



PROGRAM

***Please enjoy the art displays throughout the day!**

Basement Gallery

8:15 – 9:00 AM

POSTER HANGING & BREAKFAST

3rd floor student lounge

Please see the check-in desk for poster assignments

9:00 – 9:15 AM

MEETING INTRODUCTION

Basement Auditorium

9:15 – 10:30 AM

SESSION 1 TALKS

Basement Auditorium

Dr. Joshua Bembenek, *Wayne State University*

“Cell Cycle Control of Vesicle Trafficking in Oocyte Meiosis”

Dr. Amanda Erwin, *University of Michigan*

“Molecular Visualization of Neuronal TDP43 Pathology *In Situ*”

Dr. Jaap van Buul, *University of Amsterdam*

“Cortical tensile actin bundles rule: How the actin cytoskeleton regulates the endothelial barrier function”

10:30 – 10:45 AM

COFFEE BREAK

Basement Hallway

10:45 AM – 12:00 PM

SESSION 2 TALKS

Basement Auditorium

Dr. Allen Liu, *University of Michigan*

“Mechanobiology of Cell Invasion and Migration”

Dr. Aniruddha Ray, *University of Toledo*

“Democratizing Biomedical Measurements”

Sasha Demeulenaere, *Loyola University Chicago*

“Reciprocal control of plasticity and contractility in vascular smooth muscle cells”

12:00 – 1:00 PM	LUNCH <i>3rd floor student lounge</i> Seating can be found in the corridors and studios. Please feel free to browse CellulART's gallery and the museum grounds.
1:00 – 1:15 PM	PROMEGA <i>Basement Auditorium</i>
1:15 – 2:00 PM	SCIENTIST/ARTIST TALK <i>Basement Auditorium</i> Dr. Alex Ritter <i>Senior Scientist, Altos Labs</i> "Visualizing Killer Immune Cells with Microscopes and Hot Sculpted Glass"
2:00 – 4:00 PM	POSTER SESSION AND ART PRESENTATIONS <i>3rd floor student lounge</i> Coffee provided <i>Basement Gallery</i> Pieces presented by Department of Art students and Dr. Alex Ritter Student artwork information provided below
4:00 – 5:00 PM	SESSION 3 TALKS <i>Basement Auditorium</i> Katherine Koning, <i>University of Michigan</i> "Mechanoaccumulation of zyxin family LIM domain proteins at tricellular junctions" Electra Coffman, <i>The Ohio State University</i> "Arvcf stabilizes adherens junction and Ankyrin-B protein within the lens fiber cell membrane" Dr. Benjamin Perrin, <i>Indiana University</i> "Myosins shape the tips of developing stereocilia on auditory sensory cells"
5:00 – 5:10 PM	CELLULART IMAGE COMPETITION SPONSORED BY IBIDI AWARDS ANNOUNCED <i>Basement Auditorium</i>
5:10 – 6:10 PM	KEYNOTE PRESENTATION <i>Basement Auditorium</i> Dr. Christopher Chen <i>Founding Director, Biological Design Center</i> <i>William F. Warren Distinguished Professor of Boston University</i> <i>The Wyss Institute for Biologically Inspired Engineering at Harvard University</i> "Forces & Complexes & Adhesions. Oh My!"
6:10 – 9:00 PM	TOLEDO MUSEUM OF ART EXPLORATION Attendees may visit the galleries of the Toledo Museum of Art, connected to the Center for the Visual Arts, for free (donation is encouraged) until 9:00 pm.

Underskin

Blake Bialorucki

Archival on canvas
16" diameter

Pink Goes Good with Green

Faith Rison

Archival paint, assorted plastic and glass
beads, and E6000 glue on canvas
6" x 6"

zwierzę (fauna)

Mikayla Maziarz

Felt

8" x 5"

roślina (flora)

Mikayla Maziarz

Felt

8" x 5"

The Smiles of Sandgrass

Ladi'Anna Ritsema

Acrylic on canvas

16" x 16"

Illumination

Sabreen Hamdah

Graphite on paper

24" x 18"

Contractions

Erilyn Spooner

Acrylic paint, metallic paint, UV glue,
gems, cotton fabric, plastic pearls, and
sealed with modge-podge on canvas.

10" x 18"

Untitled

Molly Thomas

Acrylic paint on canvas

10" x 10"

Window

Molly Thomas

Colored and clear glass, glass paint,
solder, copper foil, hobby came, wire,
and chain

8" x 12"

Spore Vessel

Duncan McLean

Ceramic, Flocking, Underglaze,
Acrylic Paint
24" x 8" x 8"

Untitled

Stacia Bowers

clay, canvas, acrylic paint,
rhinestones, resin
16" x 12"

The Hidden World

Malak Ismail

Mixed media

36" x 22.5"

Stentor coeruleus

Ella Schlueter

Acrylic on canvas
16"x20"

POSTER ABSTRACTS

Poster #1

Biochemical Mechanisms Driving Migration of Apical-Out Spheroids

Skylar Lynch, Aarna Kalita, Niharika Patel, Daniel Conway

Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio

Epithelial cells, a type of cell that makes up the epidermis and lines the interiors of hollow organs, depend on apical-basal polarity for proper organization and function of epithelial tissue. Apical-basal polarity is established by segregation of different transmembrane proteins to the apical surface, which faces inward towards the hollow lumen, and the basal surface, which faces outward toward the extracellular matrix (ECM). Recent work by our group has shown increased RhoA activation causes epithelial spheroids to rapidly change apical-basal polarity, by “flipping inside out”, through rearrangement of cells, a process termed eversion, resulting in apical-out spheroids. In unpublished studies we have observed that apical-out spheroids gain the ability to migrate, whereas normal apical-in spheroids do not migrate. A hallmark of carcinomas with loss of apical-basal polarity is increased invasion, indicating a possible correlation between apical-out polarity and migration capabilities. We hypothesized that the mechanism for migration of apical-out spheroids involves altered signaling of surface receptors and proteins. To study this process, Madin-Darby canine cells (MDCK), an epithelial cell line, were used. When cultured in basement membrane MDCK cells assemble into spheroids with apical-in polarity, and can be stimulated with RhoA activation to evert, rapidly switching to apical-out polarity. We performed immunofluorescence staining for phosphorylated proteins to evaluate how apical-basal polarity switching alters signaling, enabling increased migration after eversion. Immunofluorescent staining for phosphotyrosine showed an absence of phosphorylated proteins to the apical surface in normal, apical-in spheroids. However, apical-out spheroids had a high level of phosphotyrosine immunostaining on the apical membrane, suggesting a change in signaling at the apical membrane. Analysis of migration of normal compared to apical-out spheroids shows normal spheroids rotate in place, whereas apical-out spheroids become motile and migrate within the basement membrane. We have also identified multiple human cancer cell lines with apical-in, apical-out, or a mixture of polarity when cultured in basement membrane, providing multiple cell lines to investigate changes in signaling and increase migration of apical-out cancers. In conclusion, our work suggests that apical-out polarity enables migration through altered signaling at the apical membrane. This study aims to establish a model for understanding the biochemical changes that drive migration of apical-out spheroids.

Poster #2

Investigating the mechanoaccumulation and function of the LIM domain proteins zyxin and LPP at epithelial tricellular junctions under mechanical challenge

Katherine Koning¹, Allen Liu^{1,2}, and Ann Miller^{1,3}

¹Cellular & Molecular Biology Program, University of Michigan, Ann Arbor, Michigan; ²Department of Mechanical Engineering, University of Michigan, Ann Arbor, Michigan; ³Department of Molecular, Cellular, Developmental Biology, University of Michigan, Ann Arbor, Michigan

Epithelial cells form cohesive sheets that generate barrier function to restrict the entry of harmful substances and selectively regulate permeability. Epithelia must sense and respond to mechanical challenges in order to maintain their function. One way cells can sense increased mechanical stress is by recruitment of LIM domain-containing proteins to strained actin filaments. Here, we investigate how two members of the LIM domain protein family, zyxin and LPP, help maintain epithelial integrity and barrier function at tricellular junctions (TCJs), sites which experience heightened mechanical strain compared to bicellular junctions. We find that zyxin and LPP are enriched at TCJs in the *Xenopus* embryonic epithelium under baseline tension and further mechanoaccumulate at TCJs when tension increases. To determine which domains contribute to the mechanoaccumulation of zyxin and LPP, chimeras where the LIM domains were swapped were generated. Additionally, conserved aromatic residues associated with LIM domain mechanosensitivity were mutated. We find that the chimeras and proteins with the conserved aromatic mutations mechanoaccumulate at TCJs to a lesser extent than the wildtype proteins. Currently, we are overexpressing zyxin and LPP to test their roles in regulating junctional integrity and barrier function since high levels of zyxin is associated with kidney disease. Complementary experiments in mammalian epithelial cells (MDCK II) are underway. Together, these studies will advance our understanding of the dynamic interplay between LIM domain proteins and the actin cytoskeleton in maintaining junction stability under mechanical challenge.

Poster #3

CALML4 acts as a light chain for class 7 myosins

Sadika-Tul-Jannat (Tonu) and Scott William Crawley

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

The class 7 myosins, Myo7A and Myo7B, are actin-based motor proteins essential for creating and maintaining specialized apical protrusions on transporting and sensory epithelial cells throughout the human body. Mutations in Myo7A cause several sensory disorders, most notably Usher Syndrome Type 1B, the leading cause of deaf-blindness in humans. In contrast, Myo7B genetic defects have not been directly linked to human disease. Our laboratory previously identified calmodulin-like protein 4 (CALML4) as a novel light chain for both class 7 myosins. However, the role of CALML4 in regulating myosin 7 activity within cells remained unclear. Here, we demonstrate that CALML4 functions as a calcium-independent light chain that specifically binds to the third IQ motif (IQ3) in the lever arm of both Myo7A and Myo7B. To assess binding specificity of IQ3, we tested the affinity of this motif for other potential light chain partners, including essential light chain (ELC), regulatory light chain (RLC), and conventional calmodulin (CAM). IQ3 showed no appreciable binding to these alternative light chains, indicating that this motif has evolved to be highly specific for CALML4. We subsequently identified specific mutations within IQ3 that disrupted CALML4 binding and discovered that these mutations produced strikingly different effects on Myo7A versus Myo7B function in epithelial cells. Our findings reveal that CALML4 serves distinct regulatory roles for each class 7 myosin: it acts as an inhibitory light chain for Myo7A while functioning as an obligate (essential) light chain for Myo7B. This study represents the first comprehensive characterization of the role of CALML4 in class 7 myosin biology and highlights the specialized regulatory mechanisms governing these important motor proteins.

Poster #4

Recruitment of the fission yeast pericentrin Pcp1 to the spindle pole bodies by calmodulin requires calcium

Marium Zehra¹, Debatrayee Sinha² and Qian Chen¹

^{1,3}*Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio;*

²*University of Michigan, Ann Arbor, Michigan*

Calmodulin is an evolutionally conserved eukaryotic calcium sensor. It binds calcium ions directly through each of its four EF-hand motifs. Surprisingly, the budding yeast *S. cerevisiae* calmodulin can carry out its essential functions without binding calcium (Geiser et al. 1991). In contrast, fission yeast *S. pombe* Cam1 needs to bind calcium for its essential functions, but it remains unclear why. Here, we combined genetics and quantitative microscopy to uncover such calcium-dependent functions of Cam1. We constructed a novel cam1 mutant through mutating three of its EF-hands motifs so that the mutant protein Cam1-E4 binds calcium poorly. The cam1-E4 mutant was temperature-sensitive. At the restrictive temperature, 17% of the mutant cells possessed no spindle pole bodies (SPBs). Microscopy revealed that the cam1-E4 mutation reduced the number of the essential pericentrin Pcp1 on the SPBs by 69%. This was accompanied by a 20% reduction of the polo kinase Plo1 at the mitotic SPBs. During cytokinesis, the contractile ring assembly and maturation took three times as long in the mutant than the wild type. Either supplement of extracellular calcium or overexpression of Pcp1 rescued the cam1-E4 mutant. We concluded that the essential calcium-dependent function of fission yeast Cam1 is to recruit the pericentrin Pcp1 to the SPBs, suggesting calcium is required for the assembly of SPBs.

Poster #5

Fission yeast phosphatase PP2C Ptc1 is a potential interactor of the Pkd2 ion channel

Pritha Chowdhury and Qian Chen

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Autosomal dominant polycystic kidney disease (ADPKD) is a human genetic disorder caused by mutations in either of the polycystin genes which encode cation channels. The fission yeast *Schizosaccharomyces pombe* possess a single essential polycystin homolog, Pkd2, a mechanosensitive and calcium-permissive channel localized to the plasma membrane at the cell tips and the cytokinetic cleavage furrow. While Pkd2 regulate both cell growth and cytokinesis, the signaling pathways involved the functions of this ion channel remains unclear. First, we investigated the potential interactions between pkd2 and the essential calcium sensors such as calmodulin Cam1 and calcineurin Ppb1. pkd2 and cam1 mutants interacted with each other positively. In contrast, pkd2 and ppb1 had no strong genetic interaction. Next, we took three genetic approaches to identify novel interactors of Pkd2. First, we looked for the high-copy suppressors of the pkd2 mutants. We took advantage of the previous published results of a high-copy suppressor screen using the budding yeast mutant of flc1 and flc2. Flc2, an FAD transporter and *Saccharomyces cerevisiae* homolog of pkd2, functionally complement pkd2 deletion in *S. pombe*. We selected four candidate genes from the screen. Among these, ptc1, the catalytic subunit of the fission yeast PP2C phosphatase emerged as a strong candidate. AlphaFold3 predicted that Ptc1 and Pkd2 interact with each other directly. Secondly, we carried out the RNA sequencing of the pkd2 mutants and identified a list of potential effectors of pkd2 through transcriptomic profiling. These include a few proteins required for the intracellular iron homeostasis and cell wall biosynthesis. Lastly, we isolated a revertant mutant of the temperature-sensitive pkd2 mutant and sequenced its genome. Analysis of this genomic data will help us to identify potential interactor of pkd2.

In summary, our multi-pronged genetic study identifies ptc1 as an interactor of pkd2, laying the foundation for future investigations into the molecular mechanisms of Pkd2-mediated signaling in cell growth and division.

ELUCIDATING THE AUTOINHIBITION MECHANISM OF MICROTUBULE-BINDING PROTEIN EB1

Alaa F. Abdelazziz, Shansa P.E. Jayaweera, Sayandeepa Raha, and Holly V. Goodson

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana

EB1 is a microtubule plus-end binding protein that plays an important role in many cellular processes including cell division and regulation of microtubule dynamics. Previous work has shown that EB1 undergoes autoinhibition, but the autoinhibition mechanism is still poorly defined. Because proper regulation of microtubule dynamics is important for cell division, and because some of the most effective anti-cancer drugs target microtubule dynamics, elucidating the mechanism of EB1 autoinhibition and its release may open ways to discover new cancer therapy.

Our working hypothesis is that the C-terminal domain of EB1 binds to the N-terminal domain, allowing the conversion of EB1 from the active state to the autoinhibited state. To test this hypothesis, we are using a combination of computational and experimental approaches. As a first step, we used AlphaFold to predict the autoinhibited structure of EB1 (there is no crystal structure for the whole protein). We then used AlphaFold to predict the complex of EB1 and its binding partner P150, a protein that is known to relieve the EB1 autoinhibited state by acting as an allosteric activator. The observation that P150 binds to the coiled-coil of the EB1 C-terminal domain suggests that it relieves EB1 autoinhibition. AlphaFold was able to predict the active structure of EB1 in the presence of P150 which agrees with the crystal structure of EB1 coiled coil and P150 complex. Our computational models suggest that the EB1 C-terminus (EEY) acts as a fishing hook that catches the P150 and fixes it in a position to relieve the EB1 autoinhibited state, allowing EB1 to bind with other partner proteins (via the hydrophobic cleft) and microtubules (via the calponin homology domain of the N-terminus).

To test our computational results, we are presently using isothermal titration calorimetry to study the binding between bacterially expressed EB1 fragments. We are also trying a different approach based on cross-linking the N- and C- termini using EDC and sulfo-NHS followed by protease treatment. Our combined computational and experimental approaches to characterize the autoinhibition mechanism of EB1 may offer potential paths for therapeutic intervention in cancer and other diseases.

Poster #7

Characterization of the putative mechanosensitive OSCA/TMEM63 channels of fission yeast

Varmila Kulasegaram, Abhishek Poddar, Oumou Sidibe, and Qian Chen*

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

OSCA/TMEM63 channels are a family of evolutionally conserved cation channels, found from yeast to humans. In multicellular organisms, they are essential for the responses to osmotic stresses. Mutation of human OSCA channels are mostly associated with neurodevelopmental disorders. However, their cellular functions remain unclear. Here, we investigated the cellular functions of the putative OSCA channels using the unicellular model organism fission yeast *Schizosaccharomyces pombe*. We used the amino acid sequence of the plant OSCA channel in a to identify three putative homologues. We named them Osc1, Osc2 and Rsn1 respectively. They had not been previously characterized in fission yeast. AlphaFold3 predicted that all three of them possess 11 transmembrane helices. a long intracellular loop between helix 1 and 2 that is unique to the OSCA channels and a large intracellular C-terminal domain. Therefore, the fission yeast OSCA channels are structurally similar to those mammalian and plant OSCA channels despite their relatively low sequence identity (< 20%). Through tagging the C-terminus of these three proteins with GFP at their endogenous locus, we found that Osc1-neonGreen, Osc2-GFP, and Rsn1-GFP localized to the vacuolar membrane, the plasma membrane, and the intracellular membrane compartments respectively. Osc2 was the most abundant protein among the three, while Rsn1 was the least. The localization of Osc2-GFP was altered by osmotic stress. Their localization appeared to be cell-cycle independent, except for Rsn1 which translocated to the equatorial division plane during cytokinesis. The deletion mutants of *osc1*, *osc2* and *rsn1* were viable. All three of them grew similarly as the wild type cells did under normal conditions. Their progression through cell cycle was normal, based on the staining of their DNA and septum and on FACS analysis. The *osc2* null mutant was hyper-sensitive to 100mM CaCl₂. Our preliminary work indicates that these OSCA channels may mediate adaptation of fission yeast cells against osmotic and ion stress but they are dispensable for cell proliferation under normal conditions.

Poster #8

Validating a patient-derived induced pluripotent stem cell model for studying neurofilament accumulations in Charcot-Marie-Tooth disease

Claire E. Holecek¹, Paula C. Monsma², Mehmet E. Yalvaç² & Anthony Brown²

¹Undergraduate Neuroscience Major, The Ohio State University, Columbus, Ohio; ²Department of Neuroscience, The Ohio State University, Columbus, Ohio

Charcot-Marie-Tooth (CMT) is a progressive hereditary disease of the peripheral nervous system. The type studied in our lab is CMT2E, which is caused by mutations in a protein called neurofilament light (NFL), a critical subunit of neurofilaments. Neurofilaments are necessary polymers of the nerve cell's internal cytoskeleton that provide structure and space within the axon to allow other materials to move along the axon. Induced pluripotent stem cells are an exciting technology that can be used as a model of disease. We are using induced pluripotent stem cells derived from CMT2E patients. In neurons differentiated from these cells, neurofilaments have been shown to accumulate abnormally. The goal of this study is to verify this accumulation in our laboratory. As a control, we used an isogenic cell line in which the mutation causing the disease had been corrected using gene editing. Using immunofluorescence microscopy, we quantified the neurofilament content in cell bodies and axons. We observed a statistically significant accumulation of neurofilaments in the cell bodies of the diseased cells, as well as a depletion of neurofilaments in their axons. Since neurofilaments are synthesized in the cell body and transported into and along axons, this suggests that there is a disruption in the mechanism by which the neurofilaments leave the cell body in the diseased neurons. Significantly, these experiments validate patient-derived induced pluripotent stem cells as a model to study CMT2E in cell culture.

Role of the cell-surface serine protease matriptase in corneal epithelial homeostasis

Michael Flynn^{1*}, Joseph Lundgren^{1,2}, Elizabeth Berger³, Linda Hazlett³, Mallika Somayajulu³, Ebrahim Abdul Shukkur³, Denise Bessert³, Karin List^{1,2}

¹Department of Pharmacology, ²Department of Cancer Biology, ³Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University, Detroit, MI, USA

Purpose: Maintaining a healthy corneal epithelium is vital to normal ocular health and consists of a complex network of cellular mechanisms and dynamic processes to maintain its homeostatic functions. To date, the role of extracellular proteases in ocular research has mostly centered on their harmful effects in corneal wound healing and infection. Based on our preliminary data, we hypothesize that the cell-surface serine protease matriptase, in contrast, promotes corneal epithelium homeostasis via actions involved in maintenance of barrier function and tight junction integrity.

Methods and Results: We have developed multiple in vitro and in vivo models to study the functional and molecular consequences of matriptase loss-of-function conditions. In human corneal epithelial cells (HUCL), we observe a decrease in barrier function and tight junction integrity, upon RNAi-mediated matriptase silencing by transepithelial resistance assays and immunocytochemistry. Mechanistically, we are investigating the relevance of matriptase-mediated cleavage of two transmembrane proteins TROP-2 and EpCAM for tight junction protein regulation in vitro. We previously generated a matriptase hypomorphic mouse model wherein these mice have a global reduction of matriptase expression and observed increased dendritic cell populations in the peripheral cornea. Additionally, we have generated and characterized an inducible matriptase knockout model using mice carrying loxP-flanked matriptase alleles and a ubiquitously expressed tamoxifen-inducible Cre-recombinase and have successfully achieved localized ocular matriptase gene ablation with topical eye drops containing tamoxifen.

Conclusions: The goal of this project is to gain a mechanistic understanding of matriptase in corneal epithelial homeostasis and injury and identify targetable factors/pathways for therapeutic options.

Poster #11

Vinyl Sulfones as Covalent Inhibitors for Antifungal Targeting in *Candida albicans*

Tatiana de Souza, Heather Conti, Ronald Viola

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Candida albicans is an opportunistic human fungal pathogen responsible for both mucosal and deep tissue infections. Immunocompromised individuals, such as HIV and cancer patients, are particularly vulnerable to *Candida* infections, with over 40% of nosocomial infection-related mortalities. However, the current arsenal of antifungal drugs is limited due to the challenge of developing selective agents against fungal targets without causing significant human side effects. Moreover, increasing resistance to existing antifungal drugs necessitates the exploration of novel drug targets. Aspartate semialdehyde dehydrogenase (ASADH) has emerged as a promising target for antifungal therapy due to its critical role in the aspartate pathway, responsible for the synthesis of almost 40% of essential amino acids in all known microbes. Recent studies identified 2,3-Dichloro-1,4-naphthoquinone (Inhibitor 8) as a potent *Candidal* ASADH (CaASADH) inhibitor; however, its reversible inhibition mechanism raises concerns regarding the transient nature of its interactions, which may require higher drug concentration and frequent dosing. Irreversible inhibitors, on the other hand, offer potential advantages by forming covalent adducts with their targets, leading to irreversible inactivation. Vinyl sulfones have demonstrated promising enzymatic activity, showing rapid and irreversible inhibition of the isolated *Candida albicans* ASADH (CaASADH) enzyme. Preliminary studies in our lab indicate that a series of vinyl sulfone derivatives exhibit fungistatic, fungicidal, anti-metabolic, anti-filamentation, and anti-biofilm activity against *C. albicans*. Our objective is to identify potent vinyl sulfone derivatives that selectively target *C. albicans* via covalent inhibition, offering a novel approach to antifungal therapy.

Poster #12

The Role of a Novel miRNA-2251 in Commissural Axon Guidance

Harindi Suriyaarachchi, Bhakti Khot & Guofa Liu

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Precise control of guidance receptor expression is vital for axon guidance in the developing nervous system. The spatiotemporal regulation of these receptor expression in developing neurons could function as a molecular switch to control axonal responses to extracellular guidance cues. Differential expression of Plexin A1, a repulsive receptor of Semaphorins, in spinal commissural neurons facilitates midline crossing; however, the mechanism(s) underlying this regulation remains unknown. Here, we investigate the role of microRNAs (miRNAs) in modulating Plexin A1 expression. The ectopic expression of a dual fluorescence reporter containing the full-length chicken Plexin A1 3' UTR in the developing chicken neural tubes revealed its involvement in the regulation of protein expression in developing commissural neurons, suggesting that miRNAs might play a role in this process. To identify which miRNAs are responsible for this regulation, we conducted miRNA sequencing of developing commissural neurons. Bioinformatics analysis showed that Novel miRNA-2251 is capable of binding chicken, mouse and human Plexin A1 3'UTRs. Overexpressing miRNA-2251 significantly increased commissural axons (CAs) stalled in the floor plate. Moreover, results from qRT-PCR and whole mount in-situ hybridization showed high expression levels of miRNA-2251 at stage HH 18-19, which is corroborated with our sequencing data. Altogether, these results suggest that Novel miRNA-2251 may play a role in regulating Plexin A1 expression to modulate the Semaphorin-mediated CA guidance. RT-qPCR on electroporated sides of GFP-miR-2251 versus the GFP-scramble miRNA showed no significant difference in cPlexin A1 mRNA levels, suggesting miR-2251 acts on cPlexin A1 mRNA via translational repression. To further evaluate our hypothesis, we will examine the activity of endogenous Novel miRNA-2251, the functional role of Novel miRNA-2251, and the collaborative effect of other candidate miRNAs in Semaphorin/Plexin A1-mediated CA guidance during the midline crossing.

Poster #13

Smooth muscle myosin activity drives vascular smooth muscle cell differentiation

Sasha K. Demeulenaere, Margaret A. Bennet, Patrick W. Oakes, Jordan R. Beach

Department of Cell and Molecular Physiology, Loyola University Chicago, Chicago, Illinois

Vascular smooth muscle cells (VSMCs) are highly dynamic and contractile, playing a key role in blood flow regulation. VSMCs exist on a functional spectrum between the differentiated “contractile” phenotype and the dedifferentiated “synthetic” phenotype. While their contractile nature is important for regulating blood pressure, their ability to downregulate contractility and promote migration is necessary for tissue repair in response to injury. Misregulation of these transitions can be maladaptive, contributing to diseases such as atherosclerosis through excessive VSMC proliferation and migration.

The mechanisms linking VSMC contractility and phenotype are currently not well understood. Smooth muscle myosin II (SMII) plays an essential role in VSMC contractility by assembling into bundles that bind and pull on the actin cytoskeleton. SMII expression directly correlates with VSMC dedifferentiation, and the field has suggested that the downregulation of SMII expression is an effect of this phenotype switch. However, contractility has been established throughout cell biology as causative in cell fate and differentiation. We hypothesize that SMII lies upstream of the switch from the differentiated to dedifferentiated phenotypes, and therefore helps drive VSMC phenotype.

We engineered an endogenously EGFP-labeled SMII murine model to directly observe and quantify stepwise changes in both the expression, localization, and dynamics of VSMC contractile machinery throughout phenotypic transitions. We cultured the EGFP-SMII aortic tissue ex-vivo and observed VSMCs migrating onto the culture dish within days. In this transition, we observed a notable difference in EGFP-labeled SMII filament exchange dynamics using FRAP: the native tissue ex-vivo, shortly after euthanasia (differentiated), has stabilized SMII bundles that show little exchange of monomers. The individual VSMCs isolated from the tissue and grown in culture days later (dedifferentiated) exhibit significant SMII monomer exchange. Additionally, we have seen that transduction of human primary VSMCs expressing pathology-associated SMII motor domain variants increases SMII turnover and decreases whole-cell contractility. These results suggest that SMII activity plays a causative role in driving VSMCs from one phenotype to another. To further test this hypothesis, we designed constitutively active and inactive SMII regulatory light chain constructs by modulating the phosphorylation site at Thr18/Ser19. We observed that inhibiting SMII activity via the light chain increases SMII turnover and decreases the fraction of SMII monomers assembled into filaments, while activating SMII stabilizes the filaments, further supporting our hypothesis. Our work reveals molecular insights into the regulation of VSMC differentiation and suggests that SMII expression itself plays a significant role in determining VSMC phenotype.

Poster #14

Evaluation of Novel Vinyl Sulfone Inhibitors Against Diverse Candida Species

Shreya Gupta, Tatiana De Souza, Heather Conti

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Candida species are generally harmless commensals but can cause life-threatening infections in immunocompromised individuals. Non-albicans Candida (NCAC) species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* are of particular concern due to their increasing resistance to standard antifungal therapies. This study evaluates the antifungal potential of two vinyl sulfone-based aspartate semialdehyde dehydrogenase (ASADH) inhibitors, JDD-1-44A and CFC-1-13Q, against clinically relevant NCAC species. In vitro susceptibility testing was performed to assess the growth-inhibitory effects of these compounds. Preliminary findings indicate that both inhibitors exhibit promising antifungal activity, suggesting their potential as novel therapeutic agents against drug-resistant Candida infections. Further studies are warranted to elucidate their mechanisms of action and therapeutic applicability.

Poster #15

Arvcf stabilizes adherens junctional and Ankyrin-B protein within the lens fiber cell membrane

Electra Coffman and Timothy Plageman, Jr.

College of Optometry, The Ohio State University, Columbus, Ohio

The ocular lens is an avascular transparent tissue responsible for focusing light onto the retina to produce a clear image. Uniquely, it grows throughout an organism's entire lifespan without cell turnover. Structural disruptions in these long-lived lens cells can lead to cataracts, a disease marked by lens opacification. Although age is the highest global risk factor for cataract development, how cell architectural changes contribute to its progression remains poorly characterized. We previously established that mice lacking *Arvcf*, a gene encoding a cell adhesion protein, develop disrupted adherens junctions (AJs) as soon as 1 month with premature cortical cataracts by 5 months of age. To identify important functional components of lens cell AJ's, protein lysates from control and mutant lenses were analyzed by fractionation, immunoprecipitation, western blot, and/ or mass spectroscopy. Loss of *Arvcf* induces a dramatic decrease in N-Cadherin as well as its catenin binding partners. It was also observed that N-cadherin associates well with both *Arvcf* and Ankyrin-B (AnkB) and that *Arvcf* strongly associates with the AnkB binding partner NrCAM. In vivo immunofluorescence further demonstrates that *Arvcf* facilitates the recruitment of AnkB to the short side lens fiber cell junctions by at least one month. As AnkB knock-out mutants (AnkB^{-/-}) also possess cell adhesive disruptions, we hypothesized that *Arvcf* and AnkB function in a similar pathway to maintain lens fiber cell N-Cadherin and lens transparency over age. It was observed that AnkB is required for maintaining *Arvcf* in the broad side lens fiber cell junctions as soon as E17.5. These data suggest that *Arvcf* may function in part to facilitate the association of Ankyrin-B to the cadherin-catenin complex and vice versa. These findings further suggest a shared role for *Arvcf* and AnkB in stabilizing lens fiber AJs and architecture which may provide molecular clues into cataract development.

Poster #16

Tricellular Zipping is driven by formin-mediated actin polymerization

Ashley Shahi, Babli Adhikary, Katherine Konning, and Ann L. Miller

Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan

Epithelial cells undergo changes in their topological relationships with neighboring cells during processes such as cell intercalation and rosette formation. As cells' topological relationships change, the cell-cell junctions connecting them must also remodel. Here, using the *Xenopus laevis* embryonic epithelium as a model, we describe a previously uncharacterized mode of junction remodeling, which we term tricellular zipping. In this process, a long, thin epithelial extension stretches toward a vertex where four or more cells meet. Two of these cells then extend their shared interface and zip together, while a third cell (the cell with the long extension initially) recedes, relocating the tricellular vertex to the end of the newly formed junction. This remodeling contributes to the transition from irregular epithelial geometries at blastula stage to the more regular hexagonal packing that emerges as the embryo develops from blastula to gastrula stage. To visualize cytoskeletal dynamics during this process, we used fluorescently-tagged LifeAct to monitor F-actin. Super-resolution microscopy with mosaic labeling revealed distinct actin behaviors in receding versus zipping cells. In the receding cell, F-actin displayed pulsatile dynamics near the zipping front, whereas in the zipping cells, actin accumulated at the remodeling tricellular vertex. Actin filament polymerization is often regulated by Arp2/3 and/or formins, which generate branched actin networks and unbranched actin filaments, respectively. Preliminary imaging revealed little Arp2/3 accumulation at sites of tricellular zipping, suggesting that this process may instead be formin mediated. To test this hypothesis, we inhibited formin activity with SMIFH2 in blastula-stage embryos. Blocking formin-mediated actin assembly disrupted tricellular zipping, resulting in four characteristic outcomes: failure to initiate zipping, stalled remodeling vertices, bulbous geometry at the tip of the long cellular extension, or complete—but slower—zipping events. We further quantified these differences by measuring the length of newly formed interface. Our ongoing work is focused on identifying which formins are specifically regulating this process. Together, these findings indicate that formin-mediated actin polymerization is critical for tricellular zipping.

Molecular Visualization of Neuronal TDP43 Pathology In Situ

Amanda Erwin^{1,2}, Martin Fernandez^{1,3}, Matthew Chang¹, Durga Attili^{1,2,4}, Michael Bekier⁴, Emile Pinarbasi^{4,5}, Jennifer Russ⁶, Renaldo Sutanto^{1,2}, Dafydd Thomas⁵, Xinfei Shen⁷, Ryan Baldrige^{6,8}, Elizabeth Tank⁴, Sami Barnada⁴, Shyamal Mosalaganti^{1,2,3,6}

¹Life Sciences Institute, University of Michigan, Ann Arbor, Michigan; ²Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan; ³Department of Biophysics, College of Literature, Science and the Arts, University of Michigan, Ann Arbor, Michigan; ⁴Department of Neurology, University of Michigan, Ann Arbor, Michigan; ⁵Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor, Michigan; ⁶Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan; ⁷Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; ⁸Cellular and Molecular Biology Program, University of Michigan Medical School, Ann Arbor, Michigan

Transactive response DNA/RNA binding protein of 43 kDa (TDP43) is a ubiquitously expressed nuclear protein essential for RNA metabolism. Its nuclear exclusion and cytosolic aggregation are signature molecular events in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), yet the mechanisms driving TDP43 mislocalization and the in situ architecture of TDP43 inclusions within intact human neurons have remained elusive. Emerging evidence implicates RNA dyshomeostasis as a contributing factor to pathological TDP43 mislocalization and aggregate formation.

Here, we establish that the addition of small, GU-rich RNA oligonucleotides to human iPSC-derived neurons (iNeurons) robustly recapitulates hallmarks of ALS/FTLD pathology, including TDP43 nuclear efflux, cryptic splicing, neurotoxicity, and formation of cytosolic, phosphorylated TDP43 inclusions. Crucially, we employ an innovative cryogenic correlative light and electron microscopy (cryo-CLEM) pipeline—integrating the molecular specificity of fluorescence imaging with the nanometer-scale resolution of cryo-electron tomography (cryo-ET) - to capture, for the first time, the ultrastructure of mislocalized, endogenous TDP43 at nanometer resolution within a physiologically intact cellular context.

Our imaging reveals that cytosolic TDP43 first accumulates in ribosome-excluded, phase-separated condensates before forming highly ordered filaments within autophagosomes and lysosomes, implicating acidification and macromolecular crowding as drivers of fibrillization. Quantitative analysis shows a size-dependent relationship between lysosomes and TDP43 fibril burden, linking lysosomal enlargement to impaired degradative capacity and aggregate accumulation. We extend these findings by combining immunoelectron microscopy, biochemical fractionation, and fluorescence imaging in both non-neuronal cell models and ALS/FTLD patient tissue, confirming that TDP43 filaments localize within autophagic and lysosomal compartments across contexts relevant to human disease.

Together, our work provides the first high-resolution visualization of TDP43 mislocalization and aggregation within human neurons and establishes a robust, tractable platform for dissecting the molecular and cellular basis of TDP43 proteinopathies. This integrated approach not only illuminates critical cell biological processes governing proteostasis and neuronal vulnerability in ALS/FTLD, but also opens new avenues for the development and assessment of therapeutic strategies targeting TDP43 mislocalization and clearance.

Poster #18

Regulation of the Cellular Distribution of the Tobacco NtNIP2;1 Silicon Transporter

Kritika Singh, Wendy Zellner, Marc Marusak, Korbin Leung, Jennifer Boldt, Scott Leisner

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Silicate minerals are abundant in the Earth's crust. Plants acquire silicon in the form of silicic acid (SA). SA supplementation permits plants to better deal with a wide variety of stress. We recently discovered a tobacco gene (NtNIP2;1) encoding a Si transporter. This gene is a member of the NIP subfamily of aquaporins (AQPs). While AQPs generally permit water movement, NIPs also transport solutes. For example, the NIP2 subfamily can transport SA. Little is known about the trafficking or regulation of Si transport proteins. We hypothesized: 1) that the NtNIP2;1 protein is transported to the cell surface 2) this transport is influenced by SA and post-translational modifications (PTM). To investigate the transport of NtNIP2;1 within tobacco cells, we generated plant expression constructs that tagged NtNIP2;1 with red fluorescent protein (RFP), used agrobacterium infiltration to deliver the constructs into *Nicotiana benthamiana* cells, and spinning disk confocal microscopy to visualize NtNIP2;1. Our data showed that NtNIP2;1 localized to the cell surface and accumulated in a compartment adjacent to the nucleus. Additionally, we discovered an increase in fluorescence intensity of NtNIP2;1-RFP at the cell surface, and a decrease around the nucleus when plants were supplemented with SA. To determine how much time is needed for the SA relocation response, plants were treated with SA for three time points (3, 7 and 18 days). Confocal microscopy showed that an increase in NtNIP2;1-RFP redistribution to the cell surface was observed after 18 days of SA treatment. To determine if PTM plays a role in NtNIP2;1 localization, we examined its amino acid sequence. Eukaryotic Linear Motif (ELM) predicted three potential phosphorylation sites. We individually generated mutants preventing phosphorylation at each of these three sites by changing serine to alanine. Each of these mutants showed reduced protein stability. Additional single amino acid substitution mutations at each of these sites to aspartic acid were done to mimic phosphorylation. Interestingly, each of these phosphomimic mutants showed increased accumulation at the cell surface mimicking the NtNIP2;1 redistribution response in the presence of SA. Taken together, these data suggest that SA-mediated NtNIP2;1 redistribution is regulated by phosphorylation.

Poster #19

Functional analysis of the *S. cerevisiae* Flavin carrier proteins, a homologue to the TRP Channel Pkd2 in *Schizosaccharomyces pombe*

Oluwaseun Osundina and Qian Chen

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a leading cause of kidney failure, affecting approximately 1 in 1,000 individuals. Mutations in the PKD1 and PKD2 genes, which encode polycystin proteins, disrupt ion homeostasis and promote cyst formation in renal tissues. PKD2 encodes polycystin-2 (PC-2), a TRP-like cation channel, yet its molecular mechanisms remain unclear. Due to challenges in studying PC-2 in human cells, we employ yeast models, particularly *Saccharomyces cerevisiae*, *Candida albicans*, and *Schizosaccharomyces pombe*, to investigate homologous pkd2-type channel/TRP channel proteins. *S. cerevisiae* Flc2 is involved in calcium homeostasis under hypotonic conditions, and *S. pombe* pkd2 regulates calcium spikes during cytokinesis and cell wall integrity, whereas pkd2 in *Candida albicans* (referred to as CaPkd2) has been very minimally studied. This study integrates phylogenetic analysis and experimental approaches, including gene replacement, yeast transformations, and live-cell imaging, to delineate Flc2's and CaPkd2's roles in fission yeast cells and determine whether these channels can functionally replace *S. pombe* Pkd2. Our findings suggest that Flc2 and CaPkd2 can functionally compensate for pkd2 deletion in *S. pombe*, restoring viability and localizing to key cellular structures. Further investigations using confocal microscopy, fluorescence imaging, and genetic analyses will clarify Flc2's impact on cell division and calcium dynamics. These insights will enhance our understanding of PC-2 function and its implications in ADPKD pathogenesis, potentially informing therapeutic strategies.

Molecular mechanisms of IL-17 signaling in megakaryocytes during oropharyngeal candidiasis

Sydney Conrad, Dylan Launder, Heather Conti

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Megakaryocytes are most known for their role in producing platelets to maintain hemostasis, however they are also critical for host responses to different pathogens including the opportunistic human fungal pathogen *Candida albicans*. Platelets are also directly involved in the host defense against fungal pathogens and can kill *Candida* in vitro as well as recruit neutrophils during inflammation. Oral megakaryocytes have been shown to have an immune phenotype, expressing higher levels of antigen presenting cell-related molecules (MHCII and B7-2) as compared to megakaryocytes found in the bone marrow. Additionally, oral megakaryocytes respond to interleukin-17 (IL-17), with IL-17 signaling being required for expansion of megakaryocytes in the context of oropharyngeal candidiasis (OPC). An IL-17-mediated immune response is essential for protection against OPC as signaling through the IL-17 receptor leads to the upregulation of genes that control neutrophil recruitment to the site of infection and the production of antimicrobial peptides that work to clear *Candida* from the mucosa. Mechanistic studies on the IL-17 signaling cascade have previously been limited to epithelial and mesenchymal cells and have never been explored in megakaryocytes. We aim to elucidate the downstream molecular mechanisms of IL-17R signaling in megakaryocyte-mediated antifungal immunity during OPC.

Outer Dense Fiber of Sperm Tails 2 (ODF2) Has Species-Specific Localization

Derek Kluczynski¹, Laleh Yaghutian Nezhad¹, Luke Achinger¹, & Tomer Avidor-Reiss^{1,2}

¹*Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio;*

²*Department of Urology, The University of Toledo, Toledo, Ohio*

Mammalian spermatozoa have evolved significantly and developed accessory structures that aid in sperm travel. One such structure, the outer dense fibers (ODFs), has been shown to assist in spermatozoon movement. The outer dense fibers, which comprise four main ODF proteins (ODF1, ODF2, ODF3, and ODF4), are implicated in infertility if mutations occur.

The ODFs are connected rostrally at the base of the striated columns in the neck and laterally along the axoneme along ~2/3 of the principal piece. We have recently shown that during spermatozoon tail beating, both the left and right sperm necks deform, kinking (bending) the head. How the axoneme transmits force from its dynein motors to the neck and the head is unknown. Still, we hypothesize that the ODFs participate in this process because they connect the principal piece of the axoneme with the neck structures.

To study the ODFs of rabbit and human spermatozoon, we mapped outer dense fiber protein localization along the sperm tail using immunofluorescence microscopy, as the precise localization of the ODF proteins is not well characterized.

As expected, in both rabbits and humans, ODF2, the most well-studied ODF protein, localizes along the neck, midpiece, and most of the principal piece. Interestingly, there seems to be species-specific differences in the enrichment of ODF2 in the necks of sperm cells. In rabbits, ODF2 is significantly enriched in the neck compared to humans. Furthermore, unexpectedly, we detected ODF1 only in the neck of the rabbit sperm and not in the tail.

These results may indicate that the striated columns and capitulum are potentially labeled in rabbit and human spermatozoa, in line with previous immuno-EM work. In rabbit sperm necks, ODF2 localizes to every part of the neck and fills the neck. This contrasts with other sperm neck proteins that have specific localizations in the neck, such as CP110 and CEP135. Lastly, this suggests that the ODF proteins play crucial roles in sperm neck structure, and not just in the sperm's tail, suggesting that ODF proteins one through four would need further investigation across species' spermatozoa. In the future, we will use antisense oligonucleotides to knock down ODF2 mRNA in rabbit somatic and sperm cells to see head-kinking differences between control and experimental groups.

Mechanosensitive myopathy: FHL1 missense variants impair strain sensing response

Cara L. Barnett, Jordan R. Beach, Patrick W. Oakes

Loyola University Chicago, Maywood, Illinois

Cells are constantly subject to mechanical forces that challenge their ability to function. This is especially true in muscle cells, where high contractile forces propagate within and between cells. The actin cytoskeleton is the dominant mechanical element in all cells, including muscle. Actin filaments are semiflexible polymers, and as such can bend and stretch, but have limits to physical perturbations. Cells, therefore, have developed mechanisms to protect and reinforce actin filaments under load. Previous in vitro and cellular studies have identified a subset of LIM (Lin-11, Isl-1, Mec-3) domain proteins that recognize and stabilize strained actin. There are ~70 LIM domain genes in humans, and several have mutations that have been tied to different genetic diseases. The molecular mechanisms of these diseases and any contribution from actin strain sensing, however, remain poorly understood.

FHL1 (four and a half LIM domains 1) is specifically expressed in muscle. Clinically, FHL1 missense variants are characterized by progressive muscle weakness, joint contractures, cardiac arrhythmias, hypertrophic cardiomyopathy, and rarely, aortic dilation. We hypothesize these diseases derive from impaired FHL1 mechanosensing. To test this, wild-type FHL1 and pathogenic missense variants in the LIM domains (p.W138S, p.C225R, p.C240W, p.C292Y) were tagged with mScarlet and each construct was independently expressed in mouse embryonic fibroblasts stably expressing Zyxin-EGFP as a positive control. Unlike zyxin, which localizes to focal adhesions and stress fibers, FHL1 is predominantly cytoplasmic. Using our established laser photoablation assay, we measured both FHL1 and zyxin recruitment to stress fiber strain sites. Wild-type FHL1, like zyxin, consistently recognized strained actin. All four missense variants, however, demonstrated impaired strain sensing. Our future goals include testing these variants in myocytes and primary smooth muscle cells using both laser photoablation and cyclic stretch. We also plan to test the FHL1 variants' impact on cell contractility by measuring their traction forces. While the role of mechanosensing proteins in maintaining cytoskeletal integrity in muscle remains poorly defined, our findings demonstrate that disease-causing variants reduce FHL1's ability to recognize strained actin.

Mechanically-induced Septin Networks Protect Nuclear Integrity during Confinement

Margaret E. Utgaard, Alexia Caillier, Shreya Chandrasekar, Joseph J. Tidei, Jordan R. Beach, Patrick W. Oakes

Department of Cell and Molecular Physiology, Loyola University Chicago, Chicago, Illinois

Physiological environments are crowded and cells are constantly under tension from internal and external forces. The cytoskeleton allows cells to maintain their shape in these conditions. While the contributions of actin, microtubules, and intermediate filaments to cell mechanics have been explored in great detail, the role of the septin cytoskeleton has been less clear. Here we investigate the role of septins in cell mechanics. Using a SEPT2-Halo knock-in fibroblast cell line, we observed that septins preferentially localize to actin filaments under the nucleus, moving with it as the cell migrates. Enucleating the cells resulted in viable cytoplasts that lacked central actin-septin networks. Surprisingly, replacing the nucleus with a glass bead (8-12 μm) was sufficient to partially recover this septin network. As these results suggest that pressure from the nucleus can stimulate septin network assembly we used a dynamic confinement system to apply a compressive force to cells. During confinement septins accumulated specifically under the nucleus, translocating from other regions in the cell. In comparison, actin and myosin intensity increased uniformly across the cell. To test whether internally generated forces were sufficient to induce septin accumulation, we measured septin recruitment during migration through a microfluidic device. Septins consistently assembled in regions where the nucleus was pressed against the walls of the chamber. Using iPALM super-resolution microscopy, we determined that septin filaments are specifically positioned between the actin stress fibers and the membrane. This suggests septins are responding to pressure on the cortex, potentially reinforcing these regions of the cytoskeleton. To investigate this hypothesis, we tested whether loss of septins impaired transwell migration or cells' ability to withstand compression. Knockdown of SEPT7 resulted in greater nuclear deformation after migrating through a transwell, and in compression nuclei were more likely to rupture. Together these data suggest that septins may be acting as a dynamic shock absorber and protecting the cortex, and thereby the nucleus, from excessive strain. This highlights a novel mechanosensing role for septins in the cytoskeleton, and illustrates how septins contribute to maintaining cell shape and facilitate migration through complex environments.

The role of Polycystin-2 channel in the mouse kidney cell division

Dhananjaya Rankoth Gedara, Qian Chen, Wei Niu

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder in humans. A major feature of this disease is the excessive proliferation of kidney epithelial cells, leading to renal cyst formation. ADPKD is primarily caused by mutations in the PKD1 or PKD2 genes, which encode the TRP family ion channels PC1 and PC2, respectively. Both proteins are essential for the development of multicellular organisms, but their cellular functions remain poorly understood.

Here, we investigated the potential role of PC2 in cell division by characterizing Pkd2 knockout mouse inner medullary collecting duct kidney cells (iMCD3). First, we quantified mitosis in these mutant cells using time-lapse fluorescence microscopy, immunostaining with chromatin, microtubule, and tight junction markers, and fluorescence-activated cell sorting (FACS). The proportion of Pkd2-null dividing cells was comparable to that of wild-type cells. However, Pkd2 knockout cells progressed from prophase to cytokinesis 6.5% faster than wild-type cells. Next, we measured intracellular calcium concentrations using a genetically encoded ratiometric calcium reporter, GCaMP-mCherry. Pkd2 knockout cells exhibited cytoplasmic calcium increases during cell division, similar to wild-type cells. However, mutant cells showed a 37% reduction in calcium uptake under hyperosmotic shock compared to wild-type cells within the first 5 minutes of stress.

Finally, in 3D cell culture, Pkd2 knockout and wild-type kidney cysts were quantified for growth over 10 days using immunostaining and live microscopy. After 10 days, Pkd2 knockout cysts exhibited significantly greater circularity than wild-type cysts ($p = 2.72747E-11$). Taken together, these findings demonstrate that PC2 modulates mitotic progression and promotes calcium efflux under stress conditions, suggesting that this ion channel functions as a novel regulator of mitosis through ER-mediated calcium efflux.

QUANTIFYING FORCES IN AMOEBOID CANCER CELL MIGRATION

Alexia Caillier and Patrick Oakes

Department of Cell and Molecular Physiology, Loyola University of Chicago, Chicago, Illinois

Metastasis occurs when epithelial cells from the primary tumor undergo an epithelial to mesenchymal transition (EMT) which promotes migration. In the confined tumor environment, these cells begin to bleb and display amoeboid characteristics, further enhancing their ability to migrate through complex environments. They correlate with increased invasion, poor prognosis, and greater resistance to pharmacological treatment. Surprisingly, the mechanisms of amoeboid cancer cell migration in confinement remain unknown, making it challenging to block the propagation of these highly invasive cells. Contrary to mesenchymal migration, which is characterized by the presence of focal adhesions and cycles of expansion and contraction, amoeboid cancer cells are thought to push on the substrate using pressure driven blebs, migrating in an adhesion independent and friction based manner. There are three important caveats to this hypothesis, however: First, previous work on adhesion characterization was done in vitro using rigid passivated substrates. Secondly, passive glass surfaces are not perfect, since they allow a small amount of protein adsorption from the cell culture medium, resulting in some adhesive ligands remaining. Third, multiple models of adhesion independent migration have been proposed but not validated with experimental measurements. Also, other works done in 3D collagen gels have shown that amoeboid cancer cells appear to pull and align collagen fibers as they migrate, while adhesive proteins such as talin, vinculin, paxillin, PI3K and integrin B1 localize at the base of transient blebs and colocalize with collagen. These results suggest that we could expect cells to possibly use adhesion in vivo.

We have developed a deformable 3D-like environment that allows us to measure cell force generation during confined migration. This approach addresses each of the limitations mentioned above and allows us to measure the amplitude and directionality of forces (e.g. pushing or pulling) and the molecular mechanisms responsible (e.g. adhesion-dependent or non-specific friction) of amoeboid cancer cells migrating in confinement. In this project, I measure the force generation dynamics of confined amoeboid cancer cell migration. Results from these experiments will give us a better understanding of how these insidious cells manage to spread. This work could thus ultimately lay the foundation for therapies to prevent cancer treatment resistance and additional metastases.

Poster #26

Characterization of the Mechanism of Microtubule Dynamic Instability Using Artificial Intelligence

Alexander W. Simmons^{1,2}, Walter J. Scheirer³, Holly V. Goodson^{1,2}

¹University of Notre Dame, Biophysics PhD. Program, Notre Dame, Indiana; ²University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana; ³University of Notre Dame, Department of Computer Science and Engineering, Notre Dame, Indiana

Microtubules (MTs) display a surprising behavior where they switch randomly between periods of persistent growth and intermittent collapse, known as dynamic instability (DI). The transition from growth to shortening is known as “catastrophe,” while the reverse transition is called “rescue.” Dysregulation of MT DI can lead to diseases ranging from neurodegeneration to heart failure, and while drugs that target MT DI are important therapies for diseases as diverse as cancer and gout, the mechanism of DI remains mysterious, inhibiting attempts to design new drugs targeting this process. The classical explanation for DI is that, as GTP-tubulin polymerizes, delayed GTP to GDP hydrolysis results in a GTP-cap. This GTP-cap promotes polymerization. When this GTP-cap is lost, GDP-tubulin is exposed at the MT end, resulting in catastrophe. However, there are issues with this classical description of DI. First, it is unclear as to what the exact mechanisms behind catastrophe and rescue are. Moreover, multiple groups have reported evidence that additional DI phases (e.g., pauses and stutters) exist. This information implies that the current GTP-cap model is not sufficient to fully describe MT DI. Multiple groups have proposed and provided evidence that the mechanisms of catastrophe and rescue depend on various aspects of the MT’s tip structure. This project aims to elucidate the dimer-scale mechanisms behind DI by using artificial intelligence (AI) to analyze the results of a previously validated dimer-scale simulation.

We report the development of Neural Networks (NNs) capable of predicting the DI behavior of a simulated MT at a moment in time based only on the configuration of its tip structure. These NNs were trained and evaluated using datasets where tip structures were labeled as exhibiting one of three different DI behaviors: growth, shortening, or stutter. These models could predict the behavior of previously unseen MT tips with an accuracy greater than 80% accuracy. This performance exceeds what would be expected of random guessing or confusion between classes, suggesting that NNs can identify features present in the MT tip structure that correlate with DI behavior. Machine learning interpretability methods were applied to these features to identify MT tips with similar structure-behavior relationships. In the future, we plan to test examples of structures by simulating their behavior using our dimer-scale model to examine the strength of these structure-behavior relationships. This work will provide foundational knowledge for research into MT-based treatments for cancer, heart, and neurodegenerative diseases.

Poster #27

Combinatorial Coding in *C. elegans* Olfaction as a Means For Behavioral Decision Making

Chelyan Edwards, Md Zubayer Hossain Saad, Bruce Bamber

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

The nematode *Caenorhabditis elegans* uses olfaction to navigate environments with predators, mates, and food, and utilize olfactory inputs to drive appropriate chemotactic behavior. The mechanisms the worm uses for decoding olfactory inputs are, however, poorly understood. We are using whole-network Ca^{++} imaging combined with microfluidics-based behavioral assays to better understand olfactory coding in *C. elegans*. We recently showed that the repulsive odorant 1-octanol (1-oct) is encoded combinatorially in the periphery as both an attractant and a repellent and these opposing afferents are integrated centrally to determine the sensitivity and valence of the behavioral response. This combinatorial strategy differs fundamentally from labeled line coding, a widely held explanation for olfactory coding in *C. elegans*. We are now investigating whether another odorant is coded combinatorially in *C. elegans*. The odorant isoamyl alcohol (IAA) is an interesting case study because it is the canonical example of labeled line coding in *C. elegans* olfaction. We found the worms show both attraction and repulsion to a single concentration, and simultaneous activation of the attractive and repulsive neurons. Thus, our data show that a combinatorial coding mechanism is used for IAA as well. These results suggest that, like insects and mammals, *C. elegans* may make extensive use of combinatorial coding for olfactory processing.

Dissecting Cytoskeletal Dynamic Instability with PhuZ: A Viral Tubulin Homolog as a Model System

Archita Bhattacharya and Holly V. Goodson

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana

Microtubules are central to maintaining cellular architecture and division, largely through the process of dynamic instability (DI), in which filaments stochastically switch between growth, shortening, and stuttering phases. Because canonical tubulin assembly requires complex chaperones, I employ PhuZ, a bacteriophage-derived tubulin homolog that displays DI while being easily expressed in *E. coli*. PhuZ filaments, composed of three protofilaments, undergo GTP-GDP hydrolysis dependent polymerization and display unique features such as spontaneous branching, making them an excellent model for dissecting polymer dynamics.

Firstly, I characterized critical concentrations (CCs) of wild-type PhuZ and its mutants having altered amino acids involved in lateral and longitudinal bond formation at subunit interfaces. One such mutant I studied is Q297N where glutamine in 297th position was mutated to asparagine. Sedimentation assays confirmed GTP-dependent polymerization in Q297N, and steady-state absorbance assays revealed a CC NetAssembly (subunit concentration beyond which population mass persistently increases) of 1.6 μM —lower than the 2.5 μM of wild-type. Transmission electron microscopy (TEM) showed Q297N forms shorter, more numerous filaments, suggesting increased nucleation frequency which would explain the lower CCNetAssembly. Both wild-type and mutant PhuZ filaments also exhibited spontaneous branching without accessory proteins, a phenomenon I am expanding to lateral bond mutants R290K and D303E.

Secondly, I examined GMPCPP-stabilized seeds (short filaments stabilized with GMPCPP, an unhydrolysable GTP analog). Intact and sonicated seeds supported branching and filament growth when mixed with subunits at low concentration and GTP, demonstrating that branching can initiate from both nascent and pre-formed filaments even at low free subunit concentration.

Finally, I applied Statistical Tool for Automated Dynamic Instability Analysis (STADIA) to analyze kymographs (length-history plots) generated using TIRF microscopy timelapse datasets of fluorescently labeled PhuZ. STADIA's machine-learning framework recognizes subtle phases beyond traditional two-state models. Preliminary results suggest that PhuZ filaments mainly exist in sustained growth with limited catastrophes (rapid shortening event). To improve accuracy on noisy kymographs, I am developing a Python workflow that integrates Photoshop-assisted edge detection with STADIA.

Together, the biochemical, imaging, and computational approaches show that mutations in PhuZ subunit interfaces significantly alter CCs, filament morphology, and branching. By combining TEM, TIRF, and advanced DI analysis, I aim to establish PhuZ as a tractable system for dissecting cytoskeletal polymer dynamics, providing new insights into mechanisms of DI and spontaneous branching.

Poster #29

Investigating mechanisms neighboring cells use to regulate vertebrate epithelial cytokinesis

Samantha Wheeler¹ and Ann L. Miller^{1,2}

¹Cellular and Molecular Biology Ph.D Program, University of Michigan Medical School, Ann Arbor, Michigan;

²Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan

Cytokinesis poses a challenge to the integrity of epithelial tissues: as the dividing cell and its neighbors undergo significant shape changes, they must maintain their cell-cell junctions and preserve the tissue's barrier function. Using the epithelium of *Xenopus laevis* embryos as an intact vertebrate epithelial tissue model, we have demonstrated that during cytokinesis, neighboring cells respond mechanosensitively to furrow ingression by generating their own RhoA-mediated actomyosin arrays adjacent to the cleavage furrow, which plays an important role in the determining speed and success of cytokinesis. Furthermore, mechanically uncoupling the dividing cell from its neighbors disrupts cell packing following cytokinesis and leads to increased barrier leaks during cytokinesis. Here, we test the hypothesis that decreasing actomyosin contractility in neighboring cells will cause cytokinesis defects. *Xenopus* embryos were mosaically microinjected with dominant negative RhoA. At late blastula stage, we imaged cytokinesis events where one or both neighbor cells express dominant negative RhoA and the dividing cell does not. We find a higher incidence of "asymmetric" cytokinesis events in dividing cells with one dominant negative RhoA neighbor and one control neighbor. Additionally, we observed changes in cell packing post-cytokinesis and increased incidence of early cytokinesis failures. These data provide another piece of evidence supporting the idea that a mechanosensitive response in neighboring cells during cytokinesis is important for maintaining tissue homeostasis. In ongoing work, we are identifying upstream regulators of RhoA activation in neighboring cells, as well as investigating the role of branched F-actin in neighboring cells during cytokinesis.

Poster #30

Regulation of human cortical development by the autism risk gene POGZ

Chamod Dias¹, Nithini Rajakaruna¹, Daniel C. Jaklic² and Wei Niu^{1,2}

¹Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio;

²Department of Neurology, University of Michigan, Ann Arbor, Michigan

The haploinsufficiency of the pogo transposable element derived with zinc finger domains (POGZ) gene is strongly associated with autism spectrum disorder (ASD). POGZ is an HP1 α binding protein that plays a crucial role in regulating gene expression and cell cycle progression. Previous studies in mouse models demonstrated that POGZ loss disrupts cortical development, reducing intermediate progenitors, upper-layer neurons, and synapse formation. Similarly, heterozygous POGZ mutations in 2D human stem cell-derived neuronal cultures impair neural differentiation, neurite outgrowth, and branching. But still unclear how POGZ haploinsufficiency alters Neural progenitor cells proliferation and lineage at early stages. Both rodent models and 2D cultures have limitations in recapitulating human cortical development.

Here, we employed human cerebral organoids (hCOs) to investigate how POGZ haploinsufficiency affects neural progenitor cell division and differentiation. Day 10 POGZ^{+/+} (wild type) and POGZ^{+/-} (heterozygous) organoids were analyzed using immunostaining with PAX6, POGZ, phosphorylated histone Ser28 (PHH3), and acetylated Tubulin antibodies. We first confirmed that POGZ^{+/-} organoids exhibited reduced POGZ protein expression compared to WT organoids. In addition, our preliminary data shows that POGZ^{+/-} organoids exhibited less PAX6⁺ neural progenitor cell population and less PHH3⁺ mitotic cells compared to the WT organoids. Furthermore, we found that POGZ^{+/-} organoids had higher lumen-to-organoid area ratio than WT organoids. These preliminary findings suggest that POGZ haploinsufficiency impairs neural progenitor proliferation and disrupts neuroepithelial organization.

Tricellular Zipping: A Process of Epithelial Remodeling that is Driven by Pulsatile Calcium Signaling in *Xenopus* Embryos

Andrea G. Laboy-Figueroa¹, Babli Adhikary¹, Michaela Purvis^{1,2}, Adam Martin², Ann Miller¹

¹Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan; ²Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Epithelial cells form adhesive connections through cell-cell junctions, maintaining a selective barrier despite significant shape changes during development and tissue homeostasis. This is accomplished through the active remodeling of cell-cell junctions, a process largely driven by actomyosin contractility. Using live microscopy of the *Xenopus* embryonic epithelium, we have characterized a previously unobserved remodeling event, which we have called "tricellular zipping." Tricellular zipping involves the resolution of a long, thin cell extension at a higher-order junction. As two cells 'zip' together to lengthen their bicellular interface, and the other cell recedes, thus forming a new tricellular vertex at the end of zipping. Our work reveals that tricellular zipping process is accompanied by transient flashes of cytoplasmic calcium within the cell extension. Each Zipping event is accompanied by multiple flashes and an increase in rate of de-novo bicellular junction length. Preliminary study shows that a calcium flash is associated with increase in the rate of de-novo bicellular junction formation. Additionally super-resolution microscopy with mosaic labeling revealed distinct actin behaviors in receding versus zipping cells. In the receding cell, F-actin displayed pulsatile dynamics near the zipping front. Preliminary data indicate a correlation between calcium and actin pulses in the zipping extension. We hypothesize that the long tricellular extensions are under increased tension and that can activate mechanosensitive calcium channels, leading to the observed pulsatile mechanosensitive calcium signaling. Ongoing work is focused on further characterizing the correlation between pulsatile calcium and actin dynamics and how it changes the rate of de-novo junction formation. We are also working on using FLIPPER-TR to measure membrane tension at the site of zipping to understand if the long extensions are sites of higher tensions causing mechanosensitive calcium signaling. Together, these findings suggest that tension-driven, calcium-dependent signaling plays a role in tricellular junction formation.

SGEF, a RhoG-specific GEF, influences lumen morphology and collective cell migration in 3D epithelial cysts

Madeline Lovejoy, Agustin Rabino, Sahezeel Awadia, Vennela Gangasani, Sophia Durham, and Rafael Garcia-Mata

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Most internal organs consist of a polarized epithelium surrounding a central lumen, which separates the interior of cells from the external environment. The establishment of polarity is essential for epithelial cells' function, and abnormalities in this process are a hallmark of many diseases, such as cancer. One of the key regulators of polarization is the Scribble protein complex, which interacts with proteins involved in the downstream signaling of several Rho GTPases.

Our lab has previously shown that the RhoG-specific GEF SGEF can act as a scaffold to form a ternary complex with the Scribble complex proteins Scribble and Dlg1. We demonstrated that this interaction coordinates the formation and maintenance of cell-cell junctions. Specifically, we have characterized that SGEF plays an important role in the regulation of E-cadherin and ZO-1 expression in MDCK monolayers. However, in vivo, these cells do not grow in a two-dimensional environment. Therefore, it remains unknown how SGEF influences junction integrity and morphology in a three-dimensional context. Here we utilize 3D MDCK cysts embedded in Matrigel to define the molecular mechanisms of the Scribble/SGEF/Dlg1 complex that influence cell-cell junction dynamics and cell motility during organoid morphogenesis. Our results show that, when SGEF is knocked down through stable shRNA expression, the resulting mature cysts are larger in volume, have multiple lumens, and have transcriptionally decreased E-cadherin expression. The open lumen phenotype can be restored when E-cadherin and barrier function are restored. However, there is an unknown mechanism of SGEF that is responsible for lumen number.

In addition to possessing abnormal cyst morphology, we have observed with live spinning disk microscopy that SGEF KD cysts display an E-cadherin-mediated increase in cyst fusion and collective motility. To further characterize the motile behavior of the cells, we collaborated with AI4Life to assist us in using artificial intelligence to segment and track cells stably expressing a GFP nuclear marker within two-dimensional wound healing assays. With this novel method of segmentation, we were able to compare the behavior of CTRL and SGEF KD cells at both the leading edge and the remainder of the wound. Our preliminary analysis shows that individual SGEF KD cells migrate at a higher velocity and show a greater degree of intercalation at the leading edge. In the future, we plan to apply this pipeline to characterize motility and lumenogenesis in 3D cysts.

CLIPR-76 (CLIP4): A Novel Microtubule-Binding Protein and Its Role in Striated Muscle Development

Annamarie Bryant, Gergana Ugrinova, Jill Voreis McCourt, and Holly Goodson

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana

CLIPR-76 (encoded by CLIP4) is a formally uncharacterized CAP-Gly domain-containing protein with splice isoforms capable of binding both microtubules (MTs) and membranes. Bioinformatics analysis of reported mRNA sequences indicate CLIP4 has multiple splice isoforms, all sharing common elements including N-terminal ankyrin repeats and at least one CAP-Gly domain followed by a serine-rich region. Foundational work in our lab has demonstrated that exogenously expressed isoforms of CLIPR-76 have alternative subcellular localizations that appear to be isoform dependent. Isoform 1 (containing three CAP-Gly domains) and isoforms 2 and 3 (both containing two CAP-Gly domains) localize to a subset of MTs whereas isoform 4 (containing only one CAP-Gly domain and an alternative hydrophobic C-terminal tail) localizes to the endoplasmic reticulum (ER). We determined CLIP4 mRNA to be most highly expressed in striated muscle and found endogenous CLIPR-76 protein undergoes upregulation when murine C2C12 myoblast cells are induced to differentiate into skeletal muscle. These observations suggest a potential role for CLIPR-76 in the development of striated muscle, perhaps by helping direct the cytoskeletal and ER/sarcoplasmic reticulum rearrangements necessary for myogenesis. Interestingly, CLIPR-59, (encoded by CLIP3, a homolog of CLIP4) shares similar domain features to CLIPR-76, including N-terminal ankyrin repeats, CAP-Gly domains, and a hydrophobic C-terminal tail, but localizes to the trans-Golgi network (Perez et al., JCB 2002). CLIPR-59 has been posited to act as a scaffold protein with a regulatory role in glucose transport (Ren et al., JBC 2012; Sun et al., JBC 2016) and muscle development (Couesnon et al., Development 2013; Sun et al., JBC 2015). Here we utilize a combination of cell and molecular biological techniques as well as bioinformatic analysis to further study CLIPR-76 and its role in striated myogenesis, particularly during development of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Updated bioinformatic analysis of reported CLIP4 mRNAs indicates remarkable conservation across vertebrate species at both the gene and protein levels, though different methods of alternative splicing appear to be utilized between human and mouse genomes. Immunofluorescence studies demonstrate CLIPR-76 expression and localization change throughout both skeletal and cardiac muscle development and alterations to CLIP4 using CRISPR editing is correlated to perturbation in the expected developmental timeline of iPSC-CMs. Taken together, these data suggest CLIPR-76 does play a role in early myogenesis, perhaps serving as a scaffold protein for organization of MTs in critical areas of developing striated muscle.

Mitotic Checkpoint Regulation Through Posttranslational Modifications of MAD1

Raissa Songwa, Wenbin Ji, Yibo Luo, Ejaz Ahmad, Sadia H. Khan, Nauman Ahmad, Song-Tao Liu

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Equal distribution of duplicated genomes into daughter cells is the main role of mitotic cell division. The mitotic checkpoint (MC) is a signaling system that ensures proper chromosome segregation during every mitosis, preserving the genome from chromosomal instability, a cancer hallmark. During the MC signaling, kinetochores improperly attached to microtubules emit signals that lead to the formation of the Mitotic Checkpoint Complex (MCC). This MCC inhibits the Anaphase Promoting Complex/Cyclosome, preventing premature anaphase onset. A key subunit of the MCC is the protein Mitotic Arrest Deficient 2 (MAD2). MAD2 in its open conformation (O-MAD2) is predominant in interphase cells. But for the mitotic checkpoint to get activated, MAD2 is required to be in its closed conformation (C-MAD2). An important unresolved question in the field is how this MAD2 conformational change occurs. MAD1 is an evolutionary conserved core component of the mitotic checkpoint. Many studies have shown that mutations in MAD1 lead to chromosomal instability and cancer. For a long time, it has been assumed that the middle domain of MAD1 catalyzes the conversion of O to C-MAD2. However, more recent results from us and others highlighted the importance of the MAD1 C-terminal domain (CTD) in the mitotic checkpoint and specifically the catalysis of O to C MAD2 conversion. However, the underlying mechanisms are either incomplete or unknown. Human MAD1 has 718 amino acid residues, and previously we and others have demonstrated that T716 phosphorylation is important for MAD1 checkpoint activity. We hypothesize that MAD1 CTD facilitates the catalysis of the MAD2 O-C conversion through coordinating CDC20 and MAD2 binding. Therefore, this study aims to understand how key post-translational modifications on MAD1 CTD affect its catalytic activity. My results showed that deletion of the TVA tail, the last three residues of MAD1, abolishes the mitotic checkpoint, while the 2E or 3E replacement of TVA maintains the mitotic checkpoint. These results further established the importance of the CTD tail of MAD1. Biochemical reconstitution assays and in vivo experiments using MAD1 CTD mutants, CDC20 and MAD2 are ongoing and will be reported.

This study will help us understand how the mitotic checkpoint protects cells from chromosomal instabilities. It will further provide an understanding of the mechanism of action of mitotic drugs targeting cancer and will provide the foundation to explore new and safer cell division-oriented targets against cancer.

Poster #35

Investigating Stretch Force Transfer onto Keratin Networks

Sarah Seman and Daniel Conway

Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio

Keratins are the most abundant intermediate filaments found in epithelial cells. Keratins are well known for their function in barrier formation, absorption, and secretion throughout different body systems. This group consists of 54 proteins sorted into type 1 and type 2. These form heterodimers and filaments with a type 1 and a type 2 keratin pairing, such as K5/14 which is considered the most important pairing in skin epithelium. Keratins anchor to the cell membrane via desmosomes, structural cell-cell junctions. Desmosomes and their component proteins are associated with strength in cell-cell connections; loss or disruption of desmosome proteins are associated with blistering diseases such as Epidermolysis Bullosa and Pemphigus Vulgaris. These blisters appear in response to mechanical stimulation of the epithelial membranes, supporting the idea that desmosomes are critical for the mechanical strength of these tissues. Keratin localization is well documented in skin and other epithelial tissues, but the mechanical properties of keratins and mechanics of their attachment to desmosomes remains unclear.

To investigate how keratin filaments are mechanically loaded within cells, we have developed a new nanobody-based strain sensor targeting Keratin-5 and Keratin-14. The strain sensor consists of two fluorescent proteins (FPs) attached via flexible amino acid chain with a keratin binding nanobody on either end. This allows the determination of relative strain through Fluorescent Resonant Energy Transfer (FRET) measurements. We have used this sensor to investigate how keratin strain changes when a keratinocyte monolayer is subjected to multi-axis stretch. We observed stretching of the substrate results in strain on the keratin network. This indicates our biosensor works to measure changes in loading and the keratin network experiences mechanical loading with stretch. Interestingly, the response is not linear, with a plateau of keratin strain between 5% and 10% global strain. This could indicate strain hardening behavior of the keratin network, similar to that proposed of another intermediate filament, Vimentin. Additionally, we have investigated how keratin strain is affected when a mutated K14 (R125P) is present. This keratin mutation had a reduced keratin network strain at higher magnitudes of stretch, which may indicate structural failure of the keratin network under high levels of stretch. Our results indicate that keratin networks experience mechanical forces; we have developed a new tool allowing better understanding of these loads. Future work will focus on investigating if there is spatial heterogeneity of loading in keratin networks. Our results will provide biophysical insight into keratin biomechanics.

Poster #36

Oral megakaryocytes driven by IL-17 have diverse immunological functions during oropharyngeal candidiasis

Trevor Glanz, Dylan Launder, John Dillon, Heather Conti

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Megakaryocytes have multiple functions in host defense against microbes, including the opportunistic fungal pathogen *Candida albicans*. Megakaryocytes in the oral mucosa expand during oropharyngeal candidiasis (OPC), assist in neutrophil migration, and respond to inflammatory cytokines such as interleukin-17. Interleukin-17 signaling induces platelet release and is required for megakaryocyte expansion during OPC. Using a platelet depletion model of OPC, we compared the immune profile of WT and thrombocytopenic mice. Mice deficient in platelets have increased fungal burden during OPC and lacked a protective neutrophil response. Oral megakaryocytes also express increased professional antigen presenting cell markers such as MHCII and CD86 relative to bone marrow megakaryocytes, suggesting oral megakaryocytes likely work to activate adaptive immunity. However, the specifics of these processes are still undefined. Determining signaling between megakaryocytes, platelets, and other inflammatory cells is paramount to understanding overall immune defense against *Candida*. Additionally, we hope to better characterize megakaryocyte expansion and maturation in the oral mucosa to evaluate cellular activity against *Candida* in vivo. Insights into the inflammatory mechanisms mediated by megakaryocytes and platelets will lead to increased understanding of disease progression and allow for better informed treatments for fungal infections.

Poster #37

Identifying Specificity Determinants of the Carboxysome Positioning Protein, McdB

Jordan Byrne¹, Hema Swasthi¹, Longhua Hu², Chris Azaldegui³, Jian Liu², and Anthony G. Vecchiarelli¹

¹Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan;

²Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, Maryland; ³Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan

The Maintenance of Carboxysome Distribution (Mcd) system consists of proteins McdA and McdB, which are responsible for spatially distributing carboxysomes to ensure efficient carbon fixation and equal inheritance. McdA is a member of the ParA/MinD family of ATPases and forms dynamic gradients on the nucleoid to equidistantly position McdB-bound carboxysomes. McdB represents a widespread yet understudied class of partner proteins- McdB associates with McdA to drive carboxysome positioning. Here, we find that the N-terminal 20 residues of *H. neapolitanus* McdB are necessary and sufficient to interact with McdA. As indicated by predictive modeling and in vitro analysis, we identify that Lysine 7 (K7) of McdB is critical for McdB-McdA interaction, and without K7, carboxysomes aggregate at the mid-cell. However, the deletion of McdB in *H. neapolitanus* results in the aggregation of carboxysomes at the cell poles. Until now, the field has been unable to isolate the functions of cargo separation and positioning in ParA/MinD molecular positioning systems. Our results suggest that the K7 mutant of McdB weakly interacts with McdA and functionally positions aggregates rather than evenly distributing individual carboxysomes across the nucleoid. We propose that across the A/D family of ATPases, weak interactions with the cognate partner protein can be sufficient for cargo positioning but not for partitioning, where the interaction and associated pulling forces must be strong enough to counteract the self-association interactions of the cargo.

Poster #38

Myosin 7A tail splice isoforms control cargo-mediated activation of the motor in epithelial cells

Prashun Acharya, Jasvinder Bharaj, Sadika T.J. Tonu, Morgan Timms, Nicole Roeser, Katherine Holmes and Scott W. Crawley

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Myosin 7A (Myo7A) is a motor protein expressed in specialized epithelial cells of the eye and inner ear, that is essential for both vision and hearing. Mutations in Myo7A result in sensory disorders in humans, including DFNA11 and DFNB2 deafness and Usher syndrome deaf-blindness, highlighting the importance of precise regulation and function of the motor. Myo7A is made up of a motor domain which has ATPase activity, followed by a neck region which has 5 IQ motifs and a stable α -helix and lastly, a tail containing two cargo-binding MyTH4-FERM domains (MF1, MF2), with an intervening SH3 domain. Myo7A motor activity is thought to be activated upon cargo binding to its tail, which relieves tail-mediated autoinhibition. Alternative splicing generates Myo7A isoforms with different tail domain sequences, potentially altering cargo interactions. Previous studies have identified several cargo molecules that bind the tail and activate the motor, but how isoform-specific splicing events influence cargo interaction has not been looked into. The aim of this study was to determine how distinct Myo7A tail splice isoforms control cargo-mediated activation of the motor, using apical targeting of Myo7A in kidney epithelial cells as a readout for motor activity. We observed that a splice isoform deleting 38 amino acids from MF1 had no impact on Myo7A activation by either endogenous or tested exogenous cargo. In contrast, a splice isoform deleting two amino acids from MF2 blocked activation by endogenous cargo but showed differential effects across the tested exogenous cargos. Together, our results demonstrate that Myo7A tail splice isoforms can encode cargo-specific activation profiles of the motor.

Poster #39

Turning Science into Art: The Creative Process of Scientific Journal Cover Illustration

Carmen Mancuso

Department of Art & Design, Ohio Northern University, Ada, Ohio

Scientific journal cover illustration sits at the intersection of scientific concepts and visual storytelling. This presentation explores the connection between these fields through my artist-in-residence experience at Vanderbilt University's Artlab Studio last summer. With a background in the arts, I learned on the job to analyze scientific articles and translate them into visually-appealing illustrations. Motivated by a desire to attract non-scientific audiences to scientific journals, my creative process evolved into a multi-step approach that relied on collaborative critiques and scientific review. The residency culminated in two of my three completed illustrations being selected as CellPress journal covers. By reflecting on my creative process, my presentation aims to demonstrate the value of a creative mindset in the scientific field and outline explicit steps for developing cover art illustrations that engage both the artistic and scientific communities.

Poster #40

Investigating The Role of Protocadherin-19 in Early Human Cortical Development

Nikita Jadhav and Wei Niu

Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio

Protocadherin-19 (PCDH19)-clustering epilepsy (PCE) is one of the most common monogenic epilepsies, characterized by early-onset epilepsy and severe neurodevelopmental delay. It is caused by mutations in PCDH19 gene on X chromosome, which encodes a cell adhesion protein. Unlike classical loss-of-function disorders, PCE arises from mosaic expression due to random X-inactivation. As a result, PCE exclusively affects heterozygous females and mosaic males, but not hemizygous males. Although the genetic cause is established, the functions of PCDH19 in human cortical development remain poorly defined.

We employ human cortical organoids (hCOs), which model early corticogenesis and capture features of laminar organization. A key cell type is radial glial (RG) cells, which have unique apical-basal polarity and undergo symmetric divisions to expand progenitor pool or asymmetric divisions to generate neurons and glia. Our prior work in wildtype (WT) hCOs demonstrated that PCDH19 localizes to the cell body of dividing RG cells, and colocalizes with N-cadherin (NCAD) at apical junction of ventricular-like zone. In mosaic PCE hCOs, the apical-basal organization of RG cells and subcellular localization of PCDH19 and NCAD were disrupted, suggesting a critical role for PCDH19 in adhesion and polarity. We hypothesize that PCDH19 regulates cortical lamination by controlling RG cell polarity, proliferation, and neurogenesis.

First, we aim to define how PCDH19 regulates RG polarity and proliferation during early corticogenesis. We will assess the impact of mosaic PCDH19 expression on RG division patterns and differentiation using live confocal imaging and immunocytochemistry in early stage hCOs. Our preliminary data showed that PCDH19-knockout (KO) cells undergo ~30% more cell divisions than PCDH19-WT across experiments and more diverse division patterns than PCDH19-WT cells in day 6-12 PCE hCOs.

Second, we aim to determine how PCDH19 interacts with NCAD to regulate adhesion during neurogenesis. We will use biochemical assays (co-immunoprecipitation, western blotting) and RNA sequencing to define PCDH19–NCAD interactions and identify transcriptional changes associated with abnormal lamination in PCE. Our preliminary data showed that PCDH19 and NCAD expressions slightly increased in day 12 of development, and NCAD expression was relatively higher in KO and PCE than WT, suggesting a compensatory role of NCAD in adhesion and polarity in the context of PCDH19 loss or mosaicism.

In future, we will integrate imaging, biochemical, and transcriptomic methods in hCOs to define PCDH19's role in cortical development. These studies will elucidate how PCDH19 regulates progenitor polarity and cortical organization, advancing our understanding of PCE pathogenesis.

Structural, Biochemical, and Genomic Analysis of yeast mechanosensitive Pkd2 channel

Ajay Kumar Sharma¹, Bethlehem D. Abebe², Steven Z. Chou² and Qian Chen¹

¹*Department of Molecular, Cellular and Developmental Biology, The University of Toledo, Toledo, Ohio;*

²*Department of Molecular Biology & Biophysics, University of Connecticut Health Center, Farmington, Connecticut*

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the fourth leading cause of kidney failure in the United States. Nearly all cases are caused by loss-of-function mutations in two polycystin genes: PKD1 and PKD2. The PKD2 gene encodes polycystin-2 (PC-2), which is a TRP channel that forms a non-selective cation channel. Dysfunction of this channel contributes directly to the pathogenesis of ADPKD. In fission yeast, Pkd2 has been shown to trigger calcium spikes during cytokinesis, but its precise molecular mechanism—including calcium binding and channel activity—remains poorly understood across species.

Therefore, this project aims to determine the high-resolution structure of Pkd2 and explore its additional cellular roles and genomic interactions. The first phase of this project focuses on purifying high yields of Pkd2, followed by cryo-EM for structural determination, mass spectrometry to identify binding partners, and biochemical assays—such as in vitro reconstitution in biomimetic membranes—to further probe its function. We have successfully cloned Pkd2 from three yeast species—*S. pombe*, *S. japonica* and *C. albicans*—under strong actin promoter for purification. While expression and solubilization of *S. pombe* Pkd2 have been challenging, likely due to extensive cleavage during processing, we have successfully expressed the full-length Pkd2 in hosts *S. cerevisiae* and *P. pastoris*. Moving forward, we aim to optimize purification protocols to improve yield and reduce cleavage, enabling downstream structural and functional studies.

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