comprised of membrane protrusions called microvilli that are found on the apical surface of enterocytes. These protrusions are supported by a core bundle of 20 to 30 parallel actin filaments with the plus ends oriented towards the lumen. During intestinal epithelial differentiation, 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. 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. We first wanted to understand the role of the adaptor proteins in efficiently coupling the cargo-binding domain of Myo7b to the CDHR2/CDHR5 cytoplasmic domains. 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, as well as control of the proteins within the system. 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 also be enriched at filopodial tips when coexpressed with the chimeric motor. In our initial studies, we find that the chimeric motor alone can enrich CDHR2 at the distal tips of filopodia. Additionally, the motor can 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.

Model summarizing the extensive interactions within the IMAC

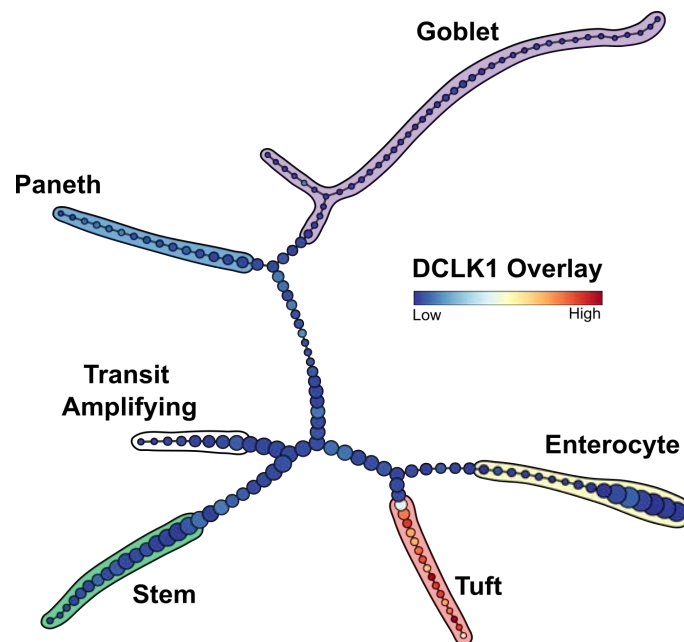


# Computational mapping of single-cell data reveals alternative tuft cell origin in the gut

Charles A. Herring, Amrita Banerjee, Eliot T. McKinley, Alan J. Simmons, Joseph T. Roland, Jeffrey L. Franklin, Michael J. Gerdes, Robert J. Coffey, Ken S. Lau

Recent progress in single-cell technologies have enabled the multiplexed sampling of cellular states within a given tissue. However, there is a dearth of computational tools for robustly analyzing multi-branching cell transitions from single-cell data, such as those found in cellular differentiation. We introduce p-Creode, a computational algorithm that borrows concepts from graph theory to infer pseudotemporal transition from single-cell data. We used p-Creode to investigate the origin of intestinal tuft cells, a chemosensory cell type recently discovered to modulate immune responses to helminths, from multiplex immunofluorescence (MxIF) data of stem cell differentiation markers. We revealed that tuft cells, in contrast to published literature, can be specified outside of the secretory lineage in the small intestine. In the colon however, their specification seems to be controlled by the master secretory cell regulator *Atoh1*. Several avenues of experimental validation support our model and demonstrate the utility of robust analysis of single-cell data to reveal insights into the complexity of multi-lineage specification.

p-Creode predicts an alternative tuft cell origin in the small intestine



# Analysis of cell-cell interaction during optic fissure closure in the developing mouse eye

Amrita Pathak, Katrina S. Hofstetter, Jonathon Kuntz, Dylan T. Burnette,  
Sabine Fuhrmann

Optic cup morphogenesis is a critical step for proper eye development. The morphogenetic process includes invagination of the ventral optic cup and the optic stalk, which leads to the formation of the optic fissure. The fissure margins subsequently fuse together leaving only a small opening for ganglion neuron axons exiting the neural retina and for blood vessels. Defects in closure of the optic fissure results in coloboma, which accounts for more than 10% of childhood blindness. Even though the importance of optic fissure closure for eye development and functioning is well appreciated, yet the cellular and molecular mechanisms underlying the closure process are still obscure.

Early electron microscopic studies suggest that thin cytoplasmic extensions arise from the fissure margins across the gap during optic fissure closure. We hypothesized that these extensions could represent filopodia-like structures. As the Rho GTPase CDC42 can induce filopodia formation, we predicted that CDC42 is critical for proper contact between the fissure margins through regulation of filopodia assembly.

To delineate the role of CDC42, we used a tamoxifen-inducible mouse line, Hes1CreERT2, for temporally controlled and tissue-specific CDC42 inactivation. CreERT2 is inserted into the Hes1 locus and has efficient activity in the ventral optic cup. Normal optic fissure starts closing around embryonic day 11 (E11) and fusion is completed by E12.5. To avoid potential effects on other functions of CDC42 such as apicobasal polarity, we disrupted CDC42 as late as possible by activating Hes1CreERT2 for 18-24 hours before analysis at E11.5.

Our immunohistochemistry data with a variety of markers show that tissue patterning and apicobasal polarity is unchanged. However, closure of the optic fissure is consistently disrupted in CDC42 mutant eyes. To observe the formation of filopodia extensions and cell-cell interactions during optic fissure closure, we used high-resolution confocal microscopy with Airy-scan. Our data in control eyes so far has revealed that we can visualize cytoplasmic bridges and filopodia-like structures extending between the optic fissure margins. Currently, we are analyzing CDC42 mutant optic cups to determine whether the formation of cytoplasmic bridges and filopodia-like structures is affected. Given the role of CDC42 in filopodia and cell adhesion, we predict that it supports optic fissure closure by filopodia formation in the cells lining the optic fissure margin.

Model figure: CDC42 supports optic fissure closure by filopodia formation in the cells lining the fissure margin. Sagittal view of optic fissure during closing process at E11.5. Red: Retinal Pigment Epithelium (RPE), Blue: retina, Green: optic fissure margins, yellow line: apical space between retina and RPE. Purple: Filopodia/ cellular bridges, Red asterisk: Cells lining the optic fissure margins.



# Multiple mechanisms drive calcium dynamics around laser-induced epithelial wounds

Erica Shannon, Aaron Stevens, Wes Edrington, Yunhua Zhao, M. Shane Hutson, Andrea Page-McCaw

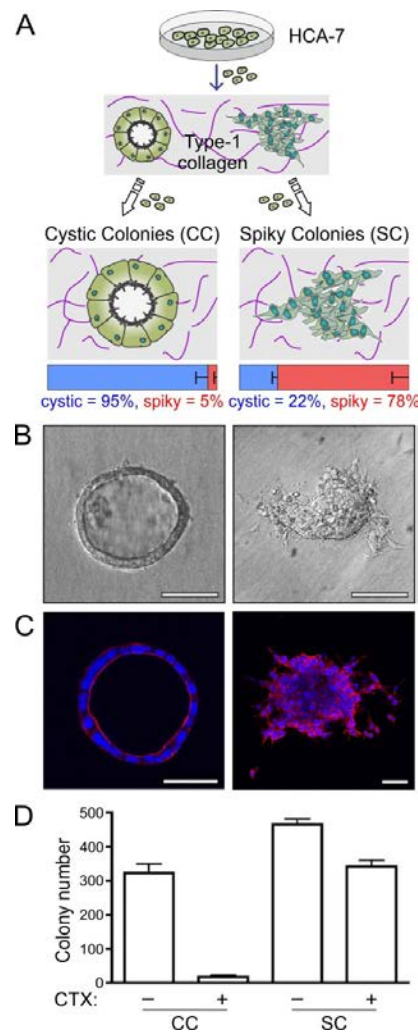
Epithelial wound healing is an evolutionarily conserved process that requires coordination across a field of cells. Studies in many organisms have shown that cytosolic calcium levels rise within a field of cells around the wound and spread to neighboring cells, within seconds of wounding. Although calcium is a known potent second messenger and master regulator of wound healing programs, it is unknown what initiates the rise of cytosolic calcium across the wound field. Here we use laser ablation, a commonly used technique for the precision removal of cells or subcellular components, as a tool to investigate mechanisms of calcium entry upon wounding. Despite its precise ablation capabilities, we find that this technique damages cells outside the primary wound via a laser-induced cavitation bubble, which forms and collapses within microseconds of ablation. This cavitation bubble damages the plasma membranes of cells it contacts, tens of microns away from the wound, allowing direct calcium entry from extracellular fluid into damaged cells. Approximately 45 seconds after this rapid influx of calcium we observe a second influx of calcium that spreads to neighboring cells beyond the footprint of cavitation. The occurrence of this second, delayed calcium expansion event is predicted by wound size, indicating that a separate mechanism of calcium entry exists, corresponding to cell loss at the primary wound. Our research demonstrates that the damage profile of laser ablation is more similar to a crush injury than the precision removal of individual cells. The generation of membrane micro-tears upon ablation is consistent with studies in the field of optoporation, which investigate ablation-induced cellular permeability. We conclude that multiple types of damage, including micro-tears and cell loss, result in multiple mechanisms of calcium influx around epithelial wounds.

# A 3D culture system identifies a new mode of cetuximab resistance

Bhuminder Singh, Cunxi Li, Ramona Graves-Deal, Gregory Daniel Ayers, Mary Kay Washington, Timothy J. Yeatman, Oliver G. McDonald, Qi Liu, and Robert J. Coffey

By culturing a human colorectal cancer (CRC) cell line (HCA-7) in 3D, we have generated two cell lines (CC and SC) with distinct morphological, genetic, biochemical, and functional properties. Although both CC and SC expressed EGF receptor (EGFR), the EGFR neutralizing monoclonal antibody, cetuximab, strongly inhibited growth of CC, whereas SC was resistant to growth inhibition, and this was coupled to increased tyrosine phosphorylation of the receptor tyrosine kinases, MET and RON. Furthermore, we found that cetuximab resistance in the SC cells can be overcome by addition of the dual MET/RON tyrosine kinase inhibitor, crizotinib. As such, this represents a potentially powerful system to identify additional therapeutic strategies and disease-relevant genes in CRC.

Cetuximab (CTX) sensitivity of HCA-7-derived CC and SC cells



# Precise control of cortical contractility maintains mechanical equilibrium during cell division

Nilay Taneja, Aidan M. Fenix, Matthew Bersi, Caleb Snider, James Cooper, Vivian Gama, David Merryman and Dylan T. Burnette

A mechanical equilibrium within the cortex is essential for controlling shape changes during cell division. Here, we show an optimum amount of cortical contractility resulting from competition between two myosin II (MII) isoforms, MIIA and MIIB, is essential to maintain cortical integrity while still allowing dynamic cortical remodeling events during cytokinesis. We found MIIA drives rapid contraction at the cleavage furrow by templating large arrays of filaments containing both MIIA and MIIB. In addition, MIIB maintains cortical tension to counterbalance the forces generated during cleavage furrow ingression. As such, loss of MIIA led to reduced rates of cleavage furrow ingression, while MIIB depletion led to intense blebbing. Depletion of either NMII isoform ultimately led to increased incidence of division failure and aneuploidy. Using Atomic Force Microscopy and biophysical perturbations using a cell indentation assay, we probed the mechanical properties of the cortex, and found opposing effects of knockdown of the two NMII isoforms. These changes were mirrored in the dynamic pools of actin in the cortex. Using fluorescence recovery after photobleaching (FRAP) and chimeras of MIIA and MIIB, we show the two isoforms compete at the cortex and exert their distinct effects due to their different motor properties and turnover rates at the cortex. Furthermore, the recruitment and activation of the two isoforms is regulated by Rho Kinase and Myosin Light Chain Kinase (MLCK). Finally, we show these mechanisms are conserved in human embryonic stem cell (hESC) colonies during interphase, suggesting this tight control on cellular contractility is vital in all cells. Given the role of mechanical forces and shape changes in stem cell differentiation and cancer, these findings have broad implications on our understanding the role of cortical mechanics during development and disease.



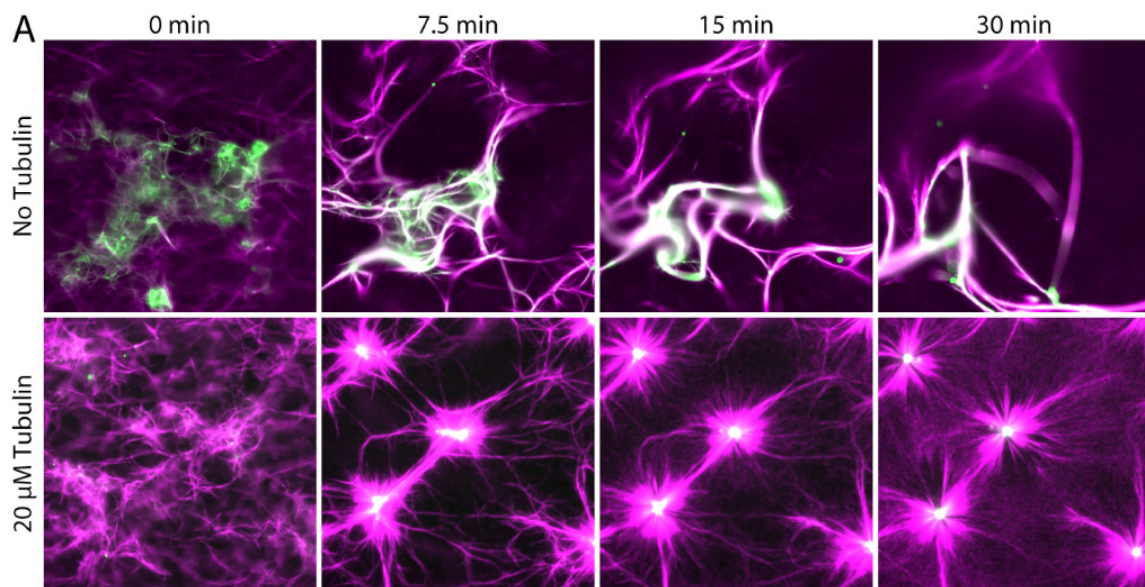
# Microtubule minus-end aster organization is driven by processive HSET-tubulin clusters

Stephen R. Norris, Claire E. Strothman, Marija Zanic\* and Ryoma Ohi\*

\*These authors contributed equally to this work.

Higher-order structures of the microtubule (MT) cytoskeleton are comprised of two structural motifs: bundles and asters. Although both are critical for cellular function, the molecular pathways that drive aster formation are poorly understood. Here, we study aster formation by human kinesin-14 (HSET/KIFC1), a molecular motor that crosslinks MTs and moves toward MT minus-ends. Similar to *Xenopus* kinesin-14 (1), we confirm that HSET forms asters from growing MTs. In contrast, we show that HSET is incapable of forming asters from pre-formed, non-growing MTs. Using single-molecule imaging we show that soluble tubulin induces HSET team formation (*i.e.*, HSET-tubulin “clusters” containing multiple motors) *via* binding to the N-terminal tail domain, thus activating motor processivity. We find that processive HSET teams rescue aster formation of non-growing MTs. This novel motor activation mechanism allows HSET to form either bundles or asters in a manner dependent on the concentration of soluble tubulin, and may contribute to HSET-driven supernumerary centrosome clustering in cancer cells. We also use time-lapse total-internal-reflection fluorescence (TIRF) microscopy to demonstrate that HSET protects growing MT minus-ends by increasing their lifetime and decreasing their catastrophe frequency in a dose-dependent fashion. To our knowledge, this work represents the first *in vitro* description of a mitotic minus-end protector.

100 nM EGFP-HSET (green) forms microtubule asters from pre-formed microtubules (magenta) after activation by soluble tubulin. Scale bar, 50  $\mu\text{m}$ .



## Post Doc / Graduate Student Breakout Sessions

### Morning Session - 9:40 a.m. – 10:55 a.m. Moderator: Karrie Dudek

<i>Speaker</i>	<i>Tagline</i>	<i>Lab</i>
Christine Jones	Examination of how phosphorylation status prevents E3 auto-ubiquitination without disrupting substrate ubiquitination	Gould
Elizabeth Lawrence	Unraveling the mechanism of CLASP-mediated microtubule stabilization	Zanic
Megan Merolla	Non-apoptotic role of Mcl-1 in the regulation of mitochondrial dynamics	Gama
Meagan Postema	Investigating the interaction between the I-BAR protein IRTKS and the actin binding protein EPS8 in microvillar growth and assembly	Tyska
Lindsey Seldin	Tissue stress and multipotency in the mammary gland	Macara

### Afternoon Session – 3:20 p.m. – 4:35 p.m. Moderator: Erick Spears

<i>Speaker</i>	<i>Tagline</i>	<i>Lab</i>
Goker Arpag	Reconstituting microtubule treadmilling in vitro	Zanic
Amrita Banerjee	Deletion of secretory cell regulator Atoh1 eliminates intestinal inflammation in a mouse model of Crohn's disease	Lau
Keyada Frye	Detyrosinated microtubule tracks in s-phase of the cell cycle facilitate the depolarization of the Golgi, decreasing cell migration velocity in preparation for mitotic entry	Kaverina
Alissa Guarnaccia	Central to my project is the oncoprotein MYC. I am investigating MYC-interacting proteins using biochemical and chromatin-based assays	Tansey
Nathaniel Hepowit	I will be discussing my recent efforts to characterize novel ubiquitin kinases and define their activities in a cellular context	MacGurn

## Poster Session I (ODD NUMBERS)

Poster #	First Name	Last Name	Poster #	First Name	Last Name
1	Mukhtar	Ahmed	53	Amanda	Halfond
3	Erin	Aho	55	Nathan	Hepowit
5	Abstract Withdrawn		57	Susan	Histed
7	Laura	Armstrong	59	Angela	Howard
9	Goker	Arpag	61	Won Jae	Huh
11	Amrita	Banerjee	63	Aja	Hyde
13	Bahnisikha	Barman	65	Brenda	Jarvis
15	Bryan	Cawthon	67	Sun Wook	Kim
17	Sierra	Cullati	69	Jennifer	Landino
19	Jessica	Do	71	Lynne	Lapierre
21	Megan	Dumas	73	Armelle	Le Guelte
23	Joey	Elsakr	75	Sora	Lee
25	Merlyn	Emmanuel	77	Abstract Withdrawn	
27	Amanda	Erwin	79	Aaron	Mason
29	Veronica	Farmer			
31	Aidan	Fenix			
33	Jonathan	Fleming			
35	Nora	Foegeding			
37	Maria	Fomicheva			
39	Audra	Foshage			
41	Keyada	Frye			
43	Hisayo	Fukuda			
45	Meredith	Giblin			
47	Laura	Glass			
49	Alissa	Guarnaccia			
51	Abstract Withdrawn				

# The Par3 Polarity Protein Is An Exocyst Receptor Essential for Mammary Cell Survival

1

Syed Mukhtar Ahmed and Ian Macara

Partition defective (Par) and associated proteins play crucial roles in controlling the processes of epithelial morphogenesis, by organizing epithelial cell membranes into apical and basolateral domains, and orienting mitosis in the plane of the epithelial sheet. Par3 acts as a scaffold protein, and contains several evolutionarily conserved domains, which are required for its function to deliver its partner proteins to the apical surface. Using a mouse mammary regeneration model, the Macara lab has shown that Par3 plays a pivotal role in mammary gland morphogenesis, where loss of Par3 in the mammary glands results in disorganized multilayered ducts and luminal lling. Depletion of Par3 in mouse mammary glands also provokes two opposing responses – apoptosis and proliferation. However, little is known about the signaling events that generate these outcomes. Here we show that we can recapitulate in vitro the apoptotic phenotype caused by loss of Par3 in 2 normal murine mammary epithelial cell lines, NMuMG and Eph4. Knockdown of Par3 using an efficient short-hairpin RNA leads to dramatic cell extrusion and apoptosis in a cell autonomous manner. Co-expression of shRNA-resistant hPar3 together with mouse Par3 shRNA rescues this phenotype. Loss of Par3-mediated apoptosis was associated with reduced pBad and pFoxo3a and increased Bim expression, concomitant with a dramatic decrease in pAKT levels. Co-expression of a constitutively active mutant of AKT (AKT-CA) rescues Par3-loss-mediated apoptosis. Loss of Par3 in NMuMG cells also leads to reduced PIP3 levels as well as defective localization of lateral membrane proteins such as E-Cadherin and Na-K-ATPase. Mechanistically we show that mislocalization of E-Cadherin and loss of pAKT is due to a defect in proper Sec6/8 exocyst delivery to its target membranes. Loss of exocyst components Sec8 or Sec10 each phenocopy the defects associated with loss of Par3, and apoptosis can be prevented by expression of AKT-CA. Furthermore, we show that Par3 associates with the exocyst via its polybasic region, which can also associate with phospholipids. Forced localization of this fragment of Par3 to the plasma membrane rescues E-Cadherin localization as well as apoptosis in cells depleted of endogenous Par3. These results suggest that Par3, independently of its role in determining apical-basal polarity of epithelial cells, is also crucial for exocyst tracking and survival of mammary epithelial cells.

## WDR5 as an epigenetic anti-leukemia target

3

Erin R. Aho, Lance Thomas, Sabine Wenzel, Shelly Lorey, Shaun Stauffer, Stephen W. Fesik, and William P. Tansey

Translocations of the Mixed Lineage Leukemia gene (MLL) are found in 70% of all infant acute lymphoblastic leukemias (ALL). ALL is a devastating and deadly disease. Most patients respond poorly to current chemotherapeutic options, and only 35% of children survive 5 years post-diagnosis. In order to improve patient survival, new, targeted therapies for ALL are urgently needed.

In ALL patients, translocation of one MLL1 allele results in the expression of an oncogenic MLL-fusion protein in addition to wild-type MLL, a histone methyltransferase capable of methylating lysine 4 of histone H3, an epigenetic mark associated with active gene transcription. The current mechanistic model for MLL-rearranged leukemogenesis posits that wild type MLL binds to target genes first and initiates gene activation while the MLL-fusions promote aberrant transcriptional elongation through the recruitment of the super-elongation complex, resulting in overexpression of genes that keep hematopoietic cells in a proliferative state. However, the intrinsic catalytic activity of MLL is very low and requires binding of WD Repeat Domain 5 (WDR5) and three other core-complex proteins for full activation. WDR5/MLL binding is particularly critical for catalytic activity. Based on this model, inhibiting the WDR5/MLL interaction would have profound implications for the possible development of a highly sought-after targeted ALL therapeutic approach.

The anti-leukemogenic potential of blocking WDR5/MLL binding has led to collaboration with the Fesik laboratory to discover small molecules that disrupt this interaction. Preliminary studies have illustrated that our WDR5/MLL inhibitors selectively reduce proliferation and viability of leukemia cell lines harboring an MLL-fusion. The purpose of our research is to define the utility of inhibiting WDR5/MLL binding in combatting leukemia by elucidating the molecular and phenotypic consequences of inhibiting WDR5/MLL, as well as to challenge the currently accepted model of MLL-leukemogenesis.

*Abstract Withdrawn*

# Heterozygous loss of TSC alters p53 signaling and stem cell reprogramming

7

Laura Armstrong, Grant Westlake, Kevin Ess

Tuberous Sclerosis Complex (TSC) is a pediatric genetic disorder which causes benign tumor growths in multiple organ systems including the brain, kidney, heart, skin, and lungs. The most debilitating symptoms are a consequence of the brain involvement leading to a high rate of epilepsy, autism spectrum disorder, and learning disabilities. Patients carry a heterozygous loss of function mutation in either the *TSC1* or *TSC2* genes, which code for the proteins hamartin and tuberin, respectively. Developmental brain abnormalities have been detected in TSC patients as early as 20 weeks gestation suggesting an important developmental role for hamartin and tuberin. The benign hamartomatous growths in the kidneys and lungs are thought to arise from a somatic mutation in the second allele of *TSC1* or *TSC2*, leading to increased mTORC1 activity and unhindered growth with surrounding heterozygous tissue functionally normal. By contrast, emerging data from surgically resected neural hamartomas demonstrate very few cells that show loss of heterozygosity. A major question remains: do heterozygous mutant cells contribute to the pathology of TSC? Patient-derived cell lines present a unique opportunity to explore this question.

In the process of generating *TSC2* mutant and wild-type induced pluripotent stem (iPS) lines from human fibroblasts, we observed an increased rate of integration of the reprogramming plasmids in mutant lines. Integrated cell lines retain the shP53/*OCT4* plasmid at a higher rate than the *KLF4/SOX2* or *L-MYC* reprogramming plasmids, suggesting that the *OCT4* gene or shP53 is driving integration of this plasmid specifically. *We hypothesize that heterozygous loss of TSC2 in human cells is sufficient to alter p53 activity thereby impairing fibroblast reprogramming and driving selection of cells integrating the shp53 reprogramming plasmid.* *TSC2*<sup>+/-</sup> fibroblasts form fewer pluripotent colonies following plasmid reprogramming. Tuberin and hamartin proteins are increased in response to UV challenge, suggesting an interaction between TSC and DNA damage response pathways. Further, *TSC2*<sup>+/-</sup> fibroblasts display increased p53 protein levels in response to DNA damage, which can be corrected with rapamycin. Heterozygous loss of *TSC2* in human cells is sufficient to alter p53 signaling and stem cell reprogramming.

# Microtubule treadmilling revealed by *in vitro* reconstitution

9

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 the total 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 through modulation of the dynamic parameter 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 by modulating tubulin concentration in the reaction solution. Our preliminary *in vitro* observations reveal treadmilling-like behavior with leading microtubule minus ends for tubulin concentrations between 8 -10  $\mu\text{M}$ . 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.



# The role of the secretory lineage in ileal inflammatory disease

11

Amrita Banerjee, Alan J. Simmons, Chuck Herring, Ken S. Lau

Global incidence of Crohn's disease (CD), a debilitating inflammatory bowel disease, has rapidly increased in the last half century. Genome-wide association studies have identified numerous CD risk alleles in genes expressed in intestinal secretory cells, including anti-microbial-secreting Paneth cells and mucus-secreting goblet cells, which contribute to chemical barrier function in the intestine. The objective of this proposal is to address the role of the epithelial secretory lineage in the pathogenesis of ileal CD. Previous studies have not systematically investigated either the underlying mechanism driving Paneth and goblet cell abnormalities during CD or the functional consequences arising from these alterations. We will conduct our investigation in the  $TNF^{\Delta ARE}$  mouse, a  $TNF-\alpha$  overexpression model that develops Crohn's-like ileitis. We have developed a model,  $Lrig1^{CreERT2}; Atoh1^{flox}; TNF^{\Delta ARE}$  in which *Cre recombinase* activation can ablate Paneth and goblet cells throughout the small and large intestine of the  $TNF^{\Delta ARE}$  model. Intriguingly, tamoxifen-treated  $Lrig1^{CreERT2}; Atoh1^{flox}; TNF^{\Delta ARE}$  animals exhibit none of the hallmarks of  $TNF-\alpha$ -induced inflammation, including blunted villi and immune cell infiltration, compared to littermate controls. Our current studies are focused on elucidating the mechanism underlying the reversal of inflammation and restoration of crypt-villus architecture in the ileum. Multiplexed immunofluorescence imaging and single cell RNA sequencing will be utilized to investigate changes to signal transduction and gene expression networks in individual epithelial cells as a result of *Atoh1* ablation. Furthermore, we will query immune cell populations, specifically CD4+ T-helper cell subclasses, to determine whether there is a causal link between the epithelial secretory lineage and the immune system in the resolution of ileal inflammation in the  $TNF^{\Delta ARE}$  model.

# Membrane contact sites regulate the biogenesis of RNA-containing Extracellular vesicles

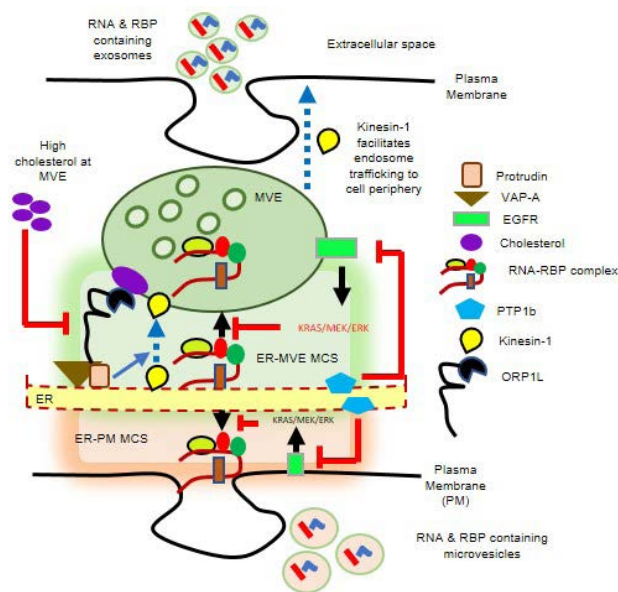
13

Bahnisikha Barman, Alissa Weaver

Exosomes and microvesicles are small extracellular vesicles (EVs) that play an important role in cell-cell communication. RNAs and RNA binding proteins (RBPs) are potentially key cargoes that can be transferred via EVs to modulate gene expression and physiology of recipient cells. The biogenesis and transfer mechanisms of RNA-containing EVs are not understood. This is an important emerging scientific area given the increasingly recognized role of EVs in paracrine communication in health and disease.

Exosomes are formed when endosomal membranes invaginate into the interior of endosomes to form multivesicular bodies (MVBs). MVBs can fuse with the plasma membrane (exocytosis) to deliver intraluminal vesicles in the extracellular space as exosomes. Microvesicles bud from the plasma membrane and are released into the extracellular space. Protein and RNA cargoes are incorporated into EVs at the time of vesicle formation.

The endoplasmic reticulum (ER) is the largest membrane-bound organelle in eukaryotic cells. ER membranes can contact other subcellular organelles in domains termed membrane contact sites (MCS). Interestingly, MCS between the ER and endosomes can control endosomal fission and transfer molecules between the organelles. As many EV-associated RBPs are typically known to localize to the ER, I will present and discuss how that ER-endosome and ER-plasma membrane MCS are a key mechanism to induce biogenesis of RNA- and RBP-containing EVs.



Proposed model of membrane contact sites regulation of RNA containing EV biogenesis.

## Understanding the role of Dally-like protein (Dlp) trafficking

15

Bryan Cawthon, Xiaoxi Wang, Indrayani Waghmare, Andrea Page-McCaw

Localization of signaling molecules is vital for proper development. In *Drosophila*, long range signaling of extracellular Wingless controls follicle stem cell divisions. It is not clear how Wingless travels from its source to the follicle stem cells in this long range signaling. Dlp is a glypican, a heparan sulfate proteoglycan with a GPI anchor, that plays a role in the spreading of Wingless (Wg) to follicle stem cells. Dlp is localized both on the cell surface and in the extracellular matrix. Previous work in our lab has shown that Dlp is negatively regulated by Mmp2. In cell culture, our lab has shown that Dlp may be cleaved by Mmp2 and possibly cause the internalization of the Dlp. Previous cell culture data suggests that once Mmp2 cleaves Dlp, Dlp no longer is in contact with Wingless. It is not understood how Dlp moves inside and outside of the cell and how this regulation is communicated across the phospholipid bilayer. We are currently investigating if cleavage of Dlp occurs *in vivo* and if it requires Mmp2. A yeast two-hybrid screen was performed for proteins that interact with Dlp to understand what causes the internalization of Dlp. We first investigated if the interactors co-localize with Dlp by overexpressing them in S2R+ cells and each of the interactors co-localize with Dlp both inside and outside of the cell. To confirm the interactors *in vitro*, we are performing a co-immunoprecipitation experiment in S2R+ cells. In addition, we will also perform crosses to RNAi lines against each of the interactors *in vivo* to see if the localization of Dlp changes. We will perform each of these experiments to determine how Dlp is internalized and thus possibly affecting how Wingless travels to follicle stem cells in long range signaling.

## Determining the significance of CK1 autophosphorylation in regulating a mitotic checkpoint

17

Sierra N. Cullati, Zachary C. Elmore, Anna S. Feoktistova, Alyssa E. Johnson, 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, and neurodegenerative disease progression. Like other multifunctional kinases, CK1 must be regulated in space and time in order to target specific subsets of its substrates in each of the pathways it participates in. For example, work from our laboratory has defined residues in the catalytic domains of Hhp1 and Hhp2, the *S. pombe* homologues of human CK1d/e, responsible for localizing Hhp1/2 to the spindle pole body, where they impose a mitotic checkpoint delay via phosphorylation of a specific spindle pole body protein.

To further elucidate the control of Hhp1/2 activity in the mitotic checkpoint, we have turned our attention to the role of autophosphorylation, a conserved property of CK1 enzymes. CK1 enzymes are known to autophosphorylate their C-terminal non-catalytic tails, which is proposed to inhibit their activity by acting as a pseudosubstrate. However, this proposed mechanism of autoinhibition has yet to be tested *in vivo* in any organism.

We have identified serine and threonine (but not tyrosine) autophosphorylation sites in the kinase domains as well as the C-terminal tails of Hhp1/2. We have tested the effects of mutating these sites on *in vitro* kinase activity and are currently investigating whether these effects are conserved in their human homologues CK1d/e. We are also testing the importance of basic patches in the kinase domain which have been proposed to interact intramolecularly with the phosphorylated C-terminus to inhibit kinase activity. Because neither enzyme is essential for cell viability in *S. pombe*, we are able to investigate the significance of each autophosphorylation event not only *in vitro* but *in vivo*. This work will lead to a greater understanding of mitotic checkpoint function, as well as elucidate conserved mechanisms of regulation for the CK1 family of enzymes.

# A *Sox10* multi-spectral allele for *in vivo* Ca<sup>2+</sup> imaging during glial cell development

19

Jessica M. Do, Jean-Marc L. DeKeyser, and E. Michelle Southard-Smith

*Sox10* is a transcription factor in the Sry-like HMG domain family that is essential in the development of the nervous system. It plays important roles in differentiation of oligodendrocytes from oligodendrocyte precursor cells (OPCs) in the central nervous system and in specification and maintenance of Schwann cells and enteric glia in the peripheral nervous system. Among these glial populations, transient fluxes of calcium promote cell communication and have been implicated in migration, cell survival, and ganglia formation. To enable live cell imaging of glial progenitor migration and interactions of mature glia with neurons and concurrent calcium flux during these processes, we have generated a multi-spectral (MS) allele of *Sox10* that encodes three separate fluorescent reporters. Our construct includes a membrane bound reporter (Src-Apple) to visualize cell extensions, a nuclear reporter (H2B-mCerule3) to localize the nucleus, and a genetically encoded calcium indicator (GCaMP5) to monitor calcium flux during cell signaling. Because homologous gene targeting disrupts the *Sox10* locus, leading to reduction of these distinct glial populations and even embryonic lethality, our new allele relies upon long-range regulatory sequences in a *Sox10* BAC to drive transgene expression. As a result, the transgenic reporter does not disrupt endogenous *Sox10* expression, and, thus, mice are phenotypically normal. Following microinjection into B6D2 F1 hybrid eggs, seven transgenic founder mice were obtained. Founders have been bred and germline transmission through to N5 progeny achieved for three distinct lines. Simple tandem repeat (STR) mapping has confirmed the integrity of the integrated construct among the various lines. Experiments are ongoing to establish that the transgene recapitulates endogenous *Sox10* gene expression by evaluating expression patterns of the *Sox10*-MS transgene in adult oligodendrocytes, Schwann cells, and mature enteric glia as well as in migrating neural crest progenitors in fetal mouse tissues. The availability of this *Sox10*-MS allele will enable studies of cell migration and communication in the context of normal glial cell development and maturation or can be coupled with other mutations for analysis of disease pathology and glial deficits.

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

21

Megan Dumas, Nicole Kendrick, Geng-Yuan Chen, 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, with many advancing to both Phase 1 and 2 clinical trials. 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, a phenomenon commonly observed 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 Eg5 and Kif15, will cripple rapidly dividing cancer cells. Therefore, we set out to perform a small molecule screen on a focused 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 in duplicate and two compounds, both containing oxindole cores, 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. VU0482674 became our lead compound, exhibiting an IC50 of 800nM. Similarly, VU0482674's IC50 in the MT gliding assay was calculated to be 734nM. Furthermore, treatment with VU0482674 on K5I resistant cells (KIRCs), whose ability to form bipolar spindles relies on Kif15, results in nearly 100% monopolar spindles. VU0482674 has no effect on mitotic progression in normal RPE-1 cells, suggesting that the compound primarily inhibits Kif15 during cell division. Mechanistically, VU0482674 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 VU0482674 is currently underway.

# The Effects of *In Utero* High Fat Diet Exposure on the Endocrine Pancreas of the Offspring

23

Joseph Elsagr, Raymond Pasek, Diana Takahashi, Kevin Grove, Al Powers, and  
Maureen Gannon

Recent evidence suggests that nearly one fourth of women in the US are obese at the time of pregnancy. Offspring born to obese mothers have an increased risk of developing metabolic syndrome and Type 2 Diabetes (T2D) later in life. In multiple animal models, maternal high fat diet (HFD) leads to a predisposition for obesity, insulin resistance, and T2D in the offspring. These offspring often have defects in the mass, function, and transcriptional profile of their pancreatic  $\alpha$  and  $\beta$  cells. Previous work from members of our group using a non-human primate (NHP) model has shown that fetuses of HFD-fed mothers have reduced  $\alpha$  cell mass, increased  $\beta$ : $\alpha$  cell ratio, and decreased  $\beta$  cell insulin content and expression of *Glut2* (the key  $\beta$  cell glucose transporter). Juvenile offspring (1-year-olds) of HFD-fed mothers maintained on HFD post-weaning (HFD/HFD) have a decrease in both  $\beta$  and  $\alpha$  cell number, increased  $\beta$ : $\alpha$  cell ratio, and a trend toward decreased *Glut2* expression relative to offspring exposed to a control diet (CTR) *in utero* and weaned onto HFD (CTR/HFD). Ongoing analysis of 3-year-old offspring is focused on characterizing  $\beta$  and  $\alpha$  cell number and proliferation as well as changes in  $\beta$  cell ultrastructure and islet gene expression and morphology. While maternal HFD did not influence levels of  $\beta$  or  $\alpha$  cell proliferation at this age, HFD/HFD offspring have a persistently elevated  $\beta$ : $\alpha$  cell ratio. We conclude that while *in utero* exposure to HFD alone results in no observable islet phenotype in this model, the negative consequences of maternal overnutrition are unmasked when the offspring are weaned onto a HFD. These results suggest that *in utero* exposure to HFD may limit the ability of offspring to adequately respond to a metabolic stressor later in life.

## Exosome secretion in stromal matrix assembly and organization

25

Merlyn Emmanuel, Bong Hwan Sung and 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 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 assembly of fibronectin (FN) by fibroblasts depends on the endolysosomal secretion of extracellular vesicles called "exosomes". Using density gradient purified exosomes derived from hTERT-immortalized human mammary fibroblasts we found that ECM molecules including fibronectin (FN), collagen I (Col I) and periostin (POSTN) as well as matrix cross linking factors like transglutaminase2 (TG2) and lysyl oxidase like protein2 (LOXL2) are specifically associated with them. Further, exosome inhibition by knock down of Rab27a (docking factor for MVBs) and Synaptotagmin7 (MVB fusion regulator) leads to a decrease in incorporation of FN and POSTN into the assembled matrix. Furthermore, exosome secretion affects matrix organization. In future studies, we plan to identify critical exosomal cargoes important for matrix 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.



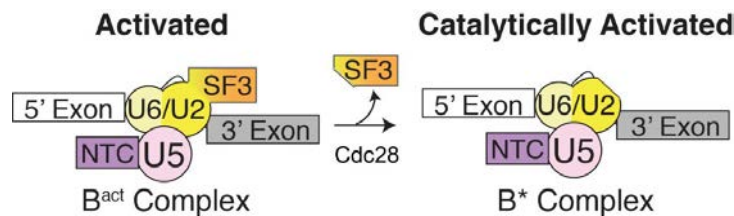
# Structural characterization of spliceosome activation

27

Amanda L. Erwin, Xue Wang, and Melanie D. Ohi

The spliceosome is an essential multi-megadalton ribonucleoprotein complex that catalyzes pre-mRNA splicing. Normal eukaryotic cell function depends on accurate splicing and aberrant splicing can result in disease. The formation of an activated spliceosome competent to accurately excise an intron and ligate two exons requires sequential conformational rearrangements of small nuclear ribonucleoproteins (snRNPs) and protein complexes that assemble on the pre-mRNA substrate in a highly regulated, stepwise manner. The complex and dynamic nature of the spliceosome has made it challenging to obtain structural information to determine the global conformational changes required for spliceosome activation. Proteomic analyses have given insight into proteins that may mediate conformational rearrangements during spliceosome activation by elucidating compositional differences in complexes throughout the splicing cycle. One major compositional change during spliceosome activation is the dissociation of the SF3 complex. SF3 is a conserved U2 snRNP associated protein complex that assembles onto the pre-mRNA prior to spliceosome activation. Proteomic analysis of pre-activated and post-activated spliceosomes indicates that SF3 is no longer associated with the spliceosome after spliceosome activation. However, the structural role of SF3 during spliceosome activation remains unclear. To gain a better understanding of the role of SF3 in spliceosome activation, we aim to determine a high-resolution cryo-electron microscopy (cryo-EM) structure of the *S. pombe* activated spliceosome ( $B^{\text{act}}$  complex). Toward this goal, we have determined a strategy to purify a robust amount of *S. pombe*  $B^{\text{act}}$  complexes. Further, we have determined vitrified ice conditions for visualization by cryo-EM. We are in the process of collecting and processing cryo-EM micrographs for high-resolution structure determination. This structure will enable insights into protein-protein and protein-RNA interactions that activate the spliceosome for subsequent splicing steps.

SF3 is dynamically associated with the spliceosome during activation.



# Regulating Microtubule Dynamics Through GTP-Hydrolysis

29

Veronica Farmer, Anika Rahman, and Marija Zanic

Dynamic instability of microtubules, the switching between phases of growth and shrinkage, enables the microtubule network to remodel throughout the cell cycle. Although much is known about the functions of the microtubule cytoskeleton, a key gap in knowledge is how the microtubule network is remodeled in cells to warrant these functions. In order to grow, a microtubule incorporates GTP-tubulin subunits at its ends. GTP is hydrolyzed into GDP, resulting in a microtubule lattice composed of GDP-tubulin, while the growing end maintains a cap of GTP-tubulin. This GTP-cap is thought to stabilize a growing microtubule. The size of the GTP-cap is dependent on microtubule growth and GTP-hydrolysis rates, and thus modulation of these parameters has the potential of producing a more or less stable microtubule. Work *in vitro*, using purified tubulin and GTP-cap marker EB1, has defined the relationship between microtubule growth rate and the size of the GTP-cap. Increasing tubulin concentrations results in increased microtubule growth rates, as well as larger comets of EB1-GFP on growing microtubule tips. Using EB1-GFP as a marker for the GTP-cap size, we demonstrate that this correlation persists when growth rates are increased to cellular rates in the presence of microtubule polymerase XMAP215. Further, we aim to modulate the GTP-hydrolysis rate *in vitro* using a number of perturbations, to change the size of the GTP-cap and investigate its impact on microtubule lifetime. Interestingly, we find that EB1-GFP comet lengths measured in cells are smaller than those *in vitro* for the corresponding growth rates. This observation suggests that the GTP-hydrolysis rate may be modulated by the cell. We therefore aim to investigate the potential correlation between microtubule lifetimes and EB1-GFP comet sizes in cells. Finally, we aim to determine whether the physiological regulators of microtubule dynamics employ modulation of the GTP-hydrolysis rate as a molecular mechanism for modulation. To do so, we will use purified microtubule-associated proteins known to change microtubule lifetime, and measure their effect on EB1-GFP comet length. In summary, this work aims to determine if the lifetime of a microtubule can be modulated by the GTP-hydrolysis rate, and has the potential to provide an understanding of how a microtubule's lifetime is determined.

# Actin arcs are essential templates for sarcomere assembly in human cardiomyocytes

31

Aidan Fenix, Mike R. Visetsouk, Nilay Taneja, Annabelle Manalo, Scott W. Crawley, David M. Bader, Matthew J. Tyska, Jennifer H. Gutzman, Dylan T. Burnette.

The sarcomere is the basic contractile unit within heart muscle cells (i.e., cardiomyocytes). The proper establishment of sarcomeres during development and their maintenance during homeostasis are critical for the contraction of the heart and pumping of blood throughout the body. How the components of sarcomeres assemble remains a major unanswered question. Here we use newly plated human induced pluripotent stem cell-derived cardiomyocytes (hiCM) combined with high-resolution microscopy to elucidate the steps of de novo sarcomere assembly. We found that sarcomere formation was preceded by bundles of actin filaments resembling so-called “actin arcs” prevalent in migrating non-muscle cells. Sarcomeres appeared along the length of these actin bundles; suggesting actin arcs are acting as a template for sarcomere assembly. Actin arc formation in non-muscle cells is dependent on the actin filament nucleator, formin, and the molecular motor, non-muscle myosin II (NMII). Inhibiting formin in hiCM stopped the formation of actin arcs and subsequent sarcomere assembly, but had little if any effect on pre-assembled sarcomeres. Two isoforms of NMII, NMIIA and NMIIB, localized to the actin arcs in hiCM. Using NMIIB as a molecular marker, we also found actin arc-like structures in mouse cardiomyocytes in vivo. Knockdown of NMIIB in hiCM resulted in a loss of sarcomere assembly. Furthermore, knockdown of NMIIB in zebrafish also resulted in a reduction of sarcomeres in vivo. Finally, we show that NMII and the muscle isoform, *b* myosin II (bMII), are found in the same filaments in hiCM and in vivo in mice and humans, suggesting individual molecular components within actin arcs could be acting as seeds for their muscle counterparts. Taken together, our data supports a view that contractile systems in cardiac muscle evolved from non-muscle contractile systems, and muscle cells still use non-muscle contractile components during de novo sarcomere assembly.

# Assembly of cerebellar circuitry requires developmental Hedgehog signaling

33

Jonathan T. Fleming, Emily Brignola, Shane Heiney, Chunqing Zhang, Timothy Warner, Jingqiong Kang, Javier Medina and Chin Chiang

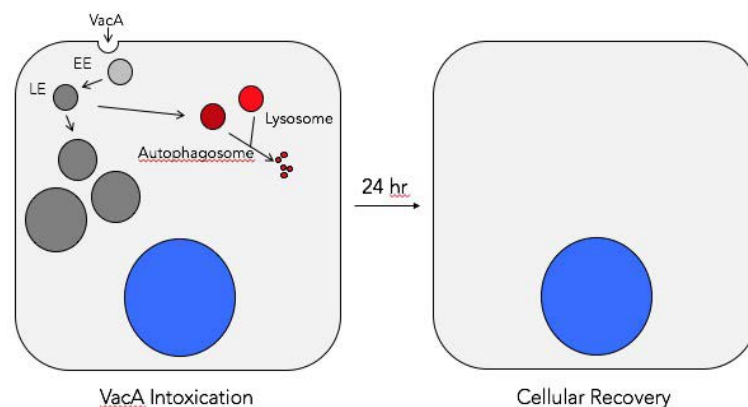
The cerebellum is a hindbrain structure whose plasticity is essential for motor learning and some forms of higher-level cognition. Many of the first descriptions of neuronal morphology, and later the notion of connectivity, came from early studies of the cerebellar cortex. Although much is known regarding the repertoire of cerebellar cell types, less is known about the developmental mechanisms responsible for generating them. We discovered that Hedgehog (Hh) signaling is active in the neonatal cerebellar white matter (PWM) where progenitors of late-born GABAergic interneurons reside. We then determined that cells expressing the transcription factor *Ptf1a* respond to Sonic hedgehog (Shh) from adjacent Purkinje neurons, but the larger pool of GABA precursor cells, marked by *Pax2* expression, do not. To ascertain the significance of this developmental signal in *Ptf1a*<sup>+</sup> cells, we used Cre-loxp technology to genetically ablate Shh-responsiveness through deletion of signal transducer, Smoothened (*Smo*). In the resulting *Ptf1a*<sup>Cre</sup>; *Smo*<sup>F/-</sup> neonates, proliferation of *Ptf1a*<sup>+</sup> cells was significantly reduced, as was the pool of *Pax2*<sup>+</sup> cells. Consistent with these findings, the cerebellar cortex of adult *Ptf1a*<sup>Cre</sup>; *Smo*<sup>F/-</sup> mutants lack significant numbers of two GABAergic inhibitory interneurons, basket and stellate cells. These interneurons subtypes represent the major source of inhibitory input to Purkinje cells, the sole projection neuron of the cerebellum, which in *Ptf1a*<sup>Cre</sup>; *Smo*<sup>F/-</sup> mutants had reduced amplitude miniature inhibitory postsynaptic spontaneous currents (mIPSCs). We are testing to what extent this loss of inhibition alters cerebellar-dependent motor learning. Because this particular form of learning is often impaired to varying degrees in individuals with autism, our work may help explain how failed assembly of neural circuitry contributes to the cerebellum's role in this disorder.

# Understanding the Structure and Function of the *H. pylori* Toxin, VacA

35

Nora J. Foegeding, Tasia M. Pyburn, Timothy L. Cover, Melanie D. Ohi

*Helicobacter pylori* persistently colonizes the gastric mucosa of more than half of the world population, with prevalence as high as 90% in developing nations. Infection with *H. pylori* causes chronic gastric inflammation and is the leading cause of stomach ulcers and gastric cancer. As a result of growing antibiotic resistance, alternative strategies are needed to disrupt *H. pylori* colonization and prevent disease. Both the ability of *H. pylori* to colonize the stomach and the risk of developing *H. pylori*-mediated disease is directly associated with the secretion of a pore-forming virulence factor called vacuolating cytotoxin A (VacA). In addition to inducing cellular vacuolation, the effect for which it is named, VacA has been reported to trigger diverse responses from both epithelial and immune cells including altering permeability of the plasma membrane, disrupting connections between cells in epithelial monolayers, altering endosomal and lysosomal function, disrupting mitochondrial function, inducing cell death, and inhibiting T lymphocyte activation. We currently do not understand how VacA triggers all of these deleterious, yet seemingly unconnected, cellular responses, nor do we understand which cellular responses lead to disease. Furthermore, although most cellular effects of VacA are dependent on its ability to form anion-selective membrane channels, there is still no molecular model for VacA pore-formation. As a result, the specific role VacA plays in contributing to *H. pylori*-mediated disease remains unclear. Our characterization of VacA trafficking has begun to clarify how cells respond to VacA intoxication. In contrast to a proposed model where VacA is trafficked to mitochondria to induce apoptosis, we do not observe VacA localization to mitochondria. Instead, we have shown that host cells recover from vacuolation and resist VacA-induced cell death by degrading VacA via autophagy. Additionally, we have developed a method to solubilize lipid-inserted VacA into detergent micelles in order to determine a high-resolution structure of the VacA pore. Altogether, our goal is to build a complete understanding of VacA toxicity using a combination of imaging, cell-based assays, and cryo-electron microscopy.



The Cellular Response to VacA Intoxication

# Investigating the mechanisms of homeostatic cell density control in mammalian epithelia

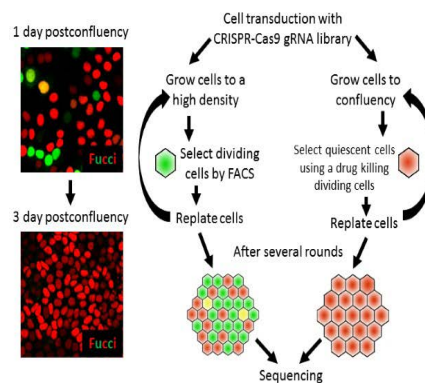
37

Maria Fomicheva, Ian G. Macara

Epithelial cells proliferate during morphogenesis and upon tissue stretching or wounding, but they stop dividing when they reach a certain density. This homeostatic mechanism differs from contact inhibition, since within the epithelial sheet cells are always in contact with one another, but proliferate only up until a pre-set density is achieved. This mechanism prevents tissue overgrowth and is lost in cancer. However, it remains poorly understood.

To find novel regulators of homeostatic cell density, we performed a genome-wide CRISPR-Cas9 gRNA knockout screen on the Eph4 mammary epithelial cell line. These cells arrest efficiently 4 days after reaching confluence. As a selection mechanism, an Eph4 line was established that expresses Fucci, which labels cells in G0-G1 with mCherry (red) and cells in G2-Mitosis with mCitrine (green). With Fucci, cycling cells can be separated from non-cycling cells by FACS. As a test, we showed that sorting a mixture of control Fucci cells and Fucci cells depleted of the CDK inhibitor p27 resulted in enrichment of p27-depleted cells.

To identify genes that are required for density-dependent arrest, we transduced the cells with the GeCKO v2.0 CRISPR-Cas9 whole genome knockout library, and selected by FACS for cells that continue to cycle (green) at high density (after 4 days). Repeated selection enriched for gRNAs that target genes essential for cell cycle control and homeostatic density control. After each round of sorting we isolated genomic DNA and performed deep sequencing of the integrated gRNA cassettes. We utilized MAGeCK software to map gRNA reads to the corresponding genes and identify the list of genes that have been enriched compared to the unsorted control. We found that the top enriched gene is NF2, a known tumor suppressor gene and regulator of Hippo signaling. We aim to validate the role of other enriched candidate genes in cell density establishment using individual siRNAs and CRISPR gRNAs. These studies will disclose novel regulators of homeostatic growth control, which is crucial for our understanding of normal epithelium maintenance and cancer prevention.



Genome-scale CRISPR-Cas9 gRNA screen strategy.

## Targeting the MYC–WDR5 Nexus in Colorectal Cancer

39

Audra M. Foshage, Lance R. Thomas, Erin R. Aho, Shaun R. Stauffer, Shelly L. Lorey, Stephen W. Fesik, and William P. Tansey

The over-expression of c-MYC is one of the most prominent alterations in colorectal cancer (CRC), featuring in roughly 70% of colorectal adenocarcinomas. Induction of the MYC oncoprotein is a critical event in the development and progression of CRC, as it causes a variety of biological responses, including cell cycle progression, cellular growth, and differentiation. Despite the pervasive involvement of MYC in CRC, and a wealth of studies in other systems demonstrating that genetic inhibition of MYC promotes frank tumor regression, no drug-like molecules have been discovered that are capable of blocking MYC function in cancer cells.

Recently, however, we devised a novel approach to target MYC, based on our observations that binding of MYC to its target genes in the context of chromatin is dependent on interaction with the chromatin-scaffolding protein WDR5. We have proposed 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 showed that interaction with WDR5 is necessary for MYC to function as a transcription factor and oncoprotein. Importantly, structural analysis revealed that MYC directly binds WDR5 by engaging a shallow, hydrophobic, cleft on the surface of WDR5 that is well-suited for drug discovery. The tractability of the MYC–WDR5 interface as a vehicle for drug discovery should make it possible to identify drug-like molecules that disrupt interaction of MYC with chromatin and diminish its tumorigenic potential.

Since the overexpression of MYC is featured in the majority of colorectal adenomas, we will investigate the requirement of the MYC–WDR5 interaction in colorectal carcinoma cell lines and in mice. An inducible mutant version of MYC will be utilized to determine the effects of loss of the MYC–WDR5 interaction in CRC cell lines and in mice with adenomatous polyps. These experiments will further elucidate the biological basis of this interaction, and establish a baseline for effects we should see from on-target small-molecule inhibitors, as we are also collaborating with the Fesik laboratory to identify, refine, and validate drug-like molecules that disrupt the MYC–WDR5 interaction. Through cellular, molecular, and genomic comparison of the effects of genetic versus chemical disruption of the MYC–WDR5 interaction, we will validate on-target action of probe compounds, providing critical information needed to select candidate molecules that will be refined for drug-like properties. Successful completion of this work will generate novel insights into MYC biology, its role in CRC, as well as first-in-class MYC–WDR5 inhibitors validated for their efficacy in CRC.

# The Role of Golgi Redistribution in Directional Cell Migration

41

Keyada Frye, Maria Fomicheva, Alexey Khodjakov, Xiaodong Zhu, and Irina Kaverina

The mammalian Golgi complex serves as a major hub for trafficking within the cell. It undergoes extensive reorganization throughout the cell cycle. One aspect of this reorganization is the process whereby the Golgi is moved from its pericentrosomal location to completely around the nucleus, which we refer to as “Golgi redistribution.” Microtubules (MTs) are highly dynamic cytoskeletal polymers that have roles such as building the mitotic spindle during cell division, serving as highways for intracellular trafficking of organelles and other cargos, and maintaining the structure and function of the Golgi complex. Preliminary data where microtubules densely encircle the nucleus when the Golgi is redistributed suggest microtubules facilitate Golgi redistribution.

We seek to elucidate the mechanism whereby Golgi redistribution is facilitated, namely by determining which microtubule tracks and molecular motors are involved. The current study presents evidence that Golgi redistribution is facilitated by stable detyrosinated microtubule (glu-microtubule) tracks that concentrate at the nucleus. We show that treatment with the drug parthenolide, which blocks detyrosination by inhibiting tubulin carboxypeptidase, results in decreased Golgi redistribution. The plus end directed motor kinesin-1 has been documented to preferentially bind detyrosinated MTs. By overexpressing the headless domain of Kin1B, pHA- $\Delta$ N332-Kin1B, which blocks kinesin activity in a dominant negative manner, we also observe decreased Golgi redistribution. These data suggest Kin1B serves to power this process.

It has been widely documented that the Golgi complex’s position at the leading edge provides directional cell migration throughout interphase. As the cell cycle progresses and the Golgi redistribution process advances, the Golgi becomes symmetric around the nucleus. At the same time, we see a decrease in cell velocity in G2 cells as compared to the velocity of G1 cells. This velocity change correlates with the time frame of Golgi redistribution.

The work presented here provides a potential mechanism and function of the Golgi redistribution process.

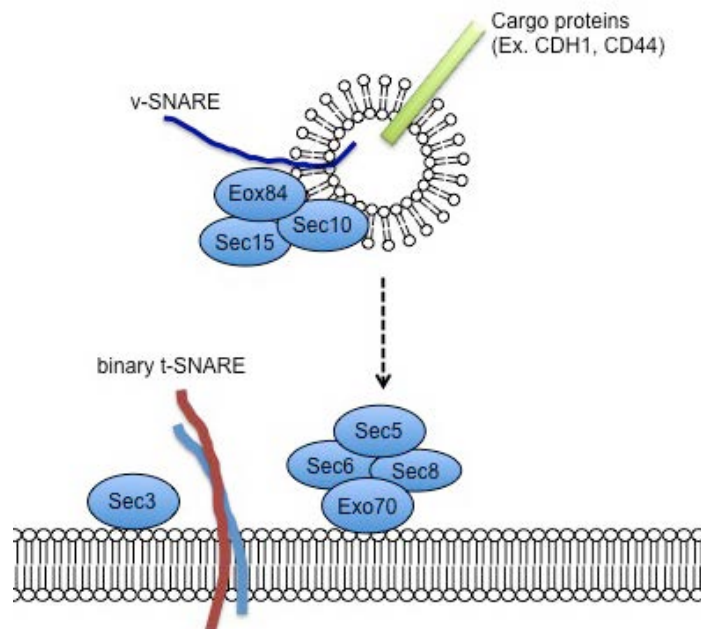


# Live cell imaging of exocyst subunits and their dynamics

Hisayo Nishida-Fukuda and Ian G. Macara

Exocytosis is a mechanism by which secretory vesicles are carried to the plasma membrane and their contents are secreted into the extracellular environment in a wide variety of physiological processes such as morphogenesis, cell cycle progression, primary ciliogenesis, cell migration and tumor invasion. The exocyst is an octameric protein complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that plays an essential role in tethering secretory vesicles to the plasma membrane. Previous studies in yeast showed that exocyst complex is intrinsically stable and contains all eight exocyst subunits with equal stoichiometry. It is therefore considered that the assembly of the all eight subunits would be required for the vesicle tethering in yeast. By contrast little is known about how subunits assemble and form the exocyst complex in mammalian cells. To examine the spatiotemporal regulation of exocyst subunits, we knocked-in a green fluorescent protein (sfGFP) into the loci of exocyst subunit genes in a mouse mammary epithelial cell line, NMuMG, by CRISPR/Cas9 system. The localization of each exocyst subunit-sfGFP fusion protein was compared with transferrin receptor-pH-sensitive red fluorescent protein (TfRpHuji), which is a marker of vesicle fusion sites at the plasma membrane. TIRF microscopic observation revealed that Exo70-sfGFP arrives at the plasma membrane 30 seconds before vesicle fusion and continues to stay at the fusion sites after the vesicle opening. On the other hand, Sec3-sfGFP localizes at the plasma membrane 30 seconds before the vesicle fusion, but it does not co-localize with TfRpHuji. These results suggest that, in mammalian cells, different exocyst subunits participate in discrete processes during multistep vesicle tethering in a sequential manner, rather than all the subunits acting as a stable octameric complex. In addition, our sfGFP and mScarlet knock-in cell lines will be valuable tools for imaging the formation of exocyst complexes, and the process of exocytosis

Predicted model of vesicle tethering by exocyst subunits.



# NFAT-dependency of IL-1 $\beta$ -induced behaviors in human retinal microvascular endothelium and Müller cells

45

Giblin, Meredith J.; Capozzi, Megan E.; McCollum, Gary W.; Penn, John S.

Early diabetic retinopathy (DR) involves chronic low-grade inflammation, characterized by elevated vitreous cytokines, such as IL-1 $\beta$ . This inflammation promotes many pathologic consequences, including the hallmark vascular changes for which DR is best known. Nuclear factor of activated T cells (NFAT) is involved in the regulation of inflammatory mediators, extracellular matrix proteins, and adhesion molecules and thus may control multiple pathogenic steps early in DR. We have examined NFAT's role in IL-1 $\beta$  auto-amplification and explored the NFAT-dependency of endothelial cell responses to IL-1 $\beta$ , including monolayer permeability and expression of targets involved in leukocyte adhesion and basement membrane thickening.

For IL-1 $\beta$  production, human Müller cells (HMC) were treated with 50pg/mL IL-1 $\beta$ , Inhibitor of NFAT-Calcineurin Association-6 (INCA, 2.5 $\mu$ M) and proper vehicles for 8 hrs before collection for qRT-PCR. For ICAM expression, human retinal microvascular endothelial cells (HRMEC) were treated with 1ng/mL IL-1 $\beta$  and 2.5 $\mu$ M INCA for 2 hrs. For collagen IV expression, HRMEC were treated with 10ng/mL IL-1 $\beta$  and 1 $\mu$ M INCA for 48 hrs. In permeability experiments, HRMEC were pre-treated with vehicle or 2.5 $\mu$ M INCA for 16 hrs before 24 hrs of exposure to 0.5ng/mL IL-1 $\beta$  with/out INCA. Transendothelial electrical resistance values were measured using the EVom2.

IL-1 $\beta$  increased HMC IL-1 $\beta$  expression by 82-fold ( $p < 0.01$ ); INCA inhibited this induction by 28% ( $p < 0.05$ ). HRMEC treatment with IL-1 $\beta$  caused a 328-fold induction of ICAM expression ( $p < 0.01$ ); INCA inhibited this induction by 20% ( $p < 0.01$ ). HRMEC treatment with IL-1 $\beta$  caused a 2-fold induction of collagen IV expression ( $p < 0.01$ ); INCA inhibited this induction by 50% ( $p < 0.01$ ). Treatment with IL-1 $\beta$  decreased HRMEC monolayer resistance by 25% (measured vs resistance at time=0,  $p < 0.01$ ), which was partially rescued (44%,  $p < 0.01$ ) by INCA treatment.

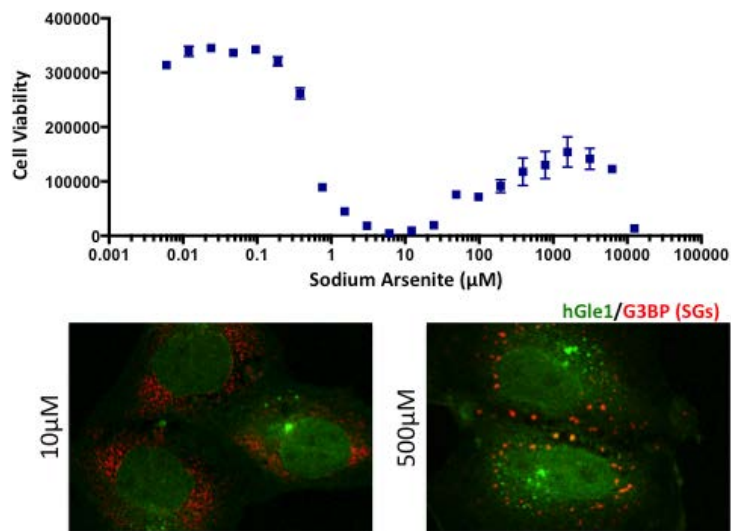
These data demonstrate the potential of NFAT as a multi-targeted therapy for retinal inflammation secondary to diabetes. NFAT inhibition not only prevented HMC IL-1 $\beta$  auto-amplification, but also inhibited the pathogenic response of HRMEC to IL-1 $\beta$ , including the expression of adhesion proteins, excessive extracellular matrix deposition and increased permeability. Future work will investigate the therapeutic potential of *in vivo* NFAT inhibition.

# GLE1-mediated stress granule formation provides a survival advantage to tumor cells

47

Laura Glass, Aditi, Susan R. Wentz

Human (h)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, generating 3 different isoforms: hGle1A, hGle1B and hGle1C, each with distinct functions. hGle1 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 this survival mechanism is mediated by stress granule (SG) formation during stress. This allows cells to evade apoptosis and survive. We recently demonstrated that hGle1A specifically mediates and supports SG function, whereas hGle1B does not. These data led us to question whether overexpression of hGle1A modulates SG biology to provide a survival advantage to tumor cells during drug treatment. Since conventional anti-cancer chemotoxic agents induce stress granule formation, we examined the long-term viability of cells exposed to multiple drugs that induce stress granules in HeLa cells. We discovered that induction of stress granule formation within 1 hour post-treatment by high doses of sodium arsenite (NaArs) produced enhanced cell viability at 72 hours of treatment. In contrast, treatment with a lower dose of NaArs, which did not induce SG formation, resulted in complete loss of viability. Importantly, we further demonstrated that knockdown of hGle1 by siRNA diminished this protective effect at the high dose, and that hGle1A specifically can rescue this survival defect. These preliminary data provide initial evidence that hGle1A-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.



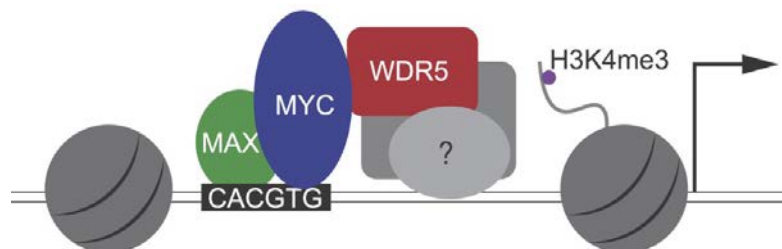
**Dose-response curve of HeLa cells with sodium arsenite after 72 hours of treatment. Doses that induce stress granule formation are associated with cell survival.**

## MYC and WDR5 in cancer

49

Alissa duPuy Guarnaccia and William P. Tansey, PhD

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. Histone modifications, DNA sequence, and interactions with other transcription factors have all been suggested to influence where MYC binds chromatin, but a consistent signature has not been defined. Our laboratory recently discovered that the chromatin regulatory protein WDR5—a core component of histone methyltransferase complexes—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, in a process we refer to as “facilitated recruitment.” In the facilitated recruitment model, the presence of WDR5 at chromatin promotes MYC binding at certain genomic loci over others. The role of WDR5 in target gene selection by MYC can explain much of the plasticity in genome-wide binding patterns of MYC that have been reported, and may provide a new avenue for therapeutically targeting MYC in cancer. 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 answer these questions, we are employing traditional and quantitative proteomics, together with biochemical approaches, to characterize the composition and stoichiometry of the WDR5-containing complex that associates with MYC. These ongoing studies demonstrate that this complex is devoid of canonical WDR5-interaction partners such as RBBP5 and HCF-1, revealing that the function of WDR5 in this setting is distinct from its well-characterized roles in histone modifications. We propose that the WDR5 complex that associates with MYC on chromatin is either entirely novel, or is a ‘ghost’ complex in which select protein components have been excluded by the direct interaction of MYC with WDR5. We continue to analyze and define the molecular details of the MYC–WDR5 complex using a combination of genetic, biochemical, and genomic approaches.



The Facilitated Recruitment Model proposes that a chromatin-bound WDR5-containing complex is required for MYC target-gene selection. I seek to define this complex.

*Abstract Withdrawn*

# Kibra and WWC2 in Hippo Signaling and Epithelial Growth Control

53

Amanda L. Halfond and Ian G. Macara, PhD.

Homeostatic control of epithelial cell density is essential to normal tissue morphogenesis, and defects in this process are important in cancer. Homeostatic growth is regulated by the cell polarity machinery and by Hippo signaling. Several proteins mediate cross-talk between apical-basal polarity complexes and the Hippo pathway, including the WW domain-containing proteins (WWC1/Kibra and WWC2). These are closely-related cytoplasmic scaffolding proteins that bind to atypical protein kinase C (aPKC) and the tumor suppressor NF2 (Merlin). We have silenced expression of Kibra and WWC2 in mammary epithelial Eph4 cells, using lentiviral RNAi, and found that aPKC – which is necessary for normal cell polarization - becomes mislocalized away from the tight junctions and apical surface. In addition, the tight junctions are severely disrupted and cell size is increased. We have also used CRISP/Cas9 gene editing to create mammary Eph4 epithelial cells deleted for either Kibra or WWC2, or both genes. These lines will be validated by genomic PCR and sequencing. Preliminary data suggests that they grow much more slowly than WT cells. We will determine whether we can rescue normal behavior by re-expression of WT Kibra or WWC2, or of mutants lacking the aPKC binding site, NF2 binding site, or other key domains in the proteins.

In *Drosophila*, Kibra binds to Merlin/NF2 to activate the Hippo pathway, which will suppress cell proliferation. Our preliminary data indicate that the opposite occurs in mammary epithelial cells, since loss of Kibra suppresses cell proliferation. This is consistent with other work suggesting that Kibra is over-expressed in some cancers, and can promote cell proliferation in prostate cancer. It will be important to dissect the roles of Kibra, aPKC and NF2 in growth control and to determine whether the regulation of Hippo signaling is the primary function for Kibra and WWC2 in mammalian epithelia.

# UBA-containing kinases of the AMPK family phosphorylate ubiquitin at the Ser57 residue

55

Nathaniel L. Hepowit and Jason A. MacGurn

Evolutionarily conserved from yeast to human, the AMPK (AMP-activated protein kinase) family of serine/threonine kinases is involved in diverse cellular processes such as metabolic sensing, cell polarity, microtubule stability, vesicular trafficking and cell cycle regulation. Distinct to some members of the AMPK kinases is the presence of ubiquitin-associated domain (UBA); however, it remains unclear whether these kinases directly mediate ubiquitin (Ub) phosphorylation or in sensing ubiquitylated proteins. Here, we identify the UBA-containing KIN1-2, GIN4, KCC4, SKS1, VHS1 and Ypl150w in yeast and their human homologs, MARK1-4, QIK and SIK, as the AMPK kinases that can phosphorylate monomeric and polymeric ubiquitin at the Ser57 residue. To facilitate the discovery on the functional roles of Ser57 phosphomodification, we use Ub phosphomimicry and phosphoinhibitory mutants in the budding yeast model system. Interestingly, cells that express only the Ser57-phosphomimetic variant of Ub have increased tolerance to heat stress, stimulated endocytic trafficking of plasma membrane proteins, and increased utilization of unconjugated Ub in response to oxidative stress. Overall, our results suggest that AMPK-mediated phosphorylation of Ub is potential to dramatically alter the ubiquitin code and its downstream signaling outcomes.

## Dissecting the molecular function of USP24

57

Susan Histed, Charles Williams, Charles Hong, Jason MacGurn

The ubiquitin system is an important player in protein stability and trafficking within eukaryotic cells. Ubiquitylation is therefore involved in regulation of many pathways in the cell, one of which is the canonical Wnt signaling pathway. There are many established regulatory roles for the ubiquitin system in Wnt signaling, such as downregulation of the Wnt co-receptors due to ubiquitylation by E3 ligases RNF43/ZNRF3 or degradation of beta-catenin due to ubiquitylation by the  $\beta$ -TRCP E3 ligase and subsequent proteasomal degradation. The MacGurn lab has found that siRNA knock down of the deubiquitylase (DUB) Usp24 causes a hyperactivation of Wnt signaling, suggesting the DUB serves as an attenuator of the pathway. Morpholino studies in zebrafish have shown that knock down of Usp24 in zebrafish embryos leads to developmental defects, including heart edemas and underdeveloped forebrain and upper jaw. My aim is to further investigate the mechanism of Usp24 attenuation of the Wnt pathway in vitro as well as its regulatory role during zebrafish development.



## Establishing a model of BM damage and analyzing its repair

59

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 system *in vivo*, as it can grow, shrink, and repair. We want to develop a system to analyze basement membrane repair in adult animals. In both mammals and flies, the gut appears to be a relatively dynamic basement membrane suggesting it could be a good system for analyzing repair.

We analyze basement membrane repair using an adult gut injury model. 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 gut, flies are fed Dextran Sodium Sulfate (DSS); DSS administration has been previously used as a model for ulcerative colitis in mice although the etiology is unknown. In *Drosophila*, DSS induces morphological changes consistent with basement membrane damage. Using both electron and structured-illumination microscopy, we observe an increase in BM thickness after DSS feeding. Furthermore, DSS becomes lodged in the gut BM, which alters the mechanical strength of the tissue. Interestingly, inhibiting or knocking down a collagen-IV crosslinking enzyme, peroxidasin, mimics the tissue changes seen in response to DSS. In addition, peroxidasin is required for the repair of basement membrane upon damage with DSS. We plan to knock down multiple basement membrane components after flies reach adulthood to test their role in repair.

# EGFR-Activated Notch Signaling Contributes to Pathogenesis of Ménétrier's Disease

61

Won Jae Huh and Robert J. Coffey

Ménétrier's disease is a rare acquired protein-losing hypertrophic gastropathy characterized by giant gastric rugal folds, decreased acid secretion, increased gastric mucus production, and hypoalbuminemia due to protein loss in the gastric mucosa. Microscopically, it is characterized by massive foveolar hyperplasia, oxyntic gland atrophy, and repatterning of cell specification. We previously reported transgenic mice overexpressing the EGF receptor (EGFR) ligand, TGF- $\alpha$ , in the stomach phenocopy Ménétrier's disease, that TGF- $\alpha$  is overexpressed in the stomach of Ménétrier's disease patients, and that the EGFR neutralizing monoclonal antibody (mAb), cetuximab, is the first effective medical therapy for Ménétrier's disease. In a proteomic analysis of gastric tissue of Ménétrier's disease patients before and after cetuximab treatment, we observed decreased levels of the Notch ligand, Jagged1, after cetuximab treatment. The aim of this study was to examine whether Notch signaling is a downstream target of EGFR signaling and whether it contributes to the pathogenesis of Ménétrier's disease.

We tested whether Notch signaling is activated in the stomach of TGF- $\alpha$  transgenic mice and whether Notch activation is reduced by MM-151, a cocktail of EGFR mAbs that blocks mouse EGFR, and/ or dibenzazepine (DBZ), a  $\gamma$ -secretase inhibitor, as determined by Hes-1 immunofluorescent staining. We also examined the level of foveolar hyperplasia and proliferation, and the number of parietal and chief cells in the gastric mucosa of these mice using markers: UEA1 (foveolar pit cell), Ki-67 (proliferation), H<sup>+</sup>/K<sup>+</sup> ATPase (parietal cell), and gastric intrinsic factor (chief cell).

Nuclear Hes-1 expression was upregulated in the pit, isthmus, and neck compartments in the stomach of TGF- $\alpha$  transgenic mice compared to wild-type mice. Number of nuclear Hes-1 positive cells were decreased by MM-151 and/ or DBZ treatment in TGF- $\alpha$  transgenic mice. Also, MM-151 and/ or DBZ treatment led to decreased foveolar hyperplasia and proliferation, and increased numbers of parietal and chief cells in the gastric mucosa of TGF- $\alpha$  transgenic mice.

These results show that Notch signaling is activated in TGF- $\alpha$  transgenic mice and activated Notch signaling contributes, at least in part, to the pathogenesis of Ménétrier's disease. Moreover, these findings suggest that blockade of Notch signaling may be a novel therapeutic strategy for Ménétrier's disease.

## STK38 is a novel Wnt agonist

63

Annastasia. S. Hyde, Lori Chiu, Rubin Baskir, Emily Crispi, Emilios Tahinci, and Ethan Lee

Serine threonine kinase 38 (STK38) is involved in apoptosis, cell cycle progression, and the Hippo signaling pathway. A high-throughput screen performed in our lab identified STK38 as a potential Wnt agonist. Injection of *STK38* mRNA in *Xenopus laevis* embryos resulted in axis duplication, indicative of active Wnt signaling. Overexpression of STK38 in a Wnt reporter cell line, HEK293STF, increased TOPflash activity and beta-catenin protein levels as compared to vector control in the presence of Wnt. These results suggest a synergistic effect for STK38 in the presence of Wnt ligand. Previously, it was shown that GSK3 phosphorylates STK38 at Ser6 and Thr7 to inhibit its activity. Double alanine and glutamate mutations were made at these sites to make a constitutively active form of STK38, as well as a phosphomimetic (constitutively inhibited) form, respectively. Overexpression of STK38<sup>S6T7A</sup> mutants resulted in increased TOPflash activity as compared to that of wildtype STK38, and STK38<sup>S6T7E</sup> mutants had markedly decreased TOPflash activity as compared to that of wildtype STK38. Injection of the phosphomutants into *Xenopus* embryos also resulted in a similar pattern, where STK38<sup>S6T7A</sup> had more duplications and STK38<sup>S6T7E</sup> had less duplications than injection of wildtype STK38. This evidence suggests that GSK3 phosphorylation of STK38 may affect STK38 activity in the Wnt signaling pathway. Localization of STK38 is nuclear, and deletion of the nuclear localization sequence (NLS) does not seem to affect its activity. However, STK38 immunoprecipitates with nuclear factors, such as TLE3 and Lef1, indicating its potential role in regulation of the Wnt pathway at the transcriptional level. Our results demonstrate that STK38 is a novel positive regulator of the Wnt signaling pathway and may possibly aid in the development of potential therapeutic targets to treat Wnt-driven cancers such as colorectal and breast cancers.

# Whole-tissue clearing and light-sheet microscope high-resolution/content analysis of juvenile human pancreas

65

Brenda Jarvis, Jeff Duryea, Matthew E. Bechard and Chris Wright

The human juvenile period (0-5 years of age) is an incredibly important developmental period in which the pancreas exhibits much plasticity and maturation in its global tissue architecture and endocrine (insulin-producing) beta-cell physiological function. Recent studies demonstrate that  $\beta$ -cell-directed autoimmunity appearing within this juvenile period is incredibly predictive of severe and early-onset type 1 diabetes (T1D) in genetically susceptible individuals. Pancreas samples have traditionally been collected in a way that prevents studies beyond relatively simple, low-content morphological examination and immunodetection of islet hormones and cell markers. We are applying advanced imaging techniques to produce a high-resolution atlas, at the cellular and organ-wide level (a scalable “Google-Earth” view), of the differentiation process and molecular signatures of islets and endocrine  $\beta$ -cells during the developmentally plastic juvenile period. We will present our progress with a passive CLARITY technique (PACT), in combination with passive immunolabeling techniques (PIT), to generate cleared, large blocks of human pancreatic tissue with preserved architecture and antigen reactivity. Using PACT-PIT is producing high-resolution organ and cellular-level analysis of islet architecture, the developing vasculature, the level of innervation, and the degree of infiltration by specific components of the immune system.

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

67

Sun Wook Kim, Jonathan Ehrman, Mok-Ryeon Ahn, Jumpei Kondo, Scott W. Crawley, James R. Goldenring, Matthew J. Tyska, Erin C. Rericha, Ken S. Lau

The flow of fluid through the gut, such as milk from a neonatal diet, generates a shear stress on the epithelial cells lining the lumen. 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 and how intestinal epithelial cells respond to shear stress is unknown.

We hypothesized that intestinal epithelial cells respond to shear stress like other epithelial cell types. We observed that exposure to fluid shear stress causes the formation of large vacuoles, containing extracellular content, within intestinal epithelial 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. We found that, structurally, intestinal epithelial microvilli play a role to sense the extracellular shear stress and to alter intracellular fluid exchange. To characterize the mechanism of shear stress-induced vacuole formation, downstream signaling pathway candidates, such as endocytosis and autophagy, were screened. Finally, we identified the autophagic flux as the intracellular signaling downstream of this response that regulates large vacuole formation. These shear stress-induced vacuoles were surrounded by autophagy markers, microtubule associated protein light chain 3 (MAP LC3) and Lamp1. In addition, loss-of-function studies by pharmaceutical inhibition and genetic modulation support our observation that shear stress-induced vacuole formation is mediated through the autophagy pathway.

To conclude, our results discovered the actin-rich microvilli as mechanosensors and the novel link among intestinal microvilli, the macroscopic transport of fluids across cells, and the autophagy signaling 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.

# Actin-based recruitment of the Chromosomal Passenger Complex to the division site during cytokinesis

69

Jennifer Landino, Stephen Norris, Ryoma Ohi

The events of mitotic exit are tightly choreographed to ensure that a replicated genome is accurately segregated. The chromosomal passenger complex (CPC) is crucial for orchestrating mitotic exit as it organizes the central spindle, specifies the division site and promotes cleavage furrow ingression. The sub-cellular localization of the CPC is tightly coupled to its mitotic functions. At the metaphase-to-anaphase transition, the CPC leaves the centromere and localizes prominently to midzone microtubule plus ends and the equatorial cortex. The transport of the CPC from the centromeres to the cell middle is thought to depend on microtubule plus-end directed transport by the kinesin Mklp2 (Kif20a). Depletion of Mklp2 causes the CPC to remain associated with chromosomes throughout mitotic exit, and these cells fail to complete cleavage furrow ingression. Our previous work indicates that the CPC interacts with actin at the equatorial cortex, through its scaffolding protein INCENP. We observed that disrupting the INCENP-actin interaction also disrupts the localization of the CPC at the division plane. We show here that actin-binding is required to fully recruit the CPC to the equatorial cortex, in combination with Mklp2-dependent transport. Actin-binding can also promote cortical enrichment in the absence of Mklp2 and this recruitment pathway is sufficient to rescue cleavage furrow ingression in Mklp2-depleted cells. By co-imaging the CPC and Mklp2 in live cells, we find that the two proteins remain associated throughout mitotic exit. Surprisingly, the Mklp2-CPC complex diffuses on the midzone and the cell cortex, suggesting that motor activity does not define the bulk of the dynamic behavior we observe during anaphase. The cortical movement of Mklp2-CPC requires F-actin and microtubules, demonstrating that these cytoskeletal networks cooperate to promote both recruitment to and diffusion along the cell cortex. Collectively, our work suggests that both actin-binding and Mklp2-dependent transport along microtubules cooperate to precisely position the CPC during mitotic exit, and that these pathways converge to ensure successful cleavage furrow ingression.

## **Interaction of Phosphorylated Rab11-FIP2 with Eps15 Regulates Apical Junction Composition**

71

Lynne A. Lapierre, Elizabeth H. Manning, Kenya M. Mitchell, Cathy M. Caldwell, and James R. Goldenring

MARK2 regulates the establishment of polarity in MDCK cells in part through phosphorylation of serine 227 of Rab11-FIP2. We have now identified Eps15 as an interacting partner of phospho-S227-Rab11-FIP2 (pS227-FIP2). During recovery from low calcium, Eps15 localized to the lateral membrane, prior to pS227-FIP2's arrival. Later in recovery, Eps15 and pS227-FIP2 co-localized at the lateral membrane. In MDCK cells expressing the pseudophosphorylated FIP2 mutant, FIP2(S227E), during recovery from low calcium, Eps15 was trapped and never localized to the lateral membrane. Mutation of any of the three NPF domains within GFP-FIP2(S227E) allowed Eps15 to localize at the lateral membrane. While expression of GFP-FIP2(S227E) induced the loss of E-cadherin and occludin, mutation of any of the NPF domains of GFP-FIP2(S227E) reestablished both proteins at the apical junctions. Knockdown of Eps15 altered the spatial and temporal localization of pS227-FIP2. Thus, an interaction of Eps15 and pS227-FIP2 at the appropriate time and location in polarizing cells is necessary for proper establishment of epithelial polarity.

# Determination of s-SHIP expressing cells fate in mammary gland by lineage tracing

73

Armelle Le Guelte & Ian Macara

The murine mammary gland is a fascinating and powerful model for the study of adult stem cells. Complex morpho-genetic changes occur in the mammary epithelium during puberty, pregnancy, lactation and involution. Remarkably, one mammary stem cell (MaSC) is able to reconstitute an entire mammary gland, but the identity of the stem cell has remained uncertain because of the absence of validated stem cell markers. However, the transgenic mouse s-SHIP-GFP expresses GFP specifically in a small population of cells in the terminal end buds of growing mammary glands, called cap cells. These cells can function as multipotent mammary stem cells when transplanted into a recipient mouse. A major unanswered question is whether mammary stem cells generate diverse progeny by asymmetric cell division, or through stochastic population asymmetry. Recent lineage-tracing experiments that used non stem cells markers such as Cyto-keratin 14 and 5 or a new MaSC Protein C receptor (ProcR) came to different conclusions about the potency of MaSCs. Here we show that tissue sections of the s-SHIP-GFP mammary glands have equally parallel and perpendicular divisions of the GFP+ cap cells in terminal end buds. We also generated the lineage tracing s-SHIP-MerCreMer-tdTomato mice. Unexpectedly, our in vivo data show that s-SHIP+ cells give rise to only myoepithelial cell layer during puberty and pregnancy. However, cells isolated from these mice and cultivated in vitro can give rise to both myoepithelial and luminal cells populations. Moreover, specific ablation of s-SHIP+ cap cells by DTA expression block the formation of mammary ducts during puberty. Therefore, these cells do not normally function as multipotent stem cells in situ, but are essential for mammary gland development and can be reprogrammed to express the stem cell fate when isolated from mice and either grown in vitro or transplanted into recipient mice. Further experiments need to be done to determinate the role of s-SHIP+ cells in the development of the mammary gland.



# Ppz phosphatases regulate endocytic trafficking in yeast

75

Lee S, MacGurn JA.

Endocytic trafficking is one of the primary mechanisms for translocation of membrane proteins, and is essential to regulate biological activity of various cargoes. The endocytic pathway of mammalian cells internalizes molecules from the plasma membrane and recycle them back to the surface, or sort them to degradation. Such endocytic downregulation is critical to human health and disease. Thus, understanding how cells regulate the abundance and activity of cell surface proteins is key to deciphering cellular strategies for balancing healthy growth of cells. Ppz is a serine/threonine protein phosphatase complex that shows in its carboxyl-terminal half about 60% identity with the catalytic subunit of mammalian and yeast protein phosphatase type I and that is involved in a variety of cell processes, including maintenance of cell integrity, in connection with the Pkc1/Mpk1 mitogen-activated protein kinase pathway, regulation of salt tolerance, and regulation of cell cycle at the G<sub>1</sub>/S transition. Yet, this phosphatase remains poorly characterized in its role in regulation of endocytic trafficking. Here, measuring the ratio of GFP signals of various cargoes expressing PM vs vacuole, we found that Ppz phosphatase deletion mutants have broad but partial endocytic trafficking defects. We also found that their catalytic activity and myristoylation are required for the trafficking and plasma membrane localization. These data indicate the regulatory roles of Ppz phosphatases for cargoes such as Mup1, Can1, and Lyp1. Taken together, this study provides valuable mechanistic insights into how Ppz phosphatases play distinct regulatory roles in membrane trafficking.

*Abstract Withdrawn*

# The Role of Self-Association in Gle1 Function

79

Aaron C. Mason, Aditi, and Susan R. Wentz

Gle1 is a conserved multidomain protein that functions in multiple aspects of gene expression including nuclear mRNA export, translation initiation, and translation termination. Genetic changes to Gle1 are linked to lethal congenital contracture syndrome-1 (LCCS1), lethal arthrogyrosis with anterior horn cell disease (LAAHD), and amyotrophic lateral sclerosis (ALS). A GLE1 mutation (*Fin<sub>major</sub>*), linked to LCCS1, perturbs the self-association of Gle1 *in vitro* and exhibits reduced activity *in vivo*. In this study, we expand efforts to understand how Gle1 self-association contributes to the multiple activities of Gle1.

Gle1 has been shown to form at least a homodimer in living cells and higher ordered homomultimers *in vitro*. The coiled-coil domain is essential for this self-association and Gle1 function. Current biochemical studies using the yeast homologue are aimed at determining which sub-domains mediate self-association and the structural details of the interaction. We have identified a region of the coiled-coil domain that is a dimer in solution, as assessed by size exclusion chromatography, and crystallization trials are underway. It is also unknown whether the three distinct hGle1 isoforms (hGle1A, hGle1B and hGle1C) oligomerize discretely or in heteromeric fashion. Independent functions and localization patterns have been well defined for hGle1A and hGle1B, with the essential mRNA export function at the NPC supported by hGle1B whereas hGle1A specifically regulates the pool of translationally active mRNPs in the cytoplasm. Using quantitative FRAP to probe homo- and heteromeric- Gle1 isoform (A, B, and C) interactions within each subcellular compartment, we seek to determine how oligomerization might regulate these distinct hGle1 functions. Lastly, the structural context of Gle1 function is also likely altered by phosphorylation events near the coiled-coil domain that occur in response to cellular stress. We have examined the structural consequences of phosphorylation on self-association using electron microscopy and also quantified a significant reduction in ATPase activation by a phosphomimetic Gle1 upon DDX3, the RNA-dependent DEAD-box ATPase involved in translation initiation and stress granule biology. Overall, our studies highlight the importance of understanding the self-association of Gle1 and begin to establish how it influences the activity of Gle1 in various cellular processes.

## Poster Session II (EVEN NUMBERS)

Poster #	First Name	Last Name	Poster #	First Name	Last Name
2	Karrie	Dudek	54	Claire	Strothman
4	Christine	Jones	56	Lakshmi	Sundararajan
6	Elizabeth	Lawrence	58	Roslin	Thoppil
8	Caitlin	McAtee	60	Kathryn	Trogden
10	Heather	McCartney	62	Erica	Tross
12	Rebecca	McWhirter	64	Jessica	Tumolo
14	Megan	Merolla	66	Gokhan	Unlu
16	Anne	Meyer	68	Indrayani	Waghmare
18	Leif	Neitzel	70	Abstract Withdrawn	
20	Victoria	Ng	72	Marcin	Wos
22	Barbara	O'Brien	74	Xiao-Dun	Yang
24	Natalya	Ortolano	76	Michael	Yarboro
26	Sierra	Palumbos	78	Xiaodong	Zhu
28	Kyungho	Park	80	Jing	Zou
30	Meagan	Postema			
32	Will	Ramos-Lewis			
34	Elaine	Ritter			
36	Leshana	Saint-Jean			
38	Kenyi	Saito-Diaz			
40	Christi	Salisbury-Ruf			
42	Anneke	Sanders			
44	Shinya	Sato			
46	Cherie	Scurrah			
48	Lindsey	Seldin			
50	Manisha	Sharma			
52	Joey	Simmons			

# Differential effects of transcription factor deletions on endocrine cell differentiation

2

Karrie D. Dudek, Anna Osipovich, Jacob Coeur, Jean-Philippe Cartailier, Guoqiang Gu and Mark A. Magnuson

Pancreatic endocrine cell identity is determined by a gene regulatory network (GRN) that is established in a step-wise manner during pancreatic organogenesis. Pancreatic endocrine cells arise from pancreatic epithelium beginning around E9.0-9.5 in the mouse, peaking around E15.5. Formation of pancreatic endocrine cells is dependent upon *Neurog3*, a helix-loop-helix transcription factor. In the absence of *Neurog3* pancreatic endocrine cells fail to form, apparently due to lack of expression of multiple downstream regulators including *Insm1*, *NeuroD1*, *Pax6*, *Isl1* and *Rfx6*, all of which have been shown to play critical roles in the formation and differentiation of endocrine cells.

Our laboratory, in collaboration with other groups, has isolated 11 different fluorescently-tagged cell populations that lie along the developmental lineage for mature  $\beta$ -cells, performed RNASeq, and derived a temporally-oriented gene correlation network (GCN) using iterative whole genome correlation network analysis (iWGCNA). This analysis, which spans from E8.0 to P60, revealed a dramatic shift in gene expression as pre-pancreatic endocrine cells give rise to pancreatic islet cells. It has also led us to identify several zinc finger protein (ZFP)-containing transcription factors that correlate strongly with the formation of endocrine cells during differentiation. However, despite having obtained over 1.5 billion mapped sequence reads, and deriving a network of over 70 million individual gene-gene correlations, only two thirds of the 15,000 genes expressed in these cell populations were categorized.

To gain a deeper understanding of the genes that contribute specifically to the formation of pancreatic endocrine cells, and to correlate modules within the GCN to specific cellular processes, we have performed additional RNASeq analysis on mice that lack either *NeuroD1* or *Ripply3*. Our findings to date indicate that disruption of these two genes impairs the gene expression profile of E15.5 pre-pancreatic endocrine cells in both distinct and similar ways.

In addition, we are developing a novel strategy for targeted gene disruption for rapid generation of gene knockouts in mice. This strategy incorporates the powerful CRISPR/Cas9 system with a highly efficient *Tol2* transposase for transgenesis. Using these platforms in tandem, we have been able to achieve gene knockouts in both a rapid and efficient manner. With this tool in hand, we plan to quickly knockout additional genes that lie downstream of *Neurog3* enabling us to assess their role in establishing the endocrine cell specific GRN. Our preliminary results from these experiments will be presented.

# Regulation of the E3 ubiquitin ligase Dma1

4

Christine M. Jones, Alyssa E. Johnson, Jun-Song Chen, and Kathleen L. Gould

Proper cell division to yield two daughter cells with identical complements of genomic material requires coordination between mitosis and cytokinesis. In the event of a mitotic error, checkpoint mechanisms must inhibit both mitotic exit and cytokinesis to ensure accurate segregation of chromosomes. In response to mitotic spindle errors, the spindle checkpoint delays cytokinesis by inhibiting the septation initiation network (SIN), a GTPase-driven signaling cascade. Specifically, the Casein Kinase I (CKI) proteins Hhp1 and Hhp2 phosphorylate the SIN scaffold protein, Sid4, recruiting the E3 ligase Dma1. Dma1 ubiquitylates Sid4, antagonizing the localization of the Polo-like kinase Plo1, and preventing phosphorylation of its downstream target Byr4. Consequently, the SIN kinase cascade and cytokinesis are delayed. Upon resolution of the mitotic spindle error, the Dma1 checkpoint signal must be withdrawn to allow continuation of the cell division cycle. However, the mechanism by which Dma1 ubiquitylation of Sid4 is stopped is not known. Furthermore, whether Dma1 itself is regulated by the checkpoint remains unclear.

Here we report that Dma1 is phosphorylated *in vivo* on seven sites, including 1 threonine and 6 serines. Preliminary data suggests that though this phosphorylation occurs throughout the cell cycle, it changes coincidentally with its activity in the checkpoint. Examination of these sites *in vitro* demonstrated that Dma1 can be phosphorylated by Cdk1, Plo1, and CK2, which each have different functions in the cell cycle. Investigation of Dma1's two known activities, Sid4 ubiquitination and Dma1 auto-ubiquitination, using phospho-ablating and phospho-mimetic mutations integrated at the endogenous *dma1* locus suggest that Dma1 is regulated by the interplay between phosphorylation and auto-ubiquitination. While all of the Dma1 phospho-mutants were catalytically active and capable of ubiquitinating Sid4, the Dma1 Cdk1-Plo1 mimetic lacked auto-ubiquitination activity.

The current data point to a model wherein Dma1 auto-ubiquitination is inhibited through phosphorylation during the checkpoint, without disrupting substrate ubiquitination. The mechanism by which this occurs is being further examined as well as the interplay between phosphorylation and auto-ubiquitination and the impact of each on Dma1's checkpoint activity.

## Rescuing Microtubules with CLASP2

6

Lawrence EJ, Kaverina I and Zanic M

Cytoplasmic Linker-Associated Proteins (CLASPs) belong to a conserved family of microtubule-associated proteins that are required for cell migration, axonal growth and cell division. In cells, CLASPs localize to microtubule tips and function to stabilize dynamically growing microtubules. However, the molecular mechanism by which CLASPs stabilize microtubules is not understood. Here, we use *in vitro* reconstitution with purified protein components and Total Internal Reflection Fluorescence (TIRF) microscopy to investigate the molecular mechanism of CLASP2-mediated microtubule stabilization. We demonstrate that CLASP2 prevents microtubule catastrophe, the switch from growth to shrinkage, and promotes rescue, the switch from shrinkage to growth, but does not affect the rates of microtubule growth or shrinkage. Strikingly, when CLASP2 is combined with EB1, a known binding partner of CLASP2, microtubules become highly stabilized and display increased rescue frequency compared to CLASP2 alone. Using a truncated EB1 protein that lacks the CLASP-binding domain, we show that the enhancement of CLASP2 activity is dependent on EB1 recruiting CLASP2 to growing microtubule ends. In summary, our data show that human CLASP2 is an anti-catastrophe and rescue factor with intrinsic microtubule-stabilizing activity that is enhanced by EB1-mediated targeting of CLASP2 to the microtubule tips.

## **Role of Exosomal Endoglin in Filopodia and Tumor Cell Motility**

8

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- $\beta$  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.



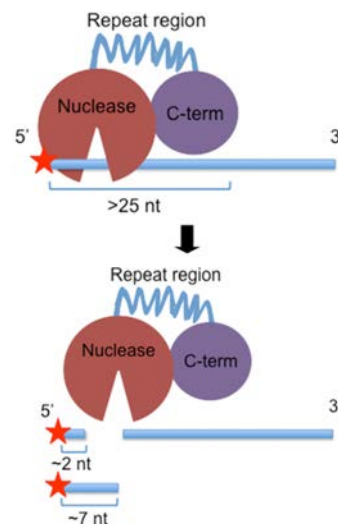
# Role of a novel Senataxin-associated nuclease in DNA repair

10

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 of greater than 15 nucleotides. 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.

Schematic of a model for SAN1 nuclease activity.



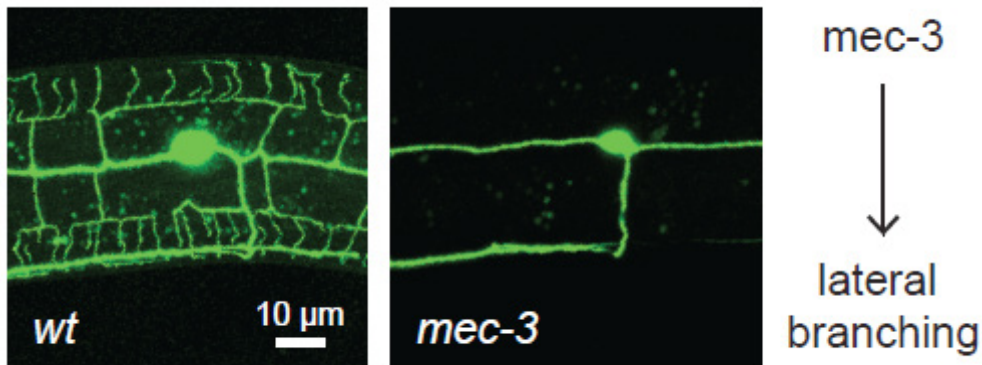
Full-length Bid maintains cristae corresponding to risk for MI.

# Neuron-specific profiling to identify dendrite branching genes

Rebecca McWhirter, Barbara O'Brien and David M. Miller

The *C. elegans* genome is completely sequenced and its developmental anatomy is defined at single cell resolution. In an effort to link gene expression to cellular identity, we are generating expression profiles of specific cell types at defined developmental stages. To accomplish this goal, we developed the SeqCel (RNA Seq of *C. elegans* cells) technique that uses FACS to isolate specific GFP-labeled for RNA-Seq analysis. To date, we have generated profiles of over 15 different cell types from larvae and adults including specific neurons and muscle cells. This approach has identified transcripts that are highly expressed in specific cells and also revealed genes with roles in important developmental processes. Here, we report the use of SeqCel to identify transcripts that drive dendritic branching in the PVD nociceptive neuron. Each PVD neuron, one on each side of the body, is defined by a single longitudinal process that gives rise to regularly-spaced menorah-like structures that envelop the animal. This structure is dramatically altered in mutations that disable the MEC-3 LIM homeodomain transcription factor. In *mec-3* mutants, PVD lateral branching fails and menorah-like structures are rarely generated. To identify MEC-3-regulated targets, we have used SeqCel to profile both wild-type (WT) and *mec-3* mutant PVD neurons during the L3 larval stage in which lateral branching normally occurs. Differentially regulated transcripts detected in a comparison of WT and *mec-3* mutant PVD SeqCel profiles will be screened by RNAi and with available genetic mutants to identify downstream determinants of dendritic branching.

Figure: Image of WT vs *mec-3* mutant PVD.



# Non-apoptotic role of Mcl-1 in mitochondrial dynamics and metabolism

14

Megan Merolla, Paul Park, Natalya Ortolano, Leigh A. Kline, Vivian Gama

Pluripotent stem cells (PSCs) maintain their unique properties of self-renewal and pluripotency through the rigorous expression of key transcriptional programs. Much effort has been devoted to the characterization of transcription factors involved in maintaining stemness. However, much less is known about other fundamental traits of PSCs and how those traits are involved in differentiation. For example, stem cells are known to have increased mitochondrial fragmentation (fission) and to rely mainly on glycolysis for energy requirements. Mitochondrial homeostasis, which is maintained by the balance of mitochondrial fission and fusion, affects 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. Mitochondrial fission is facilitated by Dynamin-related protein 1 (DRP1), while fusion is controlled by Optic atrophy 1 (OPA1) and Mitofusins 1 and 2. The mechanisms by which mitochondrial dynamics are linked to pluripotency remain unknown. My preliminary studies demonstrate that the anti-apoptotic protein Myeloid Cell Leukemia-1 (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 localizes to the outer mitochondrial membrane where it antagonizes Bak and Bax, but it is also localized to the mitochondrial matrix where its function has not been clearly elucidated. Our data show that both small molecule inhibition and siRNA-mediated downregulation of Mcl-1 induce the loss of Nanog and Oct-4, as well as dramatic changes in the mitochondrial network. Our preliminary data indicate that Mcl-1 maintains a high rate of mitochondrial fission in stem cells through its interaction with DRP1 (at the outer mitochondrial membrane) and OPA1 (at the mitochondrial matrix). These findings suggest that Mcl-1 provides a potential link between mitochondrial dynamics and metabolism, and uncovers an unexpected, non-apoptotic function for Mcl-1 in stem cell fate and pluripotency.

## CD44v9-xCT system is required for chief cell plasticity

16

Anne R. Meyer, Eunyong Choi, Amy Engevik, Takahiro Shimizu, Jim Goldenring

Gastric chief cells exhibit high cellular plasticity. In response to acute parietal cell loss, chief cells transdifferentiate and re-enter the cell cycle to generate a metaplastic cell lineage known as spasmolytic polypeptide-expressing metaplasia (SPEM). This high cellular plasticity acts as a key mechanism to promote repair and tissue regeneration, but when combined with chronic injury and inflammation it can become deleterious. CD44 variant isoform 9 (CD44v9) is a cell surface glycoprotein not found on chief cells, but CD44v9 is upregulated as chief cells begin to transdifferentiate following injury. CD44v9 interacts and stabilizes xCT, a subunit of the cystine-glutamate antiporter, increasing cystine uptake into the cell, promoting glutathione synthesis, and protecting cells against reactive oxygen species. Sulfasalazine, an inhibitor of xCT-dependent cystine transport, is known to suppress SPEM and gastric tumor growth. We therefore hypothesized that the CD44v9-xCT system is required for chief cell plasticity. To test this hypothesis we used sulfasalazine to inhibit CD44v9-xCT and determined the effect on chief cell transdifferentiation and metaplasia. We chemically induced acute parietal cell loss and metaplasia in mice by administering L635 for 3 days. Mice were treated with 10 mg of sulfasalazine per day, 2 days prior to and during L635 administration. Stomach tissue from untreated mice, or mice treated with sulfasalazine only, L635 only, or sulfasalazine and L635, was analyzed by immunofluorescence for markers of gastric lineage cells, SPEM, proliferation, and autophagy. Chief cells from mice treated with sulfasalazine and L635, like chief cells from control mice treated with L635 only, showed a loss of transcription factor *Mist1* and increased expression of the cell stress marker *clusterin*. Unlike control mice treated with L635 only, the mice treated with sulfasalazine and L635 showed a reduction in SPEM cell number and CD44v9 expression. Only a small number of chief cells in mice treated with sulfasalazine and L635 were able to re-enter the cycle, an 86% ( $p < 0.0001$ ) reduction when compared to chief cells from control mice treated with L635 only. Mice treated with sulfasalazine and L635 also showed altered expression of autophagy marker, LC3. Overall, our results suggest that without CD44v9 stabilization of xCT, chief cells lose their mature characteristics but are not able to transdifferentiate into mucous producing SPEM cells. Our results also show that sulfasalazine, an inhibitor of the CD44v9-xCT system, is an important tool that can be used to study chief cell transdifferentiation.

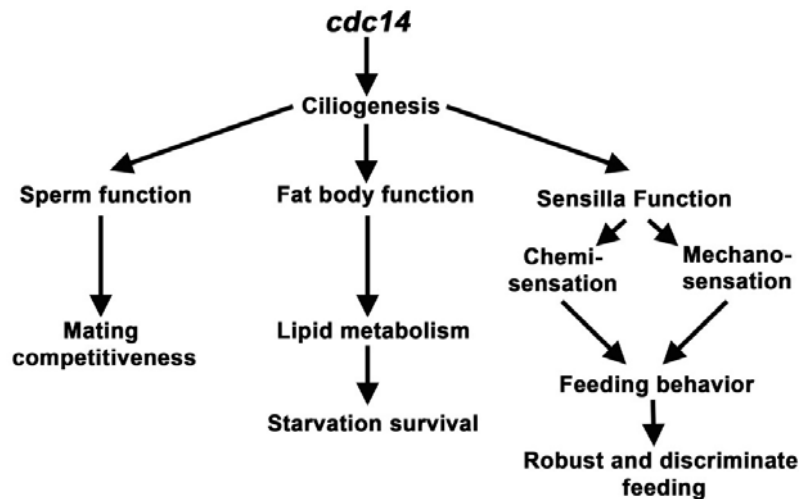
# Drosophila Cdc14 plays a conserved role in cilia function

18

Leif Neitzel, Matthew Broadus, Nailong Zhang, Emily Crispi, Leah Sawyer, Heather Wallace, William Rork, Laura Lee, Andrea Page-McCaw, Kathleen Gould, Ethan Lee

The dual specificity tyrosine phosphatase, cell division cycle 14 (Cdc14), is essential in *S. cerevisiae*. In *S. pombe*, however, knockout of the *cdc14* homolog, *clp1/ffp1*, results in no observable phenotype. Multiple *Cdc14* isoforms have been identified in metazoans that may confound loss of function studies. *Drosophila melanogaster* has a single ortholog, *cdc14*, which facilitates its loss of function analysis. Cdc14 has been reported to play roles in mitotic exit, transcriptional regulation, cytokinesis, transcriptional regulation, G1-phase length, centriole duplication, spindle stability, zygotic genome activation, DNA damage repair, and ciliogenesis. We found no role for *Drosophila cdc14* in these previously reported functions except for ciliogenesis. We demonstrate a role for *cdc14* in ciliogenesis and cilia functionality in the only two ciliated cell types in *Drosophila*, sperm and type I sensory neurons. Fertility of *cdc14* nulls is unchanged from wild-type flies although competitiveness of *cdc14* null sperm is reduced during multiple matings, suggesting a defect in sperm cilia functionality. Chemosensory and mechanosensory type I sensory neurons demonstrate decreased sensitivity to stimuli, and larvae feeding behavior is reduced and indiscriminate. We show longevity during starvation conditions is reduced and that lipid droplets in *Drosophila* fat bodies are large and irregularly shaped, suggesting defects in lipid storage and utilization. Taken together, our data support a model in which *cdc14* may reach fixation in a population due to its multiple effects on survival, breeding, and sensory defects.

Model of *cdc14* phenotypes in *Drosophila*.



# The USP46 deubiquitinase complex promotes Wnt signaling by blocking receptor turnover

20

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.

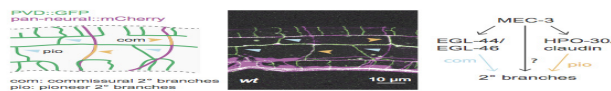
# Transcriptional regulation of dendritic branching in a *C. elegans* nociceptive neuron

22

Barbara M. J. O'Brien, Michaela Novakovic, Xueying Shang, Lakshmi Sundararajan, and David M. Miller III

The dendritic processes of nociceptive neurons transduce external signals into neurochemical cues that alert the organism to potentially damaging stimuli. The receptive field for each sensory neuron is defined by its dendritic arbor, but the mechanisms that shape dendritic architecture are poorly understood. We are using a model nociceptor, the PVD neuron in *C. elegans*, to investigate this question. Each PVD neuron, one on each side of the body, exhibits an elaborate but well-characterized dendritic arbor that is readily visible directly beneath the skin. Two types of lateral branches grow along the dorsal/ventral axis to contribute to this network: (1) pioneer dendrites that adhere to the epidermis, and (2) commissural dendrites that fasciculate with circumferential motor neuron processes. We have shown that the LIM homeodomain transcription factor MEC-3 is required for all higher order PVD branching and that one of its targets, the claudin-like membrane protein HPO-30, promotes outgrowth of pioneer branches. Here, we report that another MEC-3 target, the conserved TFIIA-like zinc finger transcription factor EGL-46, selectively promotes extension of commissural dendrites. The TEAD transcription factor EGL-44, which has been shown to function as a heteromeric binding partner of EGL-46 in other cell types, is also required for PVD commissural branch outgrowth. Double mutants of *hpo-30* and *egl-44* show strong enhancement of the lateral branching defect with decreased numbers of both pioneer and commissural dendrites. Thus, parallel acting pathways, one involving HPO-30/Claudin and the other EGL-46/EGL-44, function downstream of MEC-3 to direct PVD dendritic branching. Because the MEC-3 phenotype (i.e., absence of all PVD lateral branches) is more severe than that of *hpo-30;egl-44* double mutants, other MEC-3-regulated pathways must also contribute to lateral branching. In the future, we will use cell-specific RNA-Seq profiling to identify MEC-3 regulated transcripts as candidates for additional determinants of dendritic branching.

Figure: MEC-3 regulates multiple pathways for 2° branch development



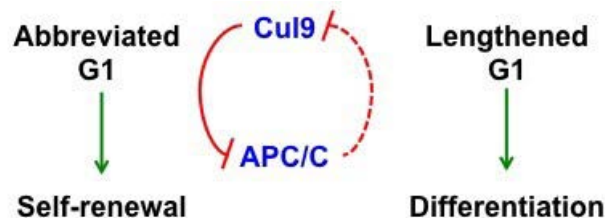
# Determining the role of Cullin9-mediated cell cycle control in stem cell fate

24

Ortolano, N.A., Kline, L.A., and Gama, V.

While there are major differences between the cell cycle of pluripotent stem cells (PSCs) and somatic cells, investigation into the unique cell cycle regulatory mechanisms of PSCs has been limited. The overall length of the PSC cell cycle in humans and mice is shorter than that of somatic cells, and the G1 phase is abbreviated. As PSCs differentiate, the G1 phase gradually lengthens, and the cell cycle begins to resemble that of a somatic cell. While novel cell cycle control mechanisms have been identified in mouse PSCs (mPSCs), the elucidation of regulatory mechanisms in the human PSC (hPSC) cycle has been unsuccessful. Our preliminary data indicates that an E3 ubiquitin ligase known as Cullin-9 (Cul9) is involved in hPSC cell cycle control. E3 ubiquitin ligases are enzymes that modify targeted proteins with ubiquitin moieties, which can signal for proteasomal degradation, or alteration of the target's localization, function, or protein interactions. Our lab previously demonstrated that Cul9 is highly expressed in post-mitotic neurons where it promotes cell survival by ubiquitin-mediated degradation of cytochrome c, delaying apoptotic execution. Despite Cul9's elevated expression in PSCs, there are currently no known Cul9 substrates in PSCs. Through an unbiased approach by mass spectrometric analysis we identified several subunits of the anaphase promoting complex/cyclosome (APC/C) as Cul9 interacting proteins in hPSCs. The APC/C is a multi-subunit E3 ubiquitin ligase that catalyzes the ubiquitin-mediated proteasomal degradation of key substrates involved in mitotic progression and maintenance of G1 phase. I will show our supporting preliminary data that Cul9 may regulate G1 length through modulation of the APC/C, thereby acting as a unique hPSC cell cycle checkpoint critical for self-renewal and differentiation.

Cul9 may play a role in control of G1 length through modulation of APC/C.





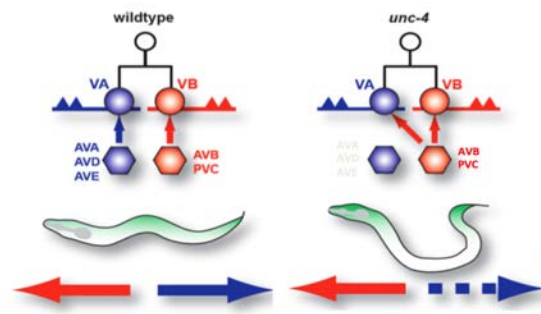
# Parallel acting signaling pathways regulate gap junction specificity in the *C. elegans* motor circuit

26

Sierra Palumbos, Rachel Skelton, Stephen E. Von Stetina, Rebecca McWhirter, David M. Miller, III

Gap junctions provide the only known form of direct electrical communication between neurons. Although much has been learned about gap junction assembly, the mechanisms that direct the formation of these electrical synapses between specific neurons are largely unknown. Here we address this question in a study to identify transcriptionally regulated components that direct the formation of gap junctions between specific neurons in the *C. elegans* motor circuit. VA and VB class motor neurons normally establish electrical synapses with different sets of presynaptic interneurons to drive either backward (VA) or forward (VB) locomotion. The UNC-4 transcription factor is expressed in VA neurons to prevent the adoption of VB-type inputs. *unc-4* mutants are unable to crawl backward because VA neurons are miswired with gap junctions from interneurons normally reserved for VBs (Fig. 1). Work in the Miller lab has shown that UNC-4 effectively preserves normal VA inputs by antagonizing two independent signaling pathways. UNC-4 blocks EGL-20/Wnt signaling as well as a G-protein-dependent pathway involving GOA-1/G $\alpha$ O that acts in parallel to promote the formation of VB-type connections in *unc-4* mutants. In both cases, however, the UNC-4 targets that effectively regulate these downstream pathways are unknown. To address this question, I used a new cell-specific profiling strategy, SeqCel (RNA-Seq of *C. elegans* cells) to identify *unc-4*-regulated transcripts in VA neurons. Genetic epistasis experiments have now identified two key *unc-4*-dependent transcripts in these data sets that are proposed to function as regulators of gap junction specificity. My results suggest that UNC-4 antagonizes the EGL-20/Wnt pathway by promoting expression of the Wnt pathway inhibitor, the secreted frizzled receptor protein SFRP-1. In addition, I hypothesize that UNC-4 blocks activation of the GOA-1/G $\alpha$ O pathway by repressing transcription of an upstream G-protein-coupled receptor, FRPR-17/GPCR. Thus, my results have uncovered a key role for a transcriptional mechanism in gap junction specificity that directs the formation of electrical synapses by regulating the sensitivity of specific neuron types to external cues.

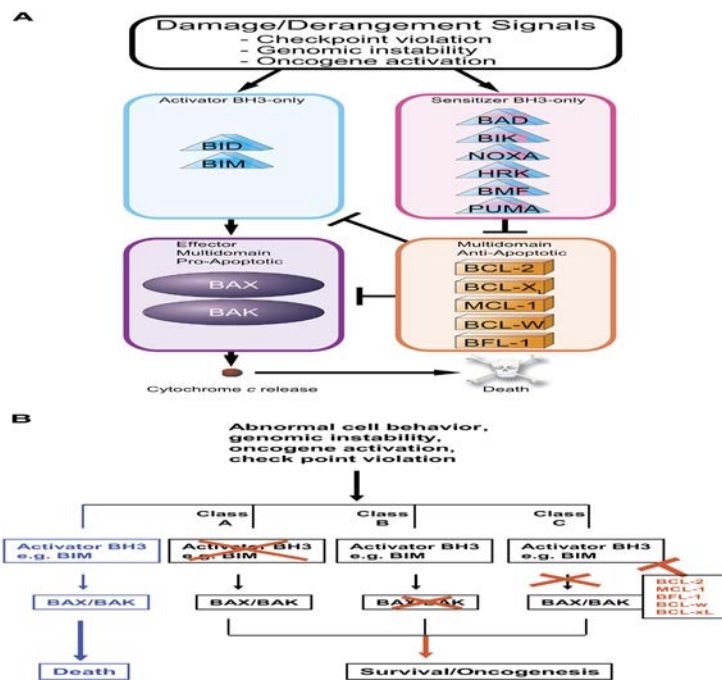
Figure 1: In *unc-4* mutants, VA inputs are replaced with inputs normally reserved for VBs, disrupting the backward circuit



# Validation of BH3 Profiling in Glioblastoma Multiforme

Kyungho P. Park, Jonathan Irish, Rebecca Ihrle and Vivian Gama

Glioblastoma (GBM) is comprised of numerous cell types a mixture of both neoplastic and non-neoplastic. A population of glioma stem-like cells (GSCs) exists in GBM and they have properties resembling normal stem cells, which are multi-potent, self-renewing, and able to initiate and maintain neoplastic clones within the tumor. Studies in adult GSCs suggest that differences in the mitochondrial network between GSCs and non-GSCs are key determinates of cell death sensitivity. To assess inter- and intra-tumoral heterogeneity based on the differential response to routinely used chemotherapeutics that are known to sensitize the mitochondria, we will use Dynamic BH3 Profiling (DBP). DBP uses live cells to measure early changes in net pro-apoptotic signaling at the mitochondria (“priming”) induced by chemotherapeutic agents and has shown to be able to predict response to therapies. We propose a single-cell approach to acquire this global profile in GBM as an important step in determining the cellular mechanisms involved in the maintenance of self-renewal and in resisting apoptosis.



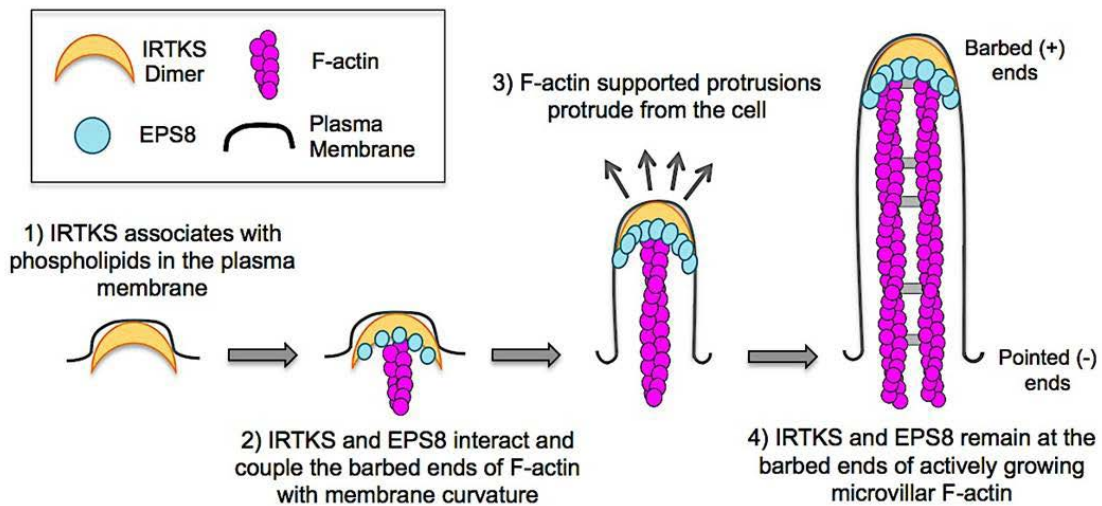
Dynamic BH3 profiling of GBM tumors as a predictor of chemo response

# Investigating the role of IRTKS in brush border assembly

M.M. Postema, N.E. Grega Larson, A.C. Neininger, B.A. Millis, M.J. Tyska

The intestinal brush border (BB) lines the apical surface of enterocytes and is composed of thousands of actin supported protrusions called microvilli that point into the gastrointestinal lumen. Microvilli function to increase the surface area of the intestines and comprise the sole site of nutrient absorption within the body. Even though properly formed microvillar protrusions are critical for maintaining intestinal homeostasis, the mechanisms underlying their formation remain unclear. Here we provide evidence that the I-BAR domain containing protein, insulin receptor tyrosine kinase substrate (IRTKS), is essential for proper microvillar growth and elongation. I-BAR proteins are scaffolds that link the plasma membrane and actin cytoskeleton in outward membrane protrusions. Although the BB is one of the most highly curved membranous organelles in biology, an I-BAR domain containing protein has yet to be investigated in this system. We found that IRTKS targets to the tips of actively growing microvilli, elongates microvilli when overexpressed, and is a necessary component in BB formation. Moreover, exogenous IRTKS promotes filopodial protrusion in cells that do not form microvilli. We also found that the SH3 domain of IRTKS binds to the actin capping and bundling protein EPS8, and that the two proteins colocalize at the distal tips of microvilli. Similar to IRTKS, EPS8 is necessary for proper BB growth. When IRTKS is knocked down, a portion of EPS8 falls out the BB and becomes cytosolic, suggesting that IRTKS is necessary to anchor EPS8 at microvillar tips. The exact relationship between IRTKS and EPS8 in microvillar growth is still being elucidated. However, we believe that the interaction between the two proteins provides a mechanism to couple outward membrane protrusion, generated by IRTKS, to the barbed end of growing actin bundles where EPS8 binds. Our current model on how the two proteins interact is depicted in Figure 1. These results provide insight into the mechanisms of microvillar growth and elongation that lead to a functional intestinal epithelium.

**Figure 1:** Model for the role of IRTKS and EPS8 in microvillar growth.



## **Basement membrane repair occurs via a similar, but not identical, mechanism as de novo assembly**

32

William Ramos-Lewis, Erica Shannon and Andrea Page-McCaw

The ability of epithelial tissue to heal upon sustaining an injury is an important feature of all animal species. Given the obvious medical implications of this topic, the cellular mechanisms that drive wound repair have been studied extensively. Despite these efforts, we still know surprisingly little about the repair of basement membrane (specialized extracellular matrix that lies on the basal surface of all epithelial cells). Transmission electron micrographs show that basement membrane is indeed repaired after sustaining damage, but the mechanism of repair has not been carefully studied. Using *Drosophila* 3<sup>rd</sup> instar larvae as a model for epidermal wound repair, we are taking advantage of the genetic tools available in this system to determine the origin of newly deposited basement membrane, whether that repair is required for re-epithelialization, and just how closely basement membrane repair mirrors de novo assembly. Of the core basement membrane proteins we're examining (laminin, collagen IV, perlecan and nidogen) we find that no single one is required for re-epithelialization. Furthermore, the fat body (an insect organ involved in regulating metabolism and immunity) is primarily responsible for secreting basement membrane into the open circulatory system of the animal, which then somehow finds its way to the site of repair. Finally, unlike previous observations of de novo basement membrane assembly, we find that laminin may not be a necessary foundation upon which basement membrane is repaired.

# Serotonin receptor 5-HT3A impacts autonomic neural development and urinary bladder function

34

K. Elaine Ritter, Dennis P. Buehler, E. Michelle Southard-Smith

The autonomic nervous system originates from the neural crest and is required for the proper function of all visceral organs, including the lower urinary tract. Despite the wide prevalence of bladder dysfunction, few effective treatment options are available. Pelvic innervation regenerative strategies are promising, but surprisingly little is known about the molecular drivers of sacral autonomic neurogenesis. In an effort to identify factors involved in the development of pelvic innervation, we conducted a microarray analysis of sacral neural crest progenitors and differentiating autonomic pelvic ganglia. We identified significant upregulation of several serotonin receptors with known roles in neurogenesis and maturation in the brain. Among these, 5-HT3A is of particular interest since we identified robust expression in early *Sox10*<sup>+</sup> progenitors and the *Htr3a* gene has a known effect in adult bladder function. Using an *Htr3a*-EGFP transgenic reporter we observed 5-HT3A expression in both sympathetic and parasympathetic autonomic pelvic ganglia neurons that is maintained through fetal and postnatal development. To probe the role of 5-HT3A in development and function of pelvic innervation we applied pharmacological and physiological approaches. We treated fetal pelvic ganglia explants cultured *in vitro* with a 5-HT3A specific agonist and observed severely impaired neurite branching and outgrowth. Conversely, bladder smooth muscle in fetal and adult 5-HT3A knockout mice is more densely innervated by cholinergic neurons compared to wildtype mice. Bladder smooth muscle contractility recordings *in vivo* and assessment of urinary voiding in 5-HT3A knockout mice demonstrate a requirement of this receptor in regulating normal bladder function. Our studies demonstrate a novel role for serotonin signaling in the development of neural crest lineages and contribute to our understanding of neurogenic causes of urinary incontinence.

# Myocardial Differentiation is Dependent on Endocardial Signaling During Early Cardiogenesis

36

Leshana Saint-Jean, Kevin L. Tompkins, H. Cristina Harmelink, Nikoloas Barkas, Rebecca J. Oakey, and H. Scott Baldwin, MD.

Congenital heart defects (CHD) affects nearly 1% of all newborns and is the leading cause of morbidity and mortality among infants. Much attention has focused on molecular components of myocardial transcriptional regulation during cardiac development as a possible cause of CHD, but not those of the endocardium. Current data demonstrates a requirement of the endocardium for proper proliferation and morphogenesis of the myocardium during the later stages of heart development (i.e. valvulogenesis and trabeculation). However, there is little data that identifies the role of endocardial:myocardial interactions in the differentiation of pro-cardiomyocytes during the initial stages of cardiogenesis. Our laboratory has determined that much of the endocardium is derived from a common cardiac progenitor cell population that also gives rise to the myocardium. We hypothesize that paracrine signaling from the endocardium to the myocardium is critical for initiating early differentiation and proliferation of myocardial cells. To test this we generated an endocardial ablation model in which mouse embryonic stem cells (ESCs) express the diphtheria toxin receptor (DTR) under the regulatory elements of the *NFATc1* genomic locus that provide endocardial specific expression. Treatment with DT during the first ten days of differentiation (D0-10) in the absence of growth factor (GF) supplementation *in vitro* significantly reduced the percentage of contracting foci within embryoid bodies (EBs) by 75%. This observation was further supported by the attenuated expression of early and late markers of myocyte differentiation and maturation. Utilizing RNASeq analysis of FACS sorted endocardial and vascular endothelial cells, we were able to identify a number of growth factors that were preferentially expressed in endocardium vs. vascular endothelium. While several of these GFs were reduced following endocardial ablation (i.e. IGF1, FGF3, and TGF- $\beta$ 1), only BMP-2 was able to partially rescue beating to approximately 50 to 60% of that seen in control ESC cultures. The increase in overt contractility was associated with a concomitant increase in the development and maturation of myocytes despite the absence of endocardial cells. Therefore, we conclude that myocardial differentiation during the early stages of development relies on BMP-2 dependent paracrine signaling between endocardium and myocardium. Our findings provide insight into early myocardial:endocardial interactions that can be explored as a means to promote myocardial development and growth.

## The DUB USP47 regulates Wnt pathway activation

38

Kenji Saito-Diaz, Victoria Ng, Alison J. Hanson, and Ethan Lee

The canonical Wnt signaling pathway regulates many fundamental processes during metazoan development and is critical for tissue homeostasis in the adult. A key event in Wnt signal transduction is the stabilization of the cytoplasmic protein  $\beta$ -catenin. In the absence of a Wnt ligand, a  $\beta$ -catenin destruction complex, composed of Axin, glycogen synthase kinase 3 (GSK3), casein kinase I  $\alpha$  (CKI $\alpha$ ), and the tumor suppressor adenomatous polyposis coli (APC), promotes phosphorylation of  $\beta$ -catenin, targeting it for ubiquitin-mediated proteasomal degradation. Binding of a Wnt ligand to its two cell-surface receptors, Frizzled (Fz) and LDL receptor related protein 5/6 (LRP5/6), results in inhibition of  $\beta$ -catenin phosphorylation and, thus, stabilization of  $\beta$ -catenin. Stabilized  $\beta$ -catenin translocates to the nucleus where it binds to TCF/Lef to activate a Wnt-specific transcriptional program. The Wnt pathway is heavily regulated by ubiquitylation. We performed a targeted RNAi screen in *Drosophila* S2 cells to identify novel deubiquitylases (DUBs) involved in Wingless (Wg, the *Drosophila* homolog of Wnt) signal transduction. We identified Ubiquitin-specific protease 64 E (Ubp64E) as a critical Wg pathway component. We found that Ubiquitin Specific Protease 47 (USP47), the human homolog of Ubp64E, is similarly required for Wnt signaling in cultured human cells. At the molecular level, downregulation of USP47 by RNAi prevents accumulation of cytoplasmic  $\beta$ -catenin and activation of the Wnt reporter TOPflash in human cells treated with Wnt3a. Interestingly, loss of USP47 also inhibits TOPflash in cells treated with the GSK3 inhibitor lithium (blocks  $\beta$ -catenin degradation). Also, we found that USP47 interacts with two Wnt pathway E3 ligases,  $\beta$ -TRCP and XIAP, as well as the transcriptional corepressor TLE3. Finally, we found that USP47 is required for primary body axis formation in *Xenopus* embryos, indicating evolutionary conservation of function. Together, these studies suggest that USP47 regulates Wnt signal transduction *in vivo* and in human cells, and it is involved in cytoplasmic and the nuclear events that lead to Wnt pathway activation.

# Bid maintains mitochondrial cristae: evidence for a protective role in human cardiac disease

40

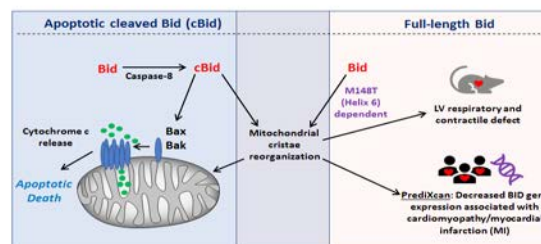
Christi Salisbury-Ruf, Clinton C. Bertram, Aurelia Vergeade, Qiong Shi, Marlene L. Heberling, W. Gray Jerome, Quinn S. Wells, Joshua Fessel, Eric R. Gamazon, and Sandra S. Zinkel

The localization of Bcl2 family proteins to the mitochondria is essential for membrane reorganization during the execution of apoptosis. During apoptosis, the BH3-only protein Bid is cleaved (cBid) and binds to family members Bax and Bak to initiate pore formation resulting in mitochondrial outer membrane permeabilization (MOMP). cBid has also been shown to reorganize mitochondria cristae. Both processes result in mobilization and release of the cytochrome c, and execution of apoptosis. Recent studies also suggest that full length Bid may transiently interact with the mitochondria. The impact of this interaction on mitochondrial structure during homeostatic conditions and the consequence for human disease has not been explored.

Our initial observations in Myeloid progenitor cells (MPCs) suggested that full-length Bid plays a role in cristae structure during homeostatic conditions. The absence of Bid results in increased nutrient deprivation sensitivity and cytochrome c mobilization independent of an apoptotic stimulus. Cristae structure of *Bid*<sup>-/-</sup> MPCs revealed loss or malformation of cristae, which can be rescued with the re-introduction of both non-apoptotic forms of Bid, BH3-mutated and caspase-8 cleavage mutated (D59A) Bid. This corresponds with decreased MPC respiration as well as left ventricular (LV) heart tissue, and decreased activity of the respiratory complexes.

To determine if our findings had implications for human disease, we used a gene-based approach applied to a large-scale biobank, validated in an independent large-scale GWAS, called PrediXcan. We observed decreased BID gene expression correlates with myocardial infarction (MI). Furthermore, we find that this result is specific to BID among other BH3-only proteins. Interestingly, at baseline and upon an acute stress, *Bid*<sup>-/-</sup> mice display left ventricular contractile dysfunction.

BioVU exome analysis revealed that coding SNP M148T, located in Bid's membrane binding domain, associates with diagnosis of MI. Helix 6 has been previously identified as important for cBid dependent cristae morphology. While Bid mutated in its apoptosis-promoting (BH3) domain restores mitochondrial respiration and cristae, it does not when combined with M148T. Our results identify a role for Bid in the regulation of mitochondrial cristae, using an innovative approach to link this novel role to human cardiac disease.





# Organization and regulation of microtubule nucleation ‘hot spots’ at Golgi stacks

42

A.A.W.M. Sanders, X. Zhu, R. Thoppil, K. Chang, I. Kaverina

MTs are polarized dynamic polymers made of  $\alpha/\beta$ -tubulin dimers that are nucleated at MT-organizing centers (MTOC), which include the centrosome and in certain cell types, the Golgi. Molecular factors that play a role in Golgi-derived microtubule (GDMT) nucleation and stability have been found on both *cis*-Golgi (e.g., AKAP450) and TGN (Trans-Golgi Network; e.g. CLASPs) membranes. How these factors can interact and how GDMTs are formed is not fully understood.

Here, we show that GDMTs are formed in both epithelial cells and fibroblasts. These GDMTs originate from a distinct site at the Golgi membrane. Quantitative image analysis combined with computational simulations reveals that GDMTs are non-randomly distributed across the Golgi membrane and are clustered at specific membrane formations we term nucleation ‘hot spots’. These nucleation ‘hot spots’ are both spatially and temporally restricted. Using super-resolution microscopy, we have resolved the precise structure and molecular composition of these ‘hot spots’.

A critical structural component of GDMT nucleation ‘hot spots’ is found at a novel *cis*-Golgi subcompartment, which only partially overlaps with *cis*-Golgi matrix protein GM130 (previously implicated in GDMT nucleation). However, AKAP450, an essential GDMT nucleator, always localizes to this subcompartment. These results further underline the importance of AKAP450 for GDMT nucleation but question the necessity of GM130 for either GDMT nucleation or AKAP450 recruitment to the Golgi. Furthermore, we have detected a second component of GDMT nucleation ‘hot spots’, which is TGN membrane tubes that extend towards the *cis*-Golgi. Combined, our data suggest that factors concentrated at both *cis*-Golgi sub-compartment and TGN participate in GDMT formation.

We have also investigated the contribution of CLASP paralogs, CLASP1 and CLASP2, to GDMT nucleation ‘hot spots’. Using siRNA-mediated depletion, we show that CLASP1 is important for non-centrosomal MT nucleation and ‘hot spot’ formation, whereas CLASP2 appears to play a role in retention of MTs at ‘hot spots’ on the Golgi membrane. These findings highlight the differential roles of CLASP paralogs in formation and maintenance of GDMT nucleation ‘hot spots’.

Taken together, this work shows that Golgi-derived microtubules are formed and retained at specific sites within the Golgi stack, which we term ‘hot spots’. AKAP450 is required but not sufficient for ‘hot spot’ formation, and nucleation and retention of GDMTs at these ‘hot spots’ is mediated differentially by CLASPs.

# The role of HNSCC-derived exosomes in driving tumor progression

44

Shinya Sato, Seema Sinha, Daisuke Hoshino, Brandee T. Brown, Micheal J. Korrer, Young Jun Kim, and Alissa 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 connected exosomes to aggressive tumor behaviors, including invasion and metastasis. Our previous data in HNSCC indicate that exosomes contribute to several hallmarks of cancer. Based on our and other data, we propose the central hypothesis that exosome secretion by HNSCC cells drives tumor aggressiveness. To test this hypothesis, we will identify specific aspects of HNSCC aggressiveness that are driven by exosomes and identify molecular exosome cargoes that drive those processes.

For clarifying the role of exosome on angiogenesis, we performed tube formation assay using HUVEC. Condition medium (CM) of HNSCC lines promoted tube formation compared with CM without exosome and control medium. Addition of HNSCC-derived exosomes in control medium facilitated tube formation. Same results were observed in tube formation assay using HMVEC-LyAd. From proteomics data of HNSCC-derived exosome, angiogenesis and lymphangiogenesis related factors such as Eph family receptors were included in exosomes. For detecting the role of tumor-derived exosomes on angiogenesis, lymphangiogenesis, tumor immunity and metastasis in vivo, we established orthotopic tongue transplantation of HNSCC cell lines. Aggressive murine HNSCC cell line, MOC2 and less-aggressive murine HNSCC cell line, MOC1 developed tongue tumor almost 1 month after tongue injection. MOC2 highly metastasized cervical lymph nodes. On the other hand, MOC1 didn't metastasize. Analysis of primary tongue tumor tissue using immunohistochemistry showed that CD31-positive microvessel density (MVD) in MOC2 tumor was significantly higher than that in MOC1. LYVE1-positive MVD in MOC2 was slightly higher than that in MOC1. Exosome secretion of MOC2 was almost 2 times higher than that of MOC1.

Our findings will address a major challenge in the field: how to connect specific molecular exosome cargoes with biological functions of cancer.

## The effects of the tumor cell-of-origin on cancer stem cell behaviors

46

Cherie' Scurrah, Alan Simmons, Robert Coffey, Ken Lau

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the United States. The high mortality rate of CRC is mostly a consequence of therapeutic resistance, tumor recurrence, and metastasis. These characteristics can be attributed to the heterogeneous nature of colon tumors. While this heterogeneity is influenced by various genetic and epigenetic mechanisms, tumor cellular heterogeneity has also been shown to be a consequence of stem-like activities of tumor initiating cells (TICs). However, the relationship of TICs with the tumor cell-of-origin, the cell that acquires the first driver mutations, remains to be defined. The commonly accepted model of CRC tumorigenesis is the “bottom-up model”, where the cell-of-origin is a normal tissue stem cell residing at the colonic crypt base. Recent publications have also suggested that non-stem cells can drive tumorigenesis in an inflammatory context known as the “top-down model”. Understanding the relationship between the origin of tumorigenesis and TICs will allow us to interpret the effects of tumor heterogeneity and facilitate the development of novel therapies targeting such heterogeneity. **I hypothesize that the tumor cell-of-origin is directly related to the TICs present in tumors that consequently arise. Specifically, tumors that arise from secretory progenitor cells in an inflammatory microenvironment produce TICs with distinct properties compared to tumors that arise from stem cells.** To test this hypothesis, I plan on utilizing mouse models of CRC that express mutated Adenomatous Polyposis Coli (*Apc*) alleles driven from either a stem cell promoter (Lrig1-Cre<sup>ERT2</sup>) or a secretory progenitor cell promoter (Mist1-Cre<sup>ERT2</sup>) to induce tumorigenesis in an inflammatory context. I will investigate the tumor-driving potential of secretory progenitors exposed to inflammation (Aim 1) and I will determine how the tumor cell-of-origin determines TIC characteristics via (Aim 2). To date, I have performed lineage tracing under homeostasis and inflammation to confirm that Mist1 is not expressed in stem cells. In the future, I plan using single cell analytic (MxIF and DISSECT-CyTOF) and *in vitro* techniques to determine the molecular underpinnings of the origin of non-stem cell driving tumorigenesis and explore the relationship between TICs, tumor cell of origins, and native tissue stem cells. Results from these studies will address how the origin of tumorigenesis influences TICs and subsequent heterogeneity in established tumors. This knowledge will expand our ability to develop therapies to counter tumor heterogeneity, which remains one of the biggest challenges in cancer biology.

# Role of oriented cell divisions in luminal breast cancer initiation

48

Lindsey Seldin and Ian Macara

The mouse mammary gland provides a robust mammalian system for investigating how epithelial behavior impacts tumorigenesis. The mammary epithelium is composed of an inner layer of milk-producing luminal cells and an outer layer of contractile myoepithelial cells. Several mammalian epithelial tissues rely on oriented cell divisions to maintain proper structure and function. Nevertheless, it remains unclear whether this mechanism plays the same important role in the mammary gland, and whether disruptions to this process promote breast cancer initiation. We hypothesize that misoriented cell divisions in the luminal layer drive cell escape from the epithelium to cause ductal filling during early breast cancer stages such as ductal carcinoma in situ (DCIS). To test this hypothesis, we designed a robust tool to drive luminal cells out of the epithelium, namely by fusing the spindle-orienting protein LGN to the apical transmembrane protein Crumbs3 to force mitotic spindles to reorient orthogonally to their normal planar orientation. When expressed in Madin-Darby Canine Kidney (MDCK) cells, this Crumbs3-LGN fusion robustly colocalizes with apical actin and causes a severe multiple lumen phenotype in 3D epithelial cyst cultures compared with single lumen controls. To express this construct exclusively in luminal cells, we created and validated a lentiviral vector that contains an EF1 $\alpha$  promoter plus multiple myoepithelial-specific miRNA target sequences within a short 3' UTR, which inhibit expression within the myoepithelial population. By transducing primary mammary cells with this construct and transplanting them into recipient mice, we can study the *in vivo* effects of misoriented luminal divisions on mammary tumor initiation and metastasis. Furthermore, using both mouse and human DCIS tissue, our preliminary studies indicate a predominance of planar luminal cell divisions in normal, bilayered tissue with hollow lumens and nonplanar cell divisions in DCIS tissue where cells are visible within the lumen. Our future studies will employ 3D organoid culture to study the consequence of misoriented divisions on cell behavior, including proliferation, delamination and migration. These studies could ultimately reveal important therapeutic targets for preventing, detecting and treating invasive breast cancers.

# Nucleocytoplasmic shuttling of Gle1 alters nascent transcript levels of immediate early genes

50

Manisha Sharma and Susan R. Wentz

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<sub>6</sub>) at the cytoplasmic NPC face stimulates Dbp5 (human DDX19) to activate mRNP remodeling, which enables directional release of the mRNP to the cytosol with the proper protein composition to direct its fate. In human cells, nuclear accumulation of mRNA is observed when either one of two seemingly paradoxical features of Gle1 are perturbed: its stable self-association at the NPC and its ability to shuttle between the nucleus and cytoplasm. Since previous genetic and cell biological studies have strongly linked Gle1 self association with mRNA export functions at the NPC, we therefore speculate that the shuttling capacity of Gle1 might alter nuclear levels of poly(A)+ RNA through a function separate from mRNA export. This study aims to address this questions by first identifying the mRNAs that accumulate in the nucleus upon disruption of hGle1 nucleocytoplasmic shuttling. Treatment of HeLa cells with a unique 39 amino acid shuttling domain (SD) peptide of Gle1 yields a two-fold disruption in poly(A)+ RNA export. Following Gle1-SD or scrambled peptide treatment, nuclear RNA was isolated for RNA-seq analysis of the poly(A)+RNA library (VANTAGE). Differential analyses comparing Gle1-SD peptide versus scrambled peptide treatments revealed that 56 protein coding mRNAs were differentially accumulated in the nucleus by greater than or equal to 2log<sub>2</sub> fold change. Notably, fifty five percent (31/56) of these mRNAs contain AU-rich elements (AREs) in their 3'UTR. These sequence elements are characteristic of immediate early response genes (IEGs), whose transcript half-lives are modulated by the complement of RNA-binding proteins targeted to the ARE. To validate the dataset, nuclear accumulation of seven ARE-containing transcripts and two non-ARE containing transcripts identified in the RNA-seq analysis upon Gle1-SD treatment 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. We hypothesize that Gle1 plays a novel essential role in transcription of IEGs. Current studies are underway to delineate this mechanism and the role of DEAD-box proteins in Gle1-mediated transcriptional modulation.

# Deciphering cellular heterogeneity from solid tissues using Mass Cytometry: Its promise and challenges

52

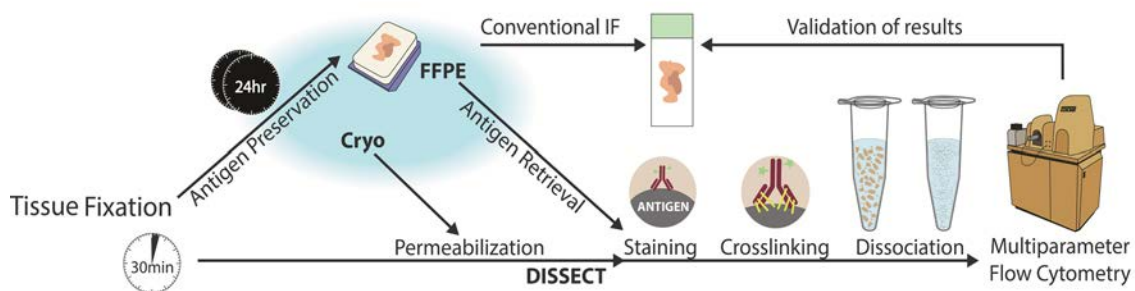
Alan J. Simmons, Amrita Banerjee, Charles A. Herring, Cherié R. Scurrah, and Ken S. Lau

Intratumoral heterogeneity is an emerging hallmark of cancer that plays significant roles in therapeutic resistance, cancer recurrence, and metastasis. Single-cell analysis holds the promise to deconstruct this heterogeneity. Current technologies enable the profiling of individual cells in a quantitative manner, followed by mathematical grouping of high-dimensional data points into cellular subsets. The functional state of a cell can be assessed by measuring the activation states of proteins central to distinct signaling pathways. Although bulk assays can achieve this goal in a multiplex fashion, a major obstacle in adapting signaling assays to single-cell studies, especially for solid tissues, is the substantial perturbation to cellular signaling pathways when single cells are disaggregated from an intact tissue. In 2015, we developed an approach called DISSECT (Disaggregation for Intracellular Signaling in Single Epithelial Cells from Tissue) for the evaluation of native activation states of signaling proteins in single epithelial cells using multi-parameter flow or mass cytometry (Fig. 1 – Bottom). Using intestinal epithelium as a stereotypical example, we demonstrated the validity of DISSECT-generated single-cell results against those from conventional bulk assays.

In a recent report in *Science Signaling*, we made additional advances with DISSECT for its application to FFPE human colon and colorectal cancer (CRC) specimens (Fig. 1 - Top). With data generated by FFPE-DISSECT coupled to mass cytometry, we used pairwise correlation between signaling analytes across all single cells in each sample to derive a metric of coordination between signaling pathways, and then used hierarchical clustering over this metric to define signaling modules. Using quantitative tree comparisons, we concluded that signaling pathways in normal human colon were organized into well-defined modules whereas this organization was lost in CRC.

We hope to continue adapting DISSECT to new tissues and instrumentation.

Figure 1: Mass cytometry-based single-cell analysis of epithelial signaling.



# Dynamics and Regulation of Microtubule Minus Ends

54

Claire Strothman, Stephen Norris, Marija Podolski, Ryoma Ohi, Marija Zanic

Microtubules are cytoskeletal polymers that serve crucial cellular functions such as intracellular transport, cell motility, and cell division. Microtubules are composed of  $\alpha\beta$ -tubulin heterodimers arranged head-to-tail so that  $\beta$ -tubulin is exposed at one end, and  $\alpha$ - at the other. This polarity defines the microtubule plus- and minus- ends. Both ends display a unique behavior in which microtubules switch between phases of growth and shrinkage, known as microtubule dynamic instability. Dynamic instability is continuously occurring in the cell, and enables rapid remodeling of the microtubule network. Specific regulation of microtubule minus ends serves critical roles in the establishment of cell polarity in epithelial cells and neurons, in addition to building and maintaining the meiotic and mitotic spindles. While numerous models have been proposed to describe plus-end dynamics, it is unknown whether these models adequately describe the minus end, especially given that dynamic instability is characteristically different at the minus end. Here, we use an *in vitro* reconstitution approach to investigate mechanisms of minus end dynamics. We find that, similar to plus-ends, the probability of minus end catastrophe increases with time spent in growth. We also find that EB1 comets marking the stabilizing cap at growing ends are defined by the growth rate, irrespective of the microtubule end. However, minus ends have lower frequency of catastrophe at corresponding growth rates, suggesting that a larger cap structure alone is not sufficient to confer protection from catastrophe. In cells, dynamic instability is regulated by a number of factors, including microtubule-associated proteins. We find that human kinesin-14 HSET regulates microtubule dynamics at the minus end by decreasing catastrophe, without affecting the growth rate. In low tubulin concentrations, HSET is also capable of inducing a polarity switch; HSET confers predominant microtubule growth to the minus end in otherwise unfavorable conditions for minus end growth. Together, these data contribute fundamental knowledge to allow us to better understand the minus end both intrinsically and in the context of regulation by an external factor, HSET.

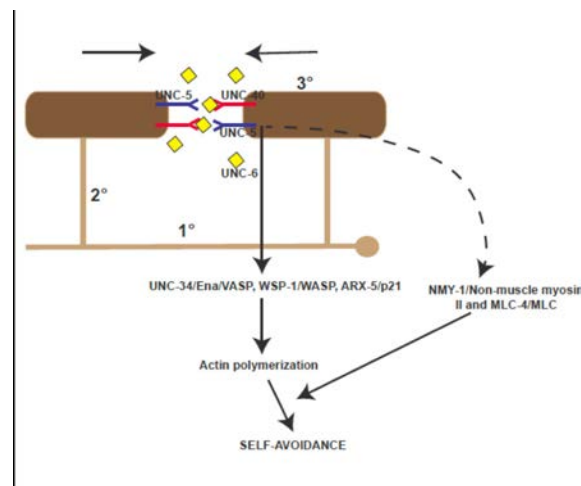
# Non-Muscle Myosin II functions with Ena/VASP and WASP to drive dendrite self-avoidance

56

Lakshmi Sundararajan, Cody Smith, Matthew Tyska and David M. Miller, III

Actin polymerization mediates cell migration and axon/dendrite outgrowth. Here, we demonstrate a novel role for actin polymerization in dendrite self-avoidance. Dendrites arising from a single neuron rarely overlap due to contact-dependent retraction. We have observed this phenomenon in the highly branched *C. elegans* nociceptive neuron, PVD, in which the sister dendrites retract upon mutual contact. We have previously shown that PVD self-avoidance is mediated by the short-range diffusible cue, UNC-6/Netrin and its receptors, UNC-40/DCC and UNC-5. A candidate genetic screen has now determined that the actin-polymerizing proteins, UNC-34/Ena/VASP and WSP-1/WASP, in concert with the actin branching Arp2/3 complex are also required for PVD self-avoidance. We used ‘pseudo-TIRF’ microscopy to detect a burst of F-actin accumulation and UNC-34 localization at the tips of retracting PVD dendrites immediately after contact. These findings are consistent with a paradoxical idea that actin polymerization drives dendrite shortening in the self-avoidance response. This model may be explained by the additional observation that the non-muscle myosin II, NMY-1, is also necessary for PVD dendrite retraction and could potentially mediate this effect by accelerating retrograde flow. This model is consistent with our finding that constitutive activation of non-muscle myosin II through expression of a phosphomimetic Myosin Regulatory Light Chain (MLC-4DD) results in PVD dendrites that are significantly shorter than wild-type.. Together, our results suggest that UNC-6/Netrin signaling triggers the local assembly of branched actin filaments, which then engage NMY-1 to elicit dendrite retraction. In addition to describing the first cell biological mechanism of dendrite self-avoidance, we also suggest that F-actin assembly and retrograde flow could be required for axonal growth cone repulsion.

Model Figure: Dendrite contact triggers assembly of branched actin filaments that then engage non-muscle myosin II to drive retraction.





# CLASP1 is Required for CLASP2 Localization at the Microtubules

58

Roslin J. Thoppil, Anneke Sanders, Irina Kaverina

CLIP-associated proteins (CLASPs) are highly conserved microtubule (MT) plus-end tracking proteins (+TIPS) that are involved in regulating MT dynamics; specifically, CLASPs were shown to promote MT rescue and enhance MT stability. Human CLASPs consists of two paralogs: CLASP1 and CLASP2, which have been found associated with the cell cortex, kinetochores, the Golgi and at the ends of growing MTs.

Although both CLASPs have been structurally and functionally characterized in depth, whether one CLASP regulates the localization/function of the other has not been studied previously. In the present study, using immunofluorescence (IF) techniques, we have observed CLASP2 highly accumulated at the Golgi and the MT plus ends in human fibroblasts. Knockdown of CLASP2 results in the loss of Golgi and MT-associated CLASP2 pool; however when CLASP1 is depleted, CLASP2 is lost at the MT plus ends, but is retained at the Golgi. These results suggest that CLASP1 may be required for CLASP2 localization at the MT plus ends; similar results were observed in a different human cell line indicating that this is not a cell-specific effect. To determine the underlying mechanism, we tested if CLASP1 was involved in regulating CLASP2 phosphorylation. Previously published data have shown that phosphorylation of CLASP2 by GSK3 $\beta$  results in CLASP2 dissociation from MTs, while inhibiting GSK3 $\beta$  leads to the increased MT association of CLASP2. Here, we found that when GSK3 $\beta$  is inhibited in CLASP1-depleted cells, CLASP2 localization to MTs was no longer impaired, suggesting that CLASP1 depletion does not affect the binding of non-phosphorylated CLASP2 to MTs. (Also, our data suggest that CLASP2 localization at the Golgi is not affected by GSK3 $\beta$  inhibition, and thus, Golgi-association of CLASP2 does not require its phosphorylation.)

Taken together, our findings represent the possibility that CLASP1 recruits CLASP2 to the MT plus ends by regulating CLASP2 dephosphorylation, while at the same time opens up several questions as to what is the relative distribution of CLASP1 protein at the Golgi vs. the MT plus ends.

## **Golgi-derived microtubule nucleation in pancreatic beta cells is regulated through cAMP signaling**

60

Kathryn P. Trogden, Guoqiang Gu, Irina N. Kaverina

How insulin release from pancreatic beta cells is regulated is poorly understood. Only a small fraction of the insulin granules present in these cells are released in response to glucose. Previous work from our lab shows that microtubules act to decrease the amount of insulin released by pancreatic beta cells in response to glucose, indicating that regulation of microtubule dynamics is important for physiological glucose metabolism. Glucose response effects microtubules in two ways: it destabilizes microtubules and increases nucleation off the Golgi membrane. Our lab has shown that in pancreatic beta cells, a vast majority of microtubules are nucleated at the Golgi, termed Golgi-derived microtubules (GDMTs). Here, we use MIN6 cells, an isolated mouse beta cell line, to show that GDMT nucleation increases in two waves in response to glucose stimulation, with no change to the nucleation at the centrosome. The GDMT nucleation curve closely resembles insulin release in both mouse islets and MIN6 cells. The first wave peaks around five minutes after stimulation with twice as many GDMTs compared to unstimulated cells and the second wave occurs between 30-50 minutes after stimulation with 1.5 times as many GDMTs compared to unstimulated cells. These experiments were done using regrowth assays where cells are depleted of microtubules and then allowed to nucleate a short amount of time before fixation. We can then count the number of microtubules nucleated off the Golgi after different times spent in high glucose was quantified. Similar results were obtained using live cell imaging of a microtubule and Golgi marker to measure microtubule nucleation at steady state. To better understand how this increase in GDMT nucleation is regulated we performed a screen of drugs that targeted different steps in the pathway between glucose uptake and insulin release. We found that both  $\text{Ca}^{2+}$  and cAMP signaling are involved in regulating these increases in nucleation. We have found that both effectors of cAMP in beta cells, PKA and EPAC2, play a role in the regulation as well. Blocking either  $\text{Ca}^{2+}$  or the cAMP effectors prevents the increase in nucleation events in response to glucose. Increasing the amount of  $\text{Ca}^{2+}$ , cAMP or activating EPAC2 increases GDMT nucleation even in cells in low glucose. These effects appear to be stronger in the first phase response to glucose stimulation which may reflect the differences in regulation of the first and second phase and how that effects GDMT nucleation. These results tie together the regulation of insulin secretion and GDMT nucleation.

# Elucidating the Gene Signature Driving Multipotency in Mammary Stem Cells

62

Erica Tross and Ian Macara, PhD

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. Important questions about mammary stem cells (MaSCs) include their identity, state of quiescence, and ability to undergo asymmetric cell divisions. A major challenge in studying MaSCs is the lack of specific markers enabling their purification. However, two recent studies describe markers that appear to be specific to MaSCs. The first study looked at a splice variant of the SH2 domain-containing 5'-inositol phosphatase (s-Ship). Transgenic mice expressing GFP from the s-SHIP promoter showed specific expression in mammary cells with multipotent potential. The second study showed that the protein C receptor (Procr) marks a separate population of activated multipotent mouse MaSCs. Important questions about these cell populations include their relationship to one another and to non-stem cell populations, their responses to extracellular signals and the gene regulatory networks that control their development and function. I will determine which transcription factors, signaling pathways, and gene networks contribute specifically to cell 'stem-ness' using FACS to isolate s-SHIP GFP<sup>+</sup> cells, Procr<sup>+</sup> cells, and non-MaSC cells from murine mammary glands and perform RNASeq analysis. The goal is to identify genes expressed specifically in one stem cell population or the other (or both) but not in the myoepithelial non-stem cells. I will perform gene regulatory network analysis to identify pathways important for maintenance of 'stem-ness'. Using lentiviral transduction and transplantation into recipient isogenic mice I will test the roles of these genes in mammary stem cell function and maintenance. Evidence has shown that myoepithelial non-stem cells grown *in vitro* can acquire multipotent MaSC identity. I will, therefore, also characterize gene expression profiles in these cells to determine if they have truly converted into MaSCs, and identify the factors that drive this conversion. Overall, my project will provide important new insights into the mechanisms that govern mammary stem cell maintenance and function.

# Hal4 and Hal5 kinases differentially regulate membrane trafficking in *Saccharomyces cerevisiae*

64

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. For example, signaling carried out by EGF receptor can cause aberrant cell proliferation, and ultimately cancer, if EGFR fails to be properly internalized and trafficked to the lysosome for degradation. Therefore, there is a critical need to understand how endocytic trafficking and signal transduction networks regulate each other. AMPK/Snf1 related kinases are generally involved in regulation of adaptive response to metabolic stress as well as cell polarity, growth and proliferation. The 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 that function redundantly in regulation of potassium transport. Hal4 and Hal5 are proposed to function redundantly in broad regulation of membrane trafficking, although significant sequence disparity among the N-terminal halves suggests unique functions. The stage of membrane trafficking impacted by these kinases, their mechanism of action, and their individual contributions in regulation are unknown. Membrane trafficking of Mup1, a methionine transporter is upregulated upon loss of either Hal4 or Hal5. Proper trafficking of Can1, an arginine transporter, requires Hal4, but not Hal5. Furthermore, trafficking of Lyp1, a lysine transporter, appears to be upregulated upon loss of Hal5, but downregulated upon loss of Hal4. These data indicate the regulatory roles of Hal4 and Hal5 may be similar, though not redundant, for some cargo such as Mup1, and unique for others. Taken together, these data suggest Hal4 and Hal5 play distinct and specific, rather than redundant and broad, regulatory roles in membrane trafficking.

# Ric1/Rgp1-Rab6 axis directs post-Golgi transport of collagen during skeletal development

66

Gokhan Unlu, Ela W. Knapik

Collagen is the primary component of extracellular matrix (ECM) that contains secreted molecules such as growth factors and signaling molecules as well as providing structural support to bones, cartilages and other organs. Defects in collagen synthesis, assembly and secretion lead to severe skeletal disorders such as brittle bone disease, osteoporosis and chondrodysplasias. Despite essential physiological functions, mechanisms by which collagen is secreted to extracellular space remain elusive. Highly oligomeric, complex fibrillar structure of collagen molecules presents a challenge for trafficking machinery. The ER-to-Golgi transport is mediated by specific COPII coat components: Sec23a, Sec24D and ER-resident accessory proteins Tango1 and cTAGE5. Strikingly, post-Golgi trafficking factors for collagen transport remain unknown.

In search for collagen post-Golgi trafficking pathways, we analyzed zebrafish mutants with defects in skeletal morphogenesis. Immunofluorescence labeling in craniofacial chondrocytes revealed that *round (rnd)* mutants fail to secrete type-II collagen and retain it intracellularly despite secreting other ECM cargoes such as matrilin and fibronectin. Positional cloning strategy identified mutations in three independent *rnd* alleles, all residing within *ric1* gene, a guanine nucleotide exchange factor (GEF) for Rab6 GTPase.

We have found that *ric1*-deficiency leads to collagen secretion defects in both chondrocytes and notochord sheath cells, in a non-cell type specific, but cargo-selective manner. In both cell types, we detected a backlog of intracellular collagen by transmission electron microscopy (TEM). TEM revealed formation of aberrant intracellular collagen fibrils, and colocalization studies with *lamp1* identified it as lysosomal vesicles. Furthermore, mosaic overexpression experiments have shown that Ric1 directs collagen transport in a Rab6- dependent manner. CRISPR/Cas9-mediated depletion of Rgp1 partner of Ric1 in Rab6 GEF complex, recapitulated collagen transport and skeletal morphogenesis defects in zebrafish. We have identified a novel procollagen-specific post-Golgi trafficking pathway and explained critical roles of Ric1/Rgp1-Rab6 trafficking axis in skeletal morphogenesis.

## Regulation of Dlp cleavage by *Drosophila* Mmp2

68

Indrayani Waghmare, Xiaoxi Wang, Bryan Cawthon, Andrea Page-McCaw

Oogenesis in *Drosophila* ovary occurs in structures called germaria that give rise to egg chambers. The apical cells of the germarium are the source of secreted ligands such as Wingless (Wg) and Hedgehog (Hh). In *Drosophila*, the spread of Wg from these anterior niche cells is required for follicle stem cell proliferation. HSPGs (Heparan Sulphate Proteoglycans) are known to regulate the distribution of secreted ligands during development. Dally like protein (Dlp) is one such cell surface HSPG. Dlp promotes long-range Wg signaling by ‘trapping’ extracellular Wg and presenting it to its cognate receptor-Frizzled (Fz) on cells that are farther away from Wg producing cells. Our previous work demonstrates that Dlp promotes long-range Wg signaling to the follicle stem cells that give rise to somatic cells which surround developing egg chambers. Using genetic tools in *Drosophila*, our lab further identified that Mmp2 inhibits Dlp mediated long range Wg signaling for follicle stem cell proliferation. Mmp2 is one of the two matrix metalloproteases found in flies and is localized at the cell surface via its glycosylphosphatidylinositol (GPI) anchor. Further, in cell-culture experiments, Dlp is cleaved by Mmp2 in the N terminal region. MMPs act on a wide range of substrates. However, they do not have consensus cleavage sites. In this study, we aim to 1) identify Mmp2 cleavage site(s) on Dlp using a proteomic approach (Mass Spec) and 2) test if Dlp cleavage occurs in vivo in an Mmp2 dependent manner. The identification of Mmp2 cleavage site(s) on Dlp, and understanding how this cleavage restricts the Wg ligand gradient will provide mechanistic insights into the regulation of long-distance signaling.

*Abstract Withdrawn*

## Role of F-BAR proteins from FCHSD subfamily in cytokinesis

72

Marcin P. Wos, Nathan A. McDonald, Kathleen L. Gould

Cytokinesis is a process in which mother cells divide cytoplasm and form two daughter cells. To accomplish that process they require to reorganize and rearrange their membranes and cytoskeleton. Proteins from F-BAR family thanks to their oligomerization, membrane binding, membrane bending and cytoskeleton interacting features are known to take part in endocytosis, cell motility and also cytokinesis. Based on their properties and domain compositions their members were split into eight subfamilies.

The FCHSD subfamily is characterized by a N-terminal F-BAR domain and two SH3 domains at the C-terminus. Members of this family are involved in endocytosis, but still there are not well known and described. We are investigating their potential role in process of cytokinesis as a scaffold integrating cell signaling, actin-myosin ring and plasma membrane. Overexpression of GFP-tagged FCHSD1 and FCHSD2 proteins resulted in their accumulation at the midbody.

Based on this result, we have constructed FCHSD1 and FCHSD2 knock-out HEK 293T cell lines using CRISPR/Cas9. Knock-out cell lines exhibit cytokinesis defects, resulting in the accumulation of multinuclear cells. Encouraged by this results we are using CRISPR/Cas9 to fluorescently tag FCHSD proteins with mNeonGreen, to visualize them in cells and with mVenusMAP, to find interacting partners by affinity purification and LC-MS/MS.

The specification of the exact role and mechanism of FCHSD proteins in membrane reorganization and cytoskeleton changes during cytokinesis may help us better understand the process and processes by which is regulated.



# The roles of Sin3 in pancreatic $\beta$ -cell function and survival

74

Xiaodun Yang, Yanwen Xu, Ruiying Hu, Guoqiang Gu

The goal of this project is to study how Sin3 regulates  $\beta$ -cell function and survival. Diabetes is caused by the insufficient function of pancreatic  $\beta$  cells, which secrete insulin to lower blood glucose level. While several pancreatic transcription factors have been well studied for directing beta cell differentiation and function, the gene regulatory networks that linking  $\beta$ -cell function, stress response, and survival remain unclear. This project focuses on the roles of a transcription regulator Sin3 (SWI-independent-3, which contains two paralogues Sin3A and Sin3B) in  $\beta$ -cell differentiation, function, and survival. Sin3A and Sin3B are frequently co-expressed within a same set of cells to assemble Sin3/histone deacetylase (HDAC) complex. By interacting with transcription factors, the Sin3/HDAC complex can target particular loci to compact chromatin and repress gene expression. On the other hand, Sin3 has also been shown to act as a co-activator to activate gene expression. One study showed that knockdown of Sin3 downregulated the genes involved in the development of muscle cells. Because of the roles of Sin3 in transcriptional regulation, it has been shown to be essential for cell proliferation and survival. For example, in one study, knockout of Sin3 in mouse embryonic fibroblasts caused cell cycle arrest. In another study, loss of Sin3 in sperm cells led to decreased survival and increased cell apoptosis. These studies indicate that Sin3 plays important roles in maintaining normal cell function and survival by regulating the expression of its target genes. Most importantly, Sin3 has been shown to closely interact with islet-enriched transcription factors in  $\beta$  cells. Consistent with previous studies, my preliminary data showed that inactivating Sin3 in pancreatic progenitors leads to substantial reduction of  $\beta$ -cell mass, partly because of increased levels of cell death. These findings led to my hypothesis that Sin3 maintains both  $\beta$ -cell function and survival by regulating genes involved in both cellular stress and function. To test the hypothesis, we will first examine the roles of Sin3 in  $\beta$ -cell differentiation, function, and survival, by measuring markers of  $\beta$ -cell differentiation, function, and survival. Then, we will examine the gene networks controlled by Sin3 in  $\beta$  cells using RNA sequencing (RNA-seq) and chromatin immunoprecipitation with massively parallel sequencing (ChIP-seq). I envision that completing these proposed studies will reveal key regulatory networks for  $\beta$ -cell gene expression, function, and survival, which may provide novel clues to battle diabetes.

# **A Novel Role for Prostaglandins in Establishing a Developmental Program in the Ductus Arteriosus**

76

Michael T. Yarboro, Ting Wong, Stanley D. Poole, Naoko Brown, Elaine L. Shelton, Jeff Reese

The ductus arteriosus (DA) is an essential vascular shunt connecting the pulmonary artery and aorta allowing oxygenated blood to bypass the developing lungs in utero. After birth, closure of the DA is required for transition to neonatal life. Cyclooxygenase (COX)-derived prostaglandins are key regulators of DA function. Prostaglandin E2 (PGE2) signaling, through the EP4 receptor, is a well-established endogenous DA dilator. Acute COX suppression typically results in constriction of the fetal and neonatal DAs, but some women treated with COX inhibitors during pregnancy have infants with persistently patent DA (PDA). Moreover, deletion of the EP4 receptor in mice counterintuitively results in offspring with PDA, rather than premature DA constriction. Thus, the role of EP4 in the DA is still unclear. We hypothesized that PGE2/EP4 signaling not only maintains DA patency in utero, but also establishes its unique identity through an uncharacterized developmental program. EP4 was the predominant DA PG receptor (by qPCR, in situ hybridization). Using isolated vessel myography we showed that pretreating wild-type DAs with an EP4 antagonist dampens PGE2-induced dilation while enhancing O2- and thromboxane-induced constriction. Furthermore, transuterine injections of this antagonist into fetal mice caused DA constriction in utero. In contrast, deletion of EP4 throughout gestation resulted in PDA. We showed vessels from EP4<sup>-/-</sup> mice are more sensitive to NO-induced dilation, suggesting alterations in this pathway during development. Strikingly, DAs from EP4<sup>-/-</sup> animals constricted when exposed to PGE2. One explanation for this is unmasking of EP1/EP3 receptor activity in the absence of EP4. Alternatively, our data indicate that chronic PGE2/EP4 signaling throughout gestation mediates a developmental program that allows the DA to appropriately respond to perinatal cues, distinct from PGE2's well-established role as an acute vasoactive mediator. Together, these data suggest a novel role for PGE2/EP4 in conferring a unique identity to the DA during development.

# Actin dynamics promotes unique Golgi organization in pancreatic beta cells for efficient insulin packaging

78

Xiaodong Zhu, Ruiying Hu, Roslin Thoppil, Guoqiang Gu, Irina Kaverina

To avoid diabetes, pancreatic beta cells must secrete insulin in response to high blood glucose stimuli. This response requires highly efficient biogenesis of insulin-containing secretory granules at the Golgi apparatus. In mammalian cells, Golgi is localized at the cell center as one extended and continuous complex. It has been proposed that an integral Golgi complex is required for coordinated cargo concentration and sorting. However, the features of the beta cell Golgi, which allow it to function under unusually high physiological demand are unknown.

In non-differentiated cells, it is very well established that cytoskeleton networks are crucial for the Golgi positioning and integrity. Microtubule depolymerization leads to disruption of the Golgi into hundreds of independent mini-stacks, which are dispersed in the cytoplasm. Surprisingly, we find that in the pancreatic beta cells, microtubule disruption leads to the rapid collapse of Golgi ribbon into a ‘donut’-shaped structure. The formation of such structure is driven and maintained by F-actin, suggesting that in beta cells F-actin serves to link Golgi ministacks into a compact structure independently of microtubules.

In non-differentiated cells, Golgi membrane nucleates only a low amount of F-actin, which has been not detected by light microscopy. However, it has been shown that actin is important for vesicle budding from the Golgi and regulation of Golgi morphology, so that F-actin disruption causes collapse of the Golgi apparatus at the cell center. Surprisingly, we find that in beta cells, disruption of F-actin dynamics causes Golgi fragmentation and dispersion, once again suggesting that F-actin maintains Golgi as a compact structure.

Our further analysis shows that beta cells have an extensive amount of F-actin nucleated by Arp2/3 surrounding the Golgi. These actin structures cannot be disrupted by BFA treatment, indicating they are not nucleated directly from the Golgi. Moreover, glucose stimulation facilitates actin dynamics and decreases F-actin concentration associated with the Golgi, allowing the Golgi to extend into a bigger area for efficient budding of insulin granules. Blocking the F-actin dynamics in beta cells leads to accumulation of proinsulin in the Golgi region. Interestingly, we also observe that in the beta cells from db/db, F-actin fails to accumulate at the Golgi region, and the Golgi is dispersed all over the cells, suggesting that the loss of F-actin around the Golgi might be involved in development of diabetes.

# Programmed Necrosis correlates with development and progression of Myelodysplastic Syndromes

80

Jing Zou, Qiong Shi, Patrice Wagner & Sandra S. Zinkel

Myelodysplastic syndrome (MDS) is a bone marrow failure disease. Multiple studies of MDS bone marrow have demonstrated excessive bone marrow cell death, and this increased cell death is thought to play an important role in the pathogenesis of the disease. Our lab has been interested in how the mechanism by which cells die impacts the cellular microenvironment, and pathogenesis of bone marrow failure.

Execution of apoptosis is driven by proteases called caspases that cause collapse of the cell in an immune silent process. Necroptosis is driven by Rip kinases and is characterized by early rupture of the plasma membrane, resulting in an immune response. Necroptosis plays a role in inflammatory diseases as well as host response to infection, and tumors.

Early studies of MDS interpreted increased cell death as apoptotic death, based on older methods that cannot distinguish between necroptosis and apoptosis. When we developed a mouse model in which necroptosis is increased in the bone marrow of mice, we find that the mice die of bone marrow failure with the salient characteristics of MDS (Wagner et al., manuscript in revision).

In this study, we ask whether necroptotic cell death is increased in MDS patient bone marrow, and whether necroptosis correlates with disease subtype. We use Rip1 and phosphorylation of Mixed Lineage Kinase domain-like protein (MLKL), as measures of necroptosis. We use activated caspase 3, a major executioner caspase, as a measure of apoptosis.

A total of 57 MDS cases and 36 control cases consisting of formalin fixed paraffin embedded core biopsies were stained with the apoptosis marker (caspase 3) and necroptosis markers (Rip1, MLKL) and visualized by immunofluorescence. The MDS specimens included cases of low-grade MDS (RCUD, RA, RARS and RCMD) and high-grade MDS (RAEB I and RAEB II), also including transformed AML. The control cases were staging lymphoma bone marrow specimens with no lymphoma infiltration.

Rip1 and MLKL positive staining was present in all cases of low-grade MDS, and more than half of all samples of RAEB. There was minimal staining of cleaved caspase 3 in all of MDS cases. Rip1 and MLKL expression was decreased in disease progression.

This study demonstrates necroptosis in human MDS clinical samples, suggesting that necroptosis but not apoptosis may play a role in the pathogenesis of MDS. Further samples are under analysis to determine whether necroptosis could be a diagnostic marker for distinguishing early MDS from normal, and to correlate changes in necroptosis with progression of disease to AML.

## Co-Sponsors for 2017

### **Integrated Biological Sciences Training in Oncology (IBSTO)**

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 exposure to current cancer research discoveries and exposure to clinical cancer treatment and translational research.

### **Program in Developmental Biology (PDB)**

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.

### **Retreat Committee**

*Steve Hann (Chair)*

*Puck Ohi*

*Ethan Lee*

*Kristi Hargrove (Program Manager)*

*Marc Wozniak*

*Erick Spears (Postdoc Representative)*

*Angela Howard and Karrie Dudek (GSA Co-Presidents)*

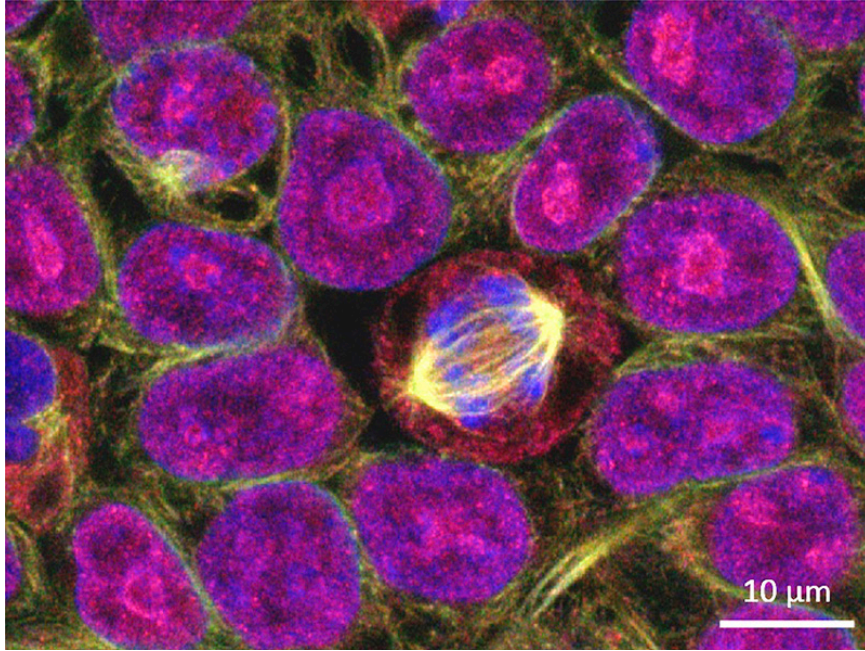
**Department of Cell and Developmental Biology**  
**Fifteenth Annual Retreat May 19<sup>th</sup>, 2017**  
**RETREAT PARTICIPANTS**

Mukhtar Ahmed	Joey Elsagr	Caleb Howard
Erin Aho	Merlyn Emmanuel	Patrick Hu
Abdalla Akef	Amy Engevik	Ruiying Hu
Parker Allen	Amanda Erwin	Won Jae Huh
Alex Andrews	Veronica Farmer	Yongliang Huo
Laura Armstrong	Aidan Fenix	Annastasia Hyde
Goker Arpag	Jonathan Fleming	Rebecca Ihrle
Justin Avila	Nora Foegeding	Brenda Jarvis
Cassandra Awgulewitsch	Maria Fomicheva	Christine Jones
Amrita Banerjee	Audra Foshage	Irina Kaverina
Bahnisikha Barman	Keyada Frye	Anne Kenworthy
Erik Beadle	Sabine Fuhrmann	Sun Wook Kim
Matt Bechard	Hisayo Fukuda	Ela Knapik
Janel Beckley	Allie Fuller	Archana Krishnamoorthy
Kai Bracey	Isabella Gaeta	Juli Krueger
Noah Bradley	Vivian Gama	Kim LaFever
Kendal Broadie	Maureen Gannon	Jennifer Landino
Dylan Burnette	Meredith Giblin	Lynne Lapierre
Manuel Castro	Laura Glass	Ken Lau
Bryan Cawthon	Guoqiang Gu	Elizabeth Lawrence
Jun-Song Chen	Alissa Guarnaccia	Armelle Le Guelte
Chin Chiang	Meena Halaka	Ethan Lee
Colbie Chinowsky	Amanda Halfond	Sora Lee
Andrea Cuentas Condori	Bing Han	Amanda Leung
Sierra Cullati	Steve Hann	Ed Levine
Renee Dawson	Cristina Harmelink	Chris Lord
Jessica Do	Yuliya Hassan	Ian Macara
Yaqing Du	Antonis Hatzopoulos	Jason MacGurn
Karrie Dudek	Nathan Hepowit	Mark Magnuson
Megan Dumas	Chuck Herring	Pallavi Manral
Jeff Duryea	Kung-Hsien Ho	Aaron Mason
Zac Elmore	Angela Howard	Caitlin McAtee

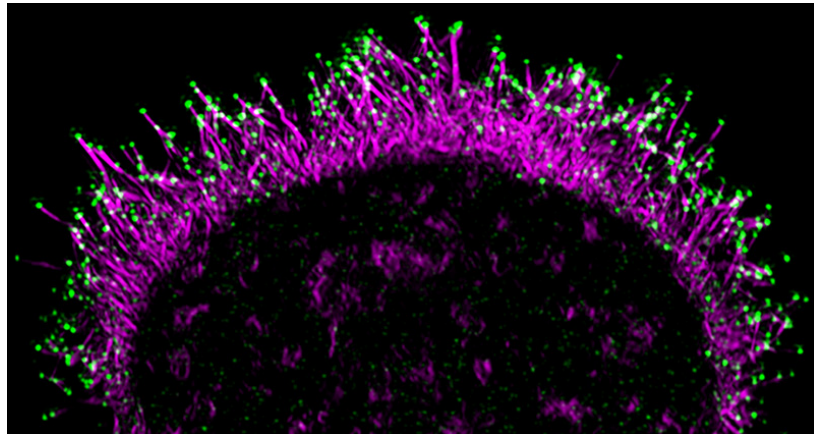
**Department of Cell and Developmental Biology**  
**Fifteenth Annual Retreat May 19<sup>th</sup>, 2017**  
**RETREAT PARTICIPANTS**

Heather McCartney	Gabrielle Rushing	Indrayani Waghmare
Rebecca McWhirter	Kenyi Saito-Diaz	Xiaoxi Wang
Anna Means	Leshana Saint-Jean	Meredith Weck
Megan Merolla	Christi Salisbury-Ruf	Annabelle Williams
Anne Meyer	Anneke Sanders	Marcin Wos
David Miller	Shinya Sato	Yanwen Xu
Bryan Millis	Leah Sawyer	Xiao-Dun Yang
Edwin Morales Obregon	Cherie Scurrah	Xu Yang
Abigail Neiningner	Jim Sears	Michael Yarboro
Leif Neitzel	Lindsey Seldin	Marija Zanic
Victoria Ng	Erica Shannon	Xiaodong Zhu
Stephen Norris	Manisha Sharma	Sandra Zinkel
Barbara O'Brien	Qiong Shi	Jing Zou
Melanie Ohi	Titan Shih	
Ryoma Ohi	Joey Simmons	
Natalya Ortolano	Bhuminder Singh	
Andrea Page-McCaw	Prashant Singh	
Sierra Palumbos	Justine Sinnaeve	
Kyungho Park	Chloe Snider	
Amrita Pathak	Michelle Southard-Smith	
Amrit Patra	Erick Spears	
Roxanne Pelletier	Claire Strothman	
John Penn	Wesley Sun	
Julia Pinette	Lakshmi Sundararajan	
Tessa Popay	Nilay Taneja	
Meagan Postema	William Tansey	
Kinsey Qi	Roslin Thoppil	
Susan Qualls	Ajit Tiwari	
Will Ramos-Lewis	Kathryn Trogden	
Michael Ray	Erica Tross	
Jeff Reese	Jessica Tumolo	
Liping Ren	Matt Tyska	
Elaine Ritter	Gokhan Unlu	

## Additional Images

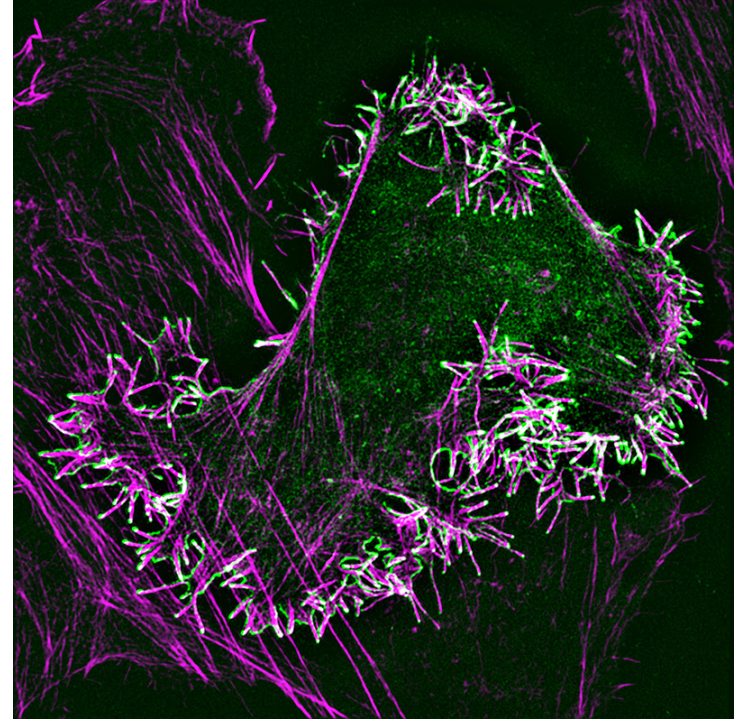
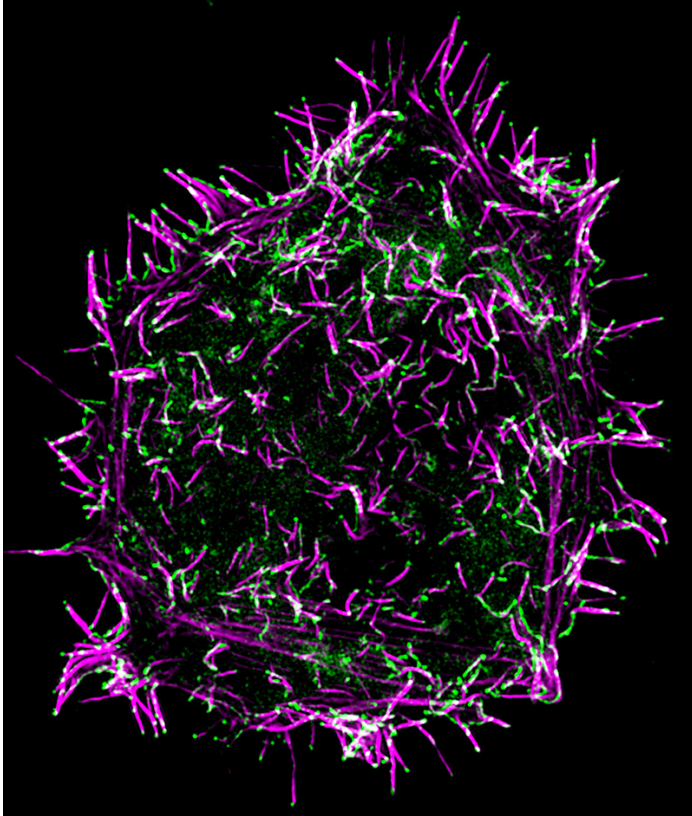


Natalya Ortolano and Megan Rasmussen (Gama Lab). Colocalization of a Cullin ubiquitin ligase (red) with the mitotic spindle (green) in human embryonic stem cells (hESCs). hESCs were stained with Hoechst and fluorescently labeled antibodies against  $\alpha$ -tubulin (green) and Cullin ubiquitin ligase (red). The image was acquired using a Zeiss 880 LSM microscope with an AiryScan detector. The software used for AiryScan image processing was Zen version 2.1 (Zeiss).



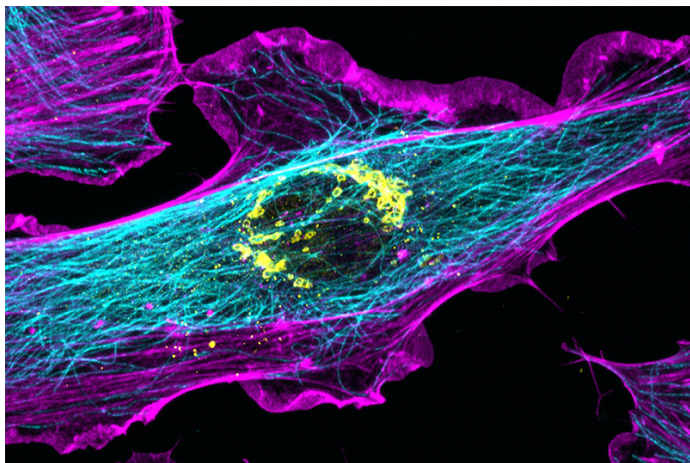
Meagan Postema (Tyska Lab). A structured illumination microscopy (SIM) image of microvilli protruding from a cultured intestinal epithelial cell. The cell is stained for the actin-binding protein EPS8 (green) and phalloidin (magenta).



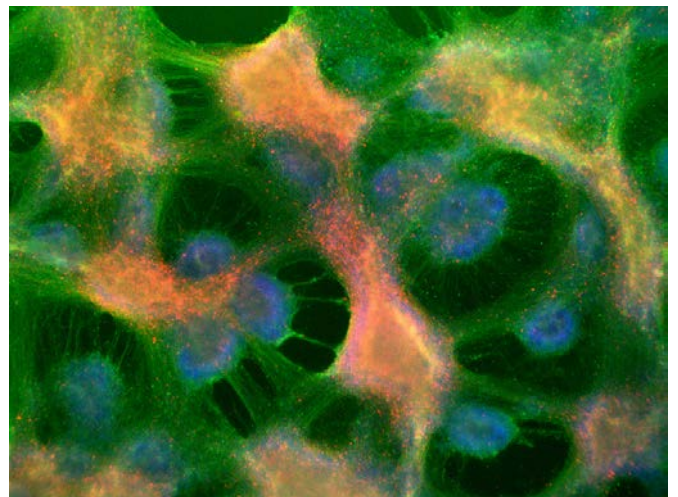


Meagan Postema (Tyska Lab). Filopodial protrusions formed from overexpression of the I-BAR domain containing protein IRTKS in a B16F1 cultured cell. EGFP-IRTKS (green) and mCherry-UtrCH (magenta).

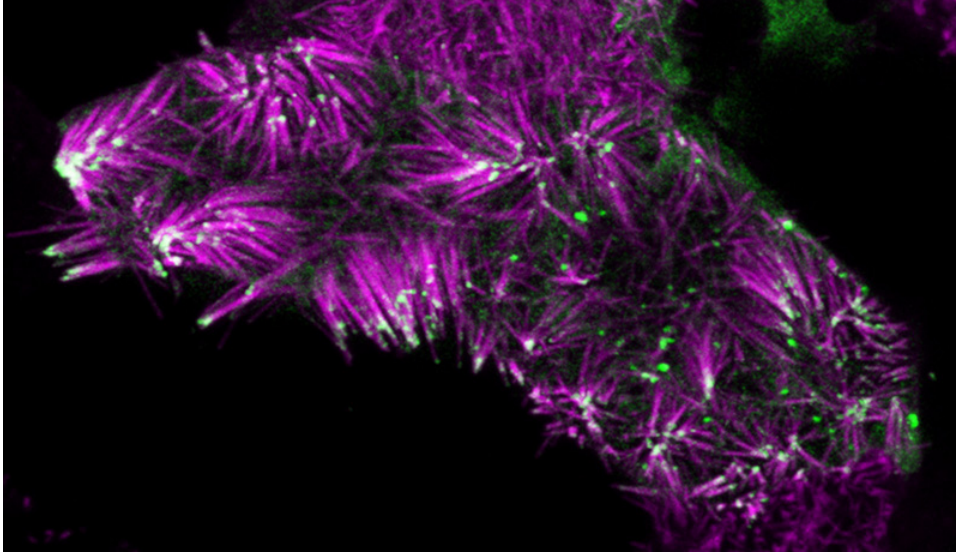
Meagan Postema (Tyska Lab). Filopodial protrusions formed from overexpression of the I-BAR domain containing protein IRTKS in a B16F1 cultured cell. EGFP-IRTKS (green) and mCherry-UtrCH (magenta).



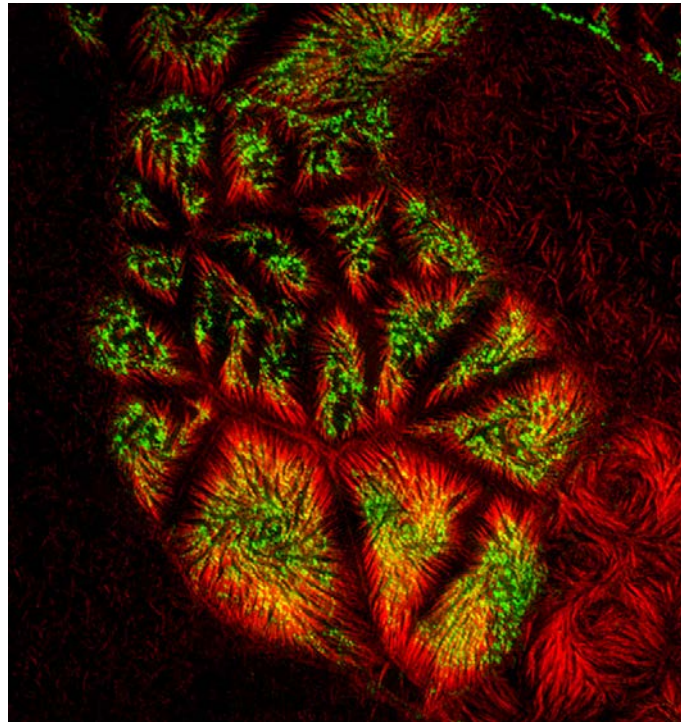
Anneke Sanders, Kaverina Lab. Maximum intensity projection of a human pulmonary artery endothelial cell immunostained for Golgi (yellow), microtubules (cyan), and actin (magenta). Acquired using laser scanning confocal microscopy.



John Snow. Immunofluorescent staining of long-term iPSC-derived neuronal culture. High-yield neuronal derivation of control human iPSCs is highlighted by clusters of GABAergic populations (red) amongst a majority of glutamatergic cells. Cells fixed at post-induction, captured at 4x magnification on an EVOS Microscope in the Ess Laboratory.



Meredith Weck (Tyska Lab). Confocal image of myosin-7b (green) targeting to the distal tips of microvilli, labeled with mCherry-espinal (magenta), in LLC-PK1-CL4 kidney epithelial cells.



Meredith Weck (Tyska Lab). SIM image of CACO-2BBE intestinal epithelial cells overexpressing myosin-7b (green) and stained for F-actin (red).