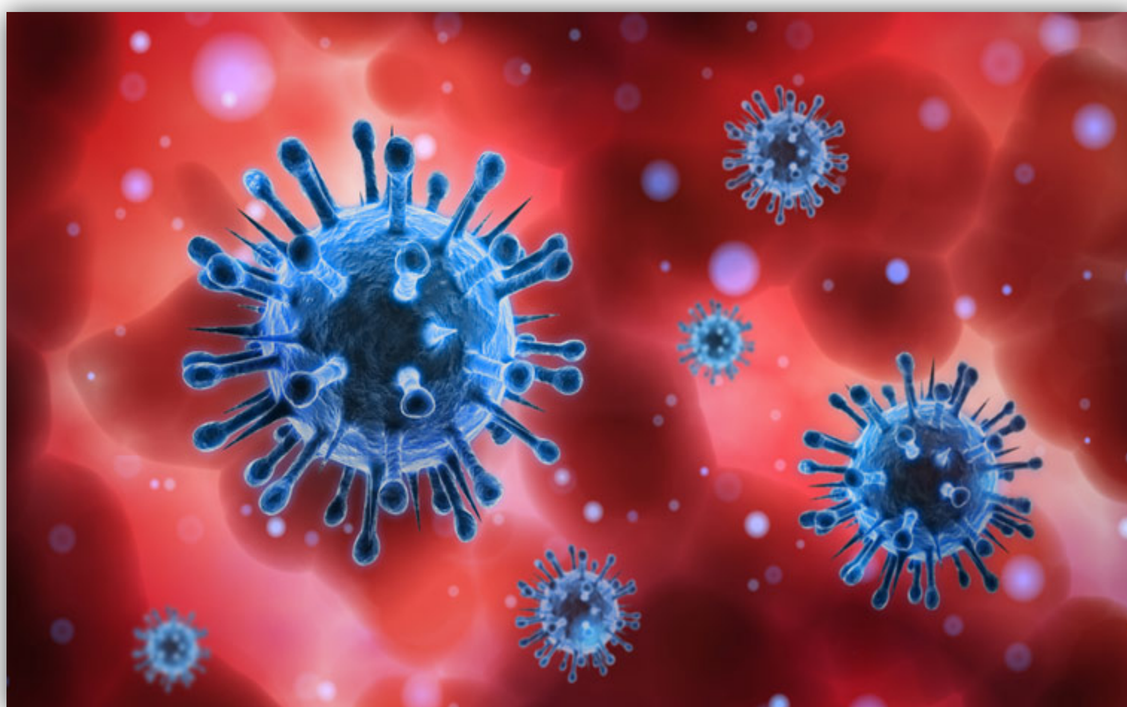
The Department of Pharmacology

Retreat 2020

October 26-30, 2020



Virtual Meeting

Retreat Chair: Dr. Beata Jastrzebska

Retreat Co-Chair: Dr. Amy Wilson-Delfosse

Monday, October 26, 2020

https://cwru.zoom.us/j/93059583509?pwd=NmF3bm02bTVUem04eHlFUHJaUnhidz09
Zoom Meeting ID: 930 5958 3509
Password: 401715

11:15-11:20am **Opening Remarks, Dr. Beata Jastrzebska**
11:20-11:30am **Chair address, Dr. Edward Yu**

11:30-12:30pm **Mini Presentations (5 min presentations + 2 min Q&A)**

Biology of Mitochondrial Fission and More

Session Moderator: Katrina Piemonte

Provocateurs: Marcin, Golczak, Paul MacDonald,

11:30-11:35am **Elizabeth O. Akinbiyi – Graduate Student (Mears Lab)**

Understanding how post-translational modifications regulate the mitochondrial fission machinery in Glioblastoma

11:37-11:42am **Brianna Bauer – Graduate Student (Mears Lab)**

Defining the mechanisms by which disease-associated mutations in Drp1 impede mitochondrial fission

11:44-11:49am **Adina Brett-Morris – Research Associate (Mears Lab)**

The effect of CDK5 on mitochondrial function in Glioblastoma Multiforme (GBM)

11:51-11:56pm **Kyle Whiddon – Graduate Student (Mears Lab)**

Functional characterization of Drp1-microtubules interactions *in vitro*

11:58-12:03pm **Kristy Rochon – Graduate Student (Mears Lab)**

Conformational changes in solution multimers of dynamin-related protein 1 (Drp1) facilitate functional assembly

12:05-12:10pm **Jackie Plau – Graduate Student (Golczak Lab)**

Identifying competitive inhibitors of CRBP1 for vitamin A metabolism modulation

12:12-12:17pm **Corey Emerson – Graduate Student (Stewart Lab)**

Complement mediated neutralization of adenovirus

Open Discussion

MYTH
Regularly rinsing your nose with saline will prevent infection.
FACT
There is no evidence to support the claim that regularly rinsing your nostrils out with saline will protect you from infection with the new coronavirus.
Source: WHO | Coronavirus: Myths vs. facts

MYTH
Gargling mouthwash will stop the spread of the virus.
FACT
Some mouthwash brands can eliminate certain microbes in your saliva for a few minutes, but it will not protect you from the coronavirus.
Source: WHO | Coronavirus: Myths vs. facts

Tuesday, October 27, 2020

https://cwru.zoom.us/j/97800472735?pwd=bG82N0RjZzRnc2N3WFZjaE5LL0F4UT09
Zoom Meeting ID: 978 0047 2735
Password: 937949

11:30-12:30pm **Mini Presentations (5 min presentations + 2 min Q&A)**

Structure of Macromolecules

Session Moderator: Linda Thomas

Provocateurs: Derek Taylor, Jason Mears

11:30-11:35am **Mitchell Moseng – Postdoc (Yu Lab)**

Cryo-EM structures of the heavy metal efflux pump CusA provide insight for a mechanism of independently operating protomers

11:37-11:42am **Meinan Lyu – Postdoc (Yu lab)**

Structural basis of transport and inhibition of the *Plasmodium falciparum* transporter PfFNT

11:44-11:49am **Chris Morgan – Postdoc (Yu Lab)**

Structural studies of the AdeB multidrug efflux pump from *Acinetobacter baumannii*

11:51-11:56pm **Avery E. Sears – Graduate Student (Palczewski Lab)**

Cryo-EM of interphotoreceptor retinoid-binding protein in complex with a monoclonal antibody

11:58-12:03pm **Pravesh Shrestha – Postdoc (Buck Lab)**

Structure and functional studies of the effects of phosphorylation on ephrin receptor tyrosine kinase, epha2, and the relationship with its SAM domain as an autoinhibitor

12:05- 12:10pm **Zhemin Zhang – Postdoc (Yu Lab)**

Cryo-electron microscopy structure of *Acinetobacter baumannii* efflux pump AdeJ

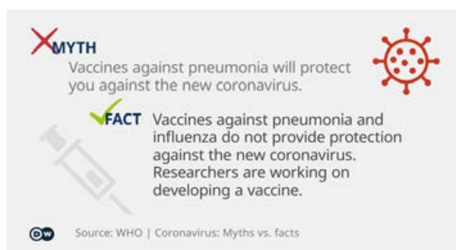
12:12-12:17pm **Alexander Day – Graduate Student (Taylor Lab)**

Filling the structural gap between PP2A A subunit isoforms

12:19-12:24pm **Sepalika Bandara – Postdoc (von Lintig Lab)**

Structural and functional analysis of Cartotenoid oxygenase 2

Open Discussion



Wednesday, October 28, 2020

https://cwru.zoom.us/j/91859217196?pwd=Z01pSkc3Y0JUQS8zWmVsbHNaRXFnZz09
Zoom Meeting ID: 918 5921 7196
Password: 074364

11:30-12:30pm **Mini Presentations (5 min presentations + 2 min Q&A)**

Molecular Therapeutics and Cancer

Session Moderator: Brianna Bauer

Provocateurs: You-Wei Zhang, Bill Schiemann

11:30-11:35am **Leslie Cuellar Vite – Graduate Student (Keri Lab)**

Targeting the MUVB complex potentiates the efficacy of CDK4/6 inhibitors in breast cancer

11:37-11:42am **Xinran Geng – Graduate Student (Zhang Lab)**

CRISPR/Cas screen of factors regulating heterochromatin liquid phase separation

11:44-11:49am **Parth Majmudar – Graduate Student (Keri Lab)**

Lis1 is necessary for TNBC cell viability and proper cell cycle progression

11:51-11:56pm **Xu Han – Graduate Student (Nieman Lab)**

Protease activated receptor 4 is a novel target associated with venous thromboembolism

11:58-12:03pm **Katrina Piemonte – Graduate Student (Keri Lab)**

GAS2L3, a novel regulator of chromosomal instability in Triple Negative Breast Cancer

12:05- 12:10pm **Yuli Buckley – Graduate Student (Mears Lab)**

Elucidating the role of Fis1 in mediating mitochondrial fission within the context of glioblastoma multiforme

12:12-12:17pm **Wilnelly Hernandez-Sanchez – Postdoc (Taylor Lab)**

Mechanistic framework for telomerase kinetics identifies regulation of telomerase by dNTP substrate inhibition

12:19-12:24pm **Franklin Mayca Pozo – Research Associate (Zhang Lab)**
b-TrCP ubiquitin ligase recognizes a DEGRON motif of Myosin-X and modulates the genomic instability in cancer cells

Open Discussion

Thursday, October 29, 2020

https://cwru.zoom.us/j/97937412426?pwd=cUR1Vy92SVRWbUF5ZUlPaWQ4NSs5Zz09
Zoom Meeting ID: 979 3741 2426
Password: 857764

10:00-11:30pm **Full Presentations (10 min presentations + 3 min Q&A)**

Cancer and Translational Therapeutics

Session Moderator: Bryan Webb

Provocateurs: Marvin Nieman, Ruth Keri

10:00-10:10am **Elizabeth Knauss – Graduate Student (Neiman Lab)**
The Crosstalk between venous thromboembolism and pancreatic cancer

10:13-10:23am **Peng Zeng – Graduate Student (Schmaier Lab)**
Ponatinib induces thrombosis through potentiating platelet activation pathway and expressing adiponectin in aorta tissue-resident immune cells

10:26-10:29am **Christine Lee-Poturalski – Graduate Student (Jankowski Lab)**
The translational landscape of patient-derived glioblastoma stem cells

10:32-10:42am **Sarah Kohrt – Graduate Student (Grabowska Lab)**
MAP3K11 as a driver of enzalutamide resistance in castration-resistant prostate cancer

10:45-10:55am **Kimberly Parker – Graduate Student (Schiemann Lab)**
The IncRNA BORG mediates alternative splicing to enhance cancer stem cell properties in TNBC

10:58-11:08am **Tawna Mangosh – Graduate Student (Taylor Lab)**
SLX4IP promotes telomere maintenance in androgen receptor- independent castration-resistant prostate cancer through ALT-like telemetric PML localization

11:11-11:30am **-BREAK-**

11:30-12:30pm **Mini Presentations (5 min presentations + 2 min Q&A)**

Animal Models of Eye Diseases

Session Moderator: Chris Sander

Provocateurs: Johannes von Lintig, Masaru Miyagi

11:30-11:35am **Elliot Choi – Graduate Student (Palczewski Lab)**
Retinal pigment epithelium-specific tamoxifen-inducible *Cre* mice

11:37-11:42am **Anahita Duruwalla – Graduate Student (Kiser Lab)**
New large animal model for RDH5-associated retinopathies

11:44-11:49am **Jennings Luu – Graduate Student (Palczewski Lab)**

Epigenetic hallmarks of age-related macular degeneration are recapitulated in a photosensitive mouse model

11:51-11:56am **Susie Suh – Graduate Student (Palczewski Lab)**

Treatment of an inherited retinal disease by *in vivo* base editing in an adult mouse model

11:58-12:03pm **Linda Thomas – Graduate Student (von Lintig Lab)**

Human β-carotene-oxygenase 2 and carotenoid metabolism

12:05- 12:10pm **Ramkumar Srinivasag – Research Associate (von Lintig Lab)**

LRAT is critical for the negative feedback control of vitamin A production in mammals

12:12-12:17pm **Joseph Ortega – Research Associate (Jastrzebska Lab)**

Protective role of flavonoids in light-induced photoreceptors' degeneration

Open Discussions

Friday, October 30, 2020

https://cwru.zoom.us/j/91603890048?pwd=eFk0Mm14emRoeTJ6bkNLeEEzSVA1UT09
Zoom Meeting ID: 916 0389 0048
Password: 367163

10:00-11:30pm **Full Presentations (10 min presentations + 3 min Q&A)**

Molecular and Structural Biology and Pharmacology

Session Moderator: Parth Majmudar

Provocateurs: Chris Dealwis, John Mieyal, Ed Yu

10:00-10:10am **Shelby Dahlen – Graduate Student (Osei-Owusu Lab)**

Fine-tuning of Gi/o signaling by RGS2 and 5 critical to ensuring normal ventricular rhythm

10:13-10:23am **Allison Grenell – Graduate Student (Anand-Apte Lab)**

Mutations in TIMP3 leads to metabolic dysregulation in retinal pigment epithelial cells

10:26-10:36am **Jean Moon – Graduate Student (von Lintig Lab)**

Vitamin A homeostasis is controlled by retinoic acid-dependent feedback mechanisms

10:39-10:49am **Christopher Sander – Graduate Student (Palczewski and Kiser Labs)**

Structural evidence for visual arrestin priming via phosphoinositol complexation

10:52-11:02am **Weiyang Zhao – Graduate Student (Taylor/Dealwis Labs)**

EM studies of multimers of human ribonucleotide reductase

11:05-11:15am **Matthew Pleshinger – Graduate Student (Adams Lab)**

Identifying novel targets to lead to 8,9 unsaturated sterol accumulation and enhancement of oligodendrocyte formation

11:20-12:00pm -BREAK-
12:00-12:15pm Retreat Awards

12:15-12:45pm **Excellence in Research Award Presentation – Wei Huang, Ph.D.**

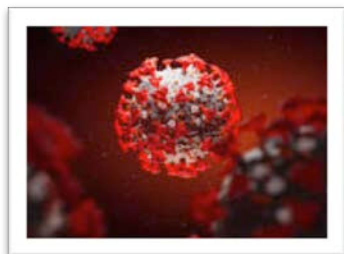
An odyssey of small molecule and biomacromolecule interactions

12:45–12:50 pm Retreat Closing

Departmental Awards

Outstanding Performance

	Student	Postdoc
1998	Minh Lam	Not Awarded
1999	Algirdas A. Velyvis	Not Awarded
2000	Joan S. Jorgensen	Christine Quirk
2001	Colleen Tagliarino	Nancy Wang
2002	Erin L. Milliken	David Kehres
2003	Philip A. Verhoef	Not Awarded
2004	Melissa C. Bentle	Not Awarded
2005	Melissa Landis	Not Awarded
2006	Vivian Gama	Not Awarded
2007	Tara Ellison	Not Awarded
2008	Ndiya Ogba	Emhonta Johnson
2009	Philip Kiser	Marcin Golczak
2010	Gina Bernardo	David Lodowski
2011	Debarshi Mustafi	Glenn Lobo
2012	Molly Taylor	Faiz Mohammed
2013	Amar Desai	Gurdeep Marwaha
2014	Andrea Boyd-Tressler	Ina Nemet
2015	William Johnson	Yuanyuan Chen
2016	Jennifer Sahni	Sylvia Gayle
2017	Sahil Gulati	Ni Made Airanthi Widjaja-Adhi
2018	Ratul Patel	Wei Huang
2019	Alyssa La Belle	Joseph T. Ortega

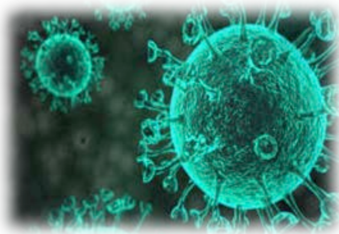


Excellence in Research

2008	Akiko Maeda
2009	Philip Kiser
2010	Gina Bernardo
2011	Debarshi Mustafi
2012	Debarshi Mustafi
2013	Ning Zhang
2014	Sanae Sakami
2015	William Johnson
2016	Jianye Zhang
2017	Deoye Tonade
2018	Ni Made Airanthi Widjaja-Adhi
2019	Philip Ropelewski
2020	Wei Huang

Nathan S. Greenfield Family Travel Award

2003 Tehnaz Parakh
2004 Molly M. Gallogly
2005 Jodi Thomson
2006 Elizabeth Sabens
2007 Vivian Gama
2008 Gina Bernardo
2009 Chris Ryder
2010 Amar Desai and Debarshi Mustafi
2011 Luke Bury
2012 Will Johnson
2013 Chris Francy
2014 Neetu Gulati
2015 Darcie Seachrist
2016 Xu Han
2017 Corey Emerson
2018 Tawna Whited (Mangosh)
2019 Kristy Rochon

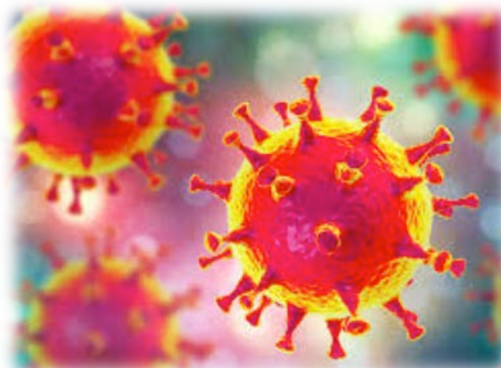


Faculty Spotlight Lectures

2000 Leslie T. Webster, Jr., MD
2002 Charles L. Hoppel, MD
2003 Clark W. Distelhorst, MD
2004 Pamela Davis, M.D.
2013 John Mieyal, Ph.D.
2014 George Dubyak, Ph.D.
2015 Ruth Keri, Ph.D.
2016 Stanton Gerson, Ph.D.
2017 Robert A. Bonomo Ph.D.
2018 Bingcheng Wang, Ph.D.

ALUMNI SPOTLIGHT LECTURES

2019 Timothy Miller, Ph.D.



Mini Oral Presentations, Monday October 26

Biology of Mitochondrial Fission and More

Presenter: Elizabeth O. Akinbiyi – Graduate Student (Mears Lab)
Title: Understanding how post-translational modifications regulate the mitochondrial fission machinery in Glioblastoma

Mitochondria are essential and dynamic organelles that maintain a delicate balance of division and fusion in response to cellular stimuli. These processes sustain mitochondrial health and function, and alterations to either fission or fusion are associated with a variety of diseases, including cancer. Glioblastoma (GBM) is an aggressive brain cancer that takes the lives of nearly 90% of patients within 5 years of diagnosis. Glioblastoma stem cells (GSCs) represent a subset of the tumor cells that can self-renew and propagate new tumors, which contributes to higher rates of recurrence. GSCs also are resistant to conventional GBM therapies, including chemotherapy and radiation. Recent studies have observed excessive mitochondrial fission in GSCs when compared to non-stem GBM cells. This finding suggests underlying bioenergetic changes, which lead to altered post-translational modifications (PTMs) of proteins in the mitochondrial fission machinery. Dynamin-related protein 1 (Drp1), the essential mediator of mammalian mitochondrial fission, is regulated by PTMs, such as phosphorylation and O-GlcNAcylation. We propose that O-GlcNAcylation of Drp1 is an activating modification that promotes Drp1 re-localization from the cytosol to the mitochondria resulting in increased mitochondrial fission. In addition, we are examining the phosphorylation status of a partner protein of Drp1, mitochondrial fission factor (Mff), to examine synergistic changes that favor mitochondrial division. Overall, our goal is to characterize the impact of PTMs on the fission machinery and assess functional changes leading to altered mitochondrial dynamics in GSCs. In doing so, we hope to identify potential therapeutic targets that alter PTMs in GSCs to prevent recurrence in GBM patients.

Presenter: Brianna Bauer – Graduate Student (Mears Lab)
Title: Defining the mechanisms by which disease-associated mutations in Drp1 impede mitochondrial fission

Mitochondria are dynamic organelles that continuously undergo fission and fusion. Fission is necessary to ensure mitochondria are properly distributed and remove damaged mitochondrial components. However, excessive fission is associated with pathogenic outcomes in cells and tissues that are especially sensitive to mitochondrial dysregulation, such as neurons. Recently, whole-exome sequencing of patients with severe dysregulation of mitochondrial dynamics has identified point mutations in dynamin-related protein 1 (Drp1), the master regulator of mitochondrial fission. Patients present with severe neurological defects, and patient-derived fibroblasts show hyperfused mitochondria. Thus, Drp1 patient mutations impair mitochondrial fission, but it is unclear how these mutations specifically disrupt Drp1 function. As a cytosolic GTPase, Drp1 self-assembles into spirals that encircle the outer mitochondrial membrane (OMM) at sites of ensuing fission. GTP hydrolysis invokes conformational changes in Drp1 oligomers that impart mechanical force and constrict the mitochondria. The critical intra- and inter-molecular interactions that govern the oligomeric state of Drp1 and the nucleation of Drp1 polymerization on the OMM are poorly defined. Because these patient mutations are located throughout the structure of Drp1, in both the catalytic and self-assembly domains, we believe these mutations represent important sites for identifying the essential features of the fission machinery. Since the complete loss of Drp1 is embryonic lethal, we propose that these mutations embody the most severe disruptions to Drp1 function possible while still allowing for survival beyond embryonic development. Individual mutations have been introduced into human Drp1 and purified by recombinant protein expression. Preliminary results indicate several mutant proteins remain capable of oligomerization and all mutants maintain GTPase activity, with some possessing a higher basal rate of GTP hydrolysis than WT. Ultimately, these studies will provide insight into the structure-function relationship of Drp1 and identify the specific defects in Drp1 activity that give rise to human pathologies.

Presenter: Adina Brett-Morris - Research Associate (Mears Lab)
Title: The effect of CDK5 on mitochondrial function in Glioblastoma Multiforme (GBM)

Glioblastoma Multiforme (GBM) is the most common and aggressive form of brain tumors. Despite conventional therapies, average patient survival remains at less than two years. GBM recurrence is in part due to self-renewing tumor initiating cells, often called glioma stem cells (GSCs), which are characterized by their proliferation and tumor formation capacity, as well as their metabolic features. Thus, mitochondrial function represents a potential link between cellular metabolism and differentiation state. Mitochondrial fission is indispensable for growing and dividing cells, and dynamin related protein 1 (Drp1) is essential for this process. There is cumulative evidence that links Drp1 with several types of cancers, including GBM, and regulation is mainly controlled by post-translational modifications (PTMs). Importantly, recent studies have shown CDK5-dependent phosphorylation of Drp1 enhances mitochondrial fission, and CDK5 plays a crucial role in brain development, neurodegenerative diseases, tumor development, and chemotherapy resistance. Therefore, our lab hypothesized that targeting CDK5 mediated Drp1 phosphorylation to inhibit mitochondrial fission could sensitize GBM to conventional therapies. Our analysis has shown that CDK5-dependent phosphorylation of Drp1 is present in GSCs and GBM cell lines but absent in non-stem tumor cells (NSTC) and astrocytes. Small-molecule CDK5 inhibitors or CDK5 knockdown through siRNA in GSCs and GBM cell lines decreased activating phosphorylation of Drp1, leading to elongation of mitochondria morphology and corresponding reductions in cell viability and cell proliferation. Additionally, CDK5 inhibition showed sensitization to radiation in GSCs and Gli36 cells. To assess the importance of Drp1 phosphorylation in GBM compared to normal tissue, CDK5 was inhibited in human neural progenitor cells (hNPC), and we found that inhibition of CDK5 did not alter Drp1 phosphorylation when compared to untreated cells. Additionally, hNPC proliferation was unchanged by these treatments. Overall, these findings suggest the important role for CDK5 and mitochondrial fission in GBM and targeting CDK5 offers a novel strategy to sensitize tumors to chemo and radiotherapies.

Presenter: Kyle Whiddon – Graduate Student (Mears Lab)
Title: Functional Ccharacterization of Drp1-microtubules interactions *in vitro*

Dynamin-related protein 1 (Drp1) is the key mediator of mitochondrial fission. Drp1 is a large (80 kDa), cytosolic GTPase that is actively recruited to the mitochondrial surface through protein and lipid interactions. Additionally, it has been shown that certain Drp1 splice variants are strongly recruited to microtubules (MT) when not targeted to the mitochondria outer membrane. It was suggested that this recruitment is driven primarily by GTP hydrolysis, but the cellular assays used in previous studies utilized a GFP-tagged Drp1 construct and a number of confounding factors may have impacted these results. We have shown that GFP-labeled Drp1 behaves differently than native Drp1 (*submitted*), so our goal is to reconstitute and characterize MT interactions with isolated Drp1 isoforms *in vitro*. A combination of biochemical and electron microscopy (EM) techniques will be used to define the regions in Drp1 and tubulin required for this interaction and to investigate the impact of GTP binding and hydrolysis on this macromolecular complex. Our initial studies have shown that different splice variants are able to bind MTs, forming regular helical polymers around these filaments. Additionally, GTP is not required for association, but hydrolysis does appear to mediates Drp1 disassembly and dissociation from MTs. Going forward, we plan to use biochemical methods to assess how MT interactions impact Drp1 activity. Mutagenesis will be used to define the specific sequence requirements for this interaction, and EM studies will elucidate the structure and function of Drp1-MT co-polymers to provide a molecular basis for understanding the impact of this association on mitochondrial and cellular physiology.

Presenter: Kristy Rochon – Graduate Student (Mears Lab)
Title: Conformational changes in solution multimers of dynamin-related protein 1 (Drp1) facilitate functional assembly

Mitochondrial dynamics is essential for cellular health, and an imbalance in this cycle has been implicated in many diseases, from heart failure to prion-related neurodegeneration. Dynamin-related protein 1 (Drp1) is the master regulator of mitochondrial fission; however, the underlying mechanisms governing this regulation are largely unclear. Drp1 exists predominantly as a mixture of dimers and tetramers in

solution, but the specific interactions that stabilize these solution forms of Drp1 and prevent the assembly of larger complexes are not known. We have observed that a disordered region in Drp1 connecting the GTPase domain and the stalk, called hinge 1, confers a high degree of flexibility in dimer populations. In larger oligomers, this flexibility is reduced, leading to more homogenous conformations. We hypothesize that these stabilized oligomers represent storage forms of the cytosolic protein, while the flexibility observed in the dimer facilitates the conformational rearrangement needed for helical assembly. Point mutations in Drp1 have been shown to enrich higher-order oligomers, and these changes are associated with defective mitochondrial fission. Conversely, separate point mutations cannot self-assemble, and Drp1 forms an obligate dimer. We have used these mutants and wild-type protein to identify structural differences in solution multimers of Drp1. By resolving high resolution cryoEM structures of the Drp1 solution dimer and tetramer, as well as helical polymers on lipid templates, we will elucidate conformational changes that facilitate functional assembly. In addition to electron microscopy, we are using molecular dynamic simulations (MDS) to identify critical residues and interfaces that regulate oligomerization and conformation flexibility to control assembly of the mitochondrial fission machinery. This analysis has identified potential cross talk between the stalk and GTPase domain providing an additional mechanism of regulation of assembly and activity.

Presenter: Jackie Plau – Graduate Student (Golczak Lab)
Title: Identifying competitive inhibitors of CRBP1 for vitamin A metabolism modulation

Cellular retinol-binding protein 1 (CRBP1) is the main vitamin A carrier protein within the body. By facilitating transport of all-trans-retinol, CRBP1 regulates levels of vitamin A and its metabolites within the cell, which are critical for optimal cell functions. Numerous studies have found evidence of CRBP1 playing an important role in the regulation of adipogenesis, regeneration of visual chromophore, regulation of immune response and have indicated an involvement in various types of cancers such as breast cancer or ovarian cancer. Thus, manipulation of the retinoid metabolism by inhibiting biological activity of CRBP1 emerges as an attractive therapeutic strategy to combat prevalent diseases, including retinal degenerations, liver cancer or certain immunological disorders.

Thereby, the main goal of this study is to identify the first in class small molecule inhibitors of CRBP1 and validate their biological activity in mouse models of human diseases. To achieve this goal, we developed a high throughput screening (HTS) methodology and investigated a small molecule library consisting of 25,000 unique chemical compounds for the interactions with CRBP1. The lead compounds were selected by specific criteria such as molecular size, fluorescence spectra, and chemical characteristics. Overall, a total of eleven compounds were selected for further binding validation by X-ray crystallography. We obtained crystals of CRBP1 in complexes with five of the tested compounds. Subsequent solving of the crystallographic structures of these complexes revealed a distinctive mode of protein-ligand interaction that outcompetes all-trans-retinol from the binding site of CRBP1. The atomic-resolution structures also provided a molecular basis for the high affinity and selectivity of these newly found inhibitors of CRBP1. Therefore, we build a solid foundation for the data-driven optimization of the binding properties of CRBP1 ligands as well as further research towards validation of CRBP1 as a valuable therapeutic target for selected diseases caused by imbalance in retinoid homeostasis.

Presenter: Corey Emerson – Graduate Student (Stewart Lab)
Title: Complement mediated neutralization of adenovirus

Engineered adenovirus (AdV) vectors are one of the most commonly used vectors in clinical trials. AdV is a promising vector for clinical applications because this virus can infect both dividing and non-dividing cells, can handle large DNA inserts without affecting the titer, and the AdV genome remains separate from the host genome. However, following intravenous administration of AdV vectors there is a strong host immune response that has limited the clinical utility of this vector. One of the important factors in the immunogenic response to AdV is the complement-mediated pathway, which has been shown to play a critical role in neutralization of AdV infections. The classical complement pathway is initiated by antigen/IgM or antigen/IgG complexes, which lead to a cascade of binding and activation of complement proteins C4, C3 and C5 and targeting of antigen/complement complexes for elimination by phagocytes. Recent studies have shown that binding of complement protein C4b, an early step in the classical complement pathway, is able to neutralize AdV independent of downstream events. Our work has

focused on using a combination of molecular modeling and molecular dynamics simulations to model how C4b might neutralize AdV infections. Our structural modeling results suggest that C4b is likely to form a covalent bond with long, flexible Arg-Gly-Asp (RGD) loops on the penton base capsid protein and that initial covalent binding leads to subsequent non-covalent interactions of C4b with multiple RGD loops. We propose that the resulting entanglement of C4b and penton base blocks conformational changes in penton base that are necessary for programmed capsid disassembly and productive cell entry. This study provides structural hypotheses that can be tested experimentally and may provide insight into the design of next-generation AdV-based vectors.

Mini Oral Presentations, Tuesday October 27

Structure of Macromolecules

Presenter: Mitchell Moseng – Postdoc (Yu Lab)
Title: Cryo-EM structures of the heavy metal efflux pump CusA provide insight for a mechanism of independently operating protomers

Gram-negative bacteria, such as *Escherichia coli*, frequently use tripartite efflux complexes in the resistance-nodulation-cell division (RND) family to expel various toxic compounds from the cell. The efflux system CusCBA is responsible for expelling biocidal Cu(I) and Ag(I) ions from the cell. Here we describe the Cryo-EM structures of the inner membrane transporter CusA in the presence of Cu(I). Binding of Cu(I) triggers significant conformational changes within the CusA protomers in both the periplasmic and transmembrane domains. These structures demonstrate that each CusA protomer within the trimer is able to function independently. On the basis of this structural information, we postulate that individual protomers of these trimeric RND pumps could bind and export substrates independently instead of operating in a synchronized fashion. Thus, each protomer may autonomously go through a sequence of conformational transitions, which lead to the extrusion of substrates through a particular protomer in which the protomers export metals independently of each other within the trimer. Our data indicates that the CusA protomers can independently progress from a closed apo state to a half open binding state and then to the open Cu(I) bound extrusion conformational state. These CusA structures provide new structural information about the HME subfamily of RND efflux pumps. The structures suggest that the metal-binding sites, formed by a three-methionine cluster, are located within the cleft region of the periplasmic domain. This cleft is closed in the apo-CusA protomers but open in the Cu(I) bound CusA protomers, which directly suggests a plausible pathway for metal ion export.

Presenter: Meinan Lyu – Postdoc (Yu Lab)
Title: Structural basis of transport and inhibition of the *Plasmodium falciparum* transporter PfFNT

Malaria is caused by the protozoan parasite *Plasmodium*, which is endemic in tropical and subtropical regions and causes up to one million deaths each year. Areas especially where *P. falciparum* predominates suffer more disease and death. And the emergence and continuing spread of drug-resistant *P. falciparum* also deepen the need for identification of novel antimalarial drugs. The intra-erythrocyte stage of *P. falciparum* relies primarily on glycolysis to generate adenosine triphosphate (ATP) and the energy required to support growth and reproduction. The major byproduct of glycolysis, lactic acid, is potentially toxic as it lowers the pH inside the parasite. *P. falciparum* formate-nitrite transporter (PfFNT), a 34-kDa transmembrane protein, has been identified as a novel drug target as it exports lactate from inside of the parasite to the surrounding parasitophorous vacuole within the erythrocyte cytosol. The structure and detailed molecular mechanism of this membrane protein are not yet available. Here we present structures of PfFNT in the apo, substrate L-lactate binding and functional inhibitor MMV007839 binding forms using single particle cryo-EM. Combined with transport assays, our data suggest a stepwise displacement mechanism for substrate transport and inhibition in an atomic-level, providing important information for novel drug design.

Presenter: Chris Morgan – Postdoc (Yu Lab)
Title: Structural studies of the AdeB multidrug efflux pump from *Acinetobacter baumannii*

The consistent rise in antibiotic resistance threatens to make standard bacterial infections difficult or

even impossible to treat. Antibiotic evasion in multi-drug resistant (MDR) organisms is achieved through a number of mechanisms such as antibiotic modifying enzymes, ribosomal modifying enzymes and multidrug efflux pumps. *Acinetobacter baumannii* is a bacterial pathogen that displays a high level of multi-drug resistance. The *A. baumannii* AdeB membrane protein is a major efflux transporter that mediates resistance to most clinically relevant antibiotics. Here, we elucidate the structure of AdeB in the presence of the substrate ethidium bromide using cryo-electron microscopy (cryo-EM). Using this technique, we have identified six distinct structures of AdeB that depict novel transient conformational states of the pump in action. This work characterizes an important antibiotic resistance mechanism and sheds light on a novel target for the development of new antibiotics.

Presenter: Avery E. Sears – Graduate Student (Palczewski Lab)
Title: Cryo-EM of interphotoreceptor retinoid-binding protein in complex with a monoclonal antibody.

Interphotoreceptor retinoid-binding protein (IRBP) is a highly expressed protein secreted by rod and cone photoreceptors that has major roles in photoreceptor homeostasis as well as retinoid and polyunsaturated fatty acid transport between the neural retina and retinal pigment epithelium. Despite two crystal structures reported on fragments of IRBP and decades of research, the overall structure of IRBP and function within the visual cycle remain unsolved. Here we studied the structure of native bovine IRBP in complex with a monoclonal antibody (mAb5) by cryo-electron microscopy, revealing the tertiary and quaternary structure at sufficient resolution to clearly identify the complex components. Complementary mass spectrometry experiments revealed the structure and locations of *N*-linked carbohydrate post-translational modifications. This work provides insight into the structure of IRBP, displaying an elongated, flexible three-dimensional architecture not seen among other retinoid-binding proteins. This work is the first step in elucidation of the function of this enigmatic protein.

Presenter: Pravesh Shrestha – Postdoc (Buck Lab)
Title: Structure and functional studies of the effects of phosphorylation on ephrin receptor tyrosine kinase, epha2, and the relationship with its SAM domain as an autoinhibitor

Erythropoietin-producing hepatocellular (Eph) receptors are the largest subfamily of the membrane-bound receptor tyrosine kinase (RTK) family. Eph receptors have significant roles during embryonic development, cell maturation, and adulthood. The role of the receptor in axon guidance and synaptogenesis is well established. While normally a repulsive signal, a non-canonical cell migration promoting activity has been observed by a non-canonical unliganded Ephrin type-A receptor 2 (EphA2) signaling mechanism. Here we probed the effects of phosphorylation on the intracellular domain (ICD) interactions of EphA2 in solution and bound to membranes [1]. Results from this study indicate that deletion of the sterile α motif (SAM) domain leads to an increased binding between kinase domains in solution [2]. Interestingly, upon oligomerization, a reduced kinase activity is observed, compared to that of the monomeric state of the EphA2 ICD. Thus, intriguingly, while deletion of the SAM domain increases oligomerization in case of phosphorylated ICD, it appears that such persistent protein-protein interactions are not required for kinase activity in vitro. A mutation study of the linker region between kinase domain and SAM domain give insight into its regulatory role for EphA2 activity. Also docking, all atom as well as coarse grained dynamics simulations only support a weakly bound kinase dimer in solution. However, at the membrane the receptors quickly dimerize in the simulations. Nevertheless, the activation likely involves an allosteric mechanism by disrupting SAM domain-kinase domain and/or SAM domain-membrane interactions.

Presenter: Zhemin Zhang – Postdoc (Yu Lab)
Title: Cryo-electron microscopy structure of *Acinetobacter baumannii* Efflux Pump AdeJ

Acinetobacter baumannii is a Gram-negative, nonfermentative bacillus, which is considered as an important nosocomial pathogen causing pneumonia, urinary tract infections, bacteremia, septicemia, and meningitis. Infected by A. baumannii is extremely difficult to cure. One major mechanism that A. baumannii uses to mediate antibiotic resistance is the expression of multidrug efflux pumps, especially

those belonging to the resistance-nodulation-cell division (RND) superfamily. The most clinically relevant RND-type multidrug efflux in A. baumannii is the AdeJ membrane protein, which extrudes multiple classes of antibiotics, such as tetracyclines, aminoglycosides, β-lactams, and fluoroquinolones. Here, we report the cryo-electron microscopy (cryo-EM) structure of the A. baumannii AdeJ multidrug efflux pump bound with eravacycline to a resolution of 2.9 Å, revealing a mechanism on how AdeJ recognize and extrude the antibiotics of the tetracycline family. These data will provide a key structural foundation for future anti-bacterial drug discovery.

Presenter: Alexander Day – Graduate Student (Taylor Lab)
Title: Filling the structural gap between the PP2A A subunit isoforms

Protein phosphatase 2A (PP2A) is a heterotrimeric serine/threonine phosphatase that is an important tumor suppressor due to its involvement in regulating many oncogenic signaling pathways. The formation of PP2A is a highly regulated process in which one of many regulatory B subunit isoforms and the catalytic C subunit bind to one of two scaffolding A subunit isoforms (Aα or Aβ). Dysregulation of this process is the most common cause of impairment in PP2A function, leading to the pathogenesis of many human cancers. The alterations (through mutation, deletion, or amplification) responsible are found in all three subunits, but most commonly occur in the A subunit, being found in ~35% of human cancers. Interestingly, mutations found in cancers resulting in deletion of the A subunit are significantly more common in the Aβ isoform compared to the Aα isoform. This suggests that deletion of Aα is less favorable for tumorigenesis compared to deletion of Aβ. Furthermore, previous research has shown that deletion of Aα actually results in an increase in Aβ expression, producing an anti-tumorigenic effect, possibly explaining this phenomenon. Despite sharing 86% sequence identity and differing primarily in a 12 amino acid N-terminus unique to Aβ, the two isoforms behave quite differently in the context of tumor suppression. This leads to the hypothesis that Aα and Aβ function differently in regulating PP2A heterotrimeric assembly, with each A isoform possibly favoring assembly with different B subunit isoforms. Structurally, the work up to this point has focused on the Aα isoform, with structural information available of the isoform in complex with many of the B subunits. This work seeks to obtain similar information in the context of the Aβ isoform to allow direct structural comparison between the isoforms that will hopefully elucidate the structural mechanisms contributing to their differing observed phenotypes.

Presenter: Sepalika Bandara – Postdoc (von Lintig Lab)
Title: Structural and functional analysis of Carotenoid oxygenase 2

Apocarotenoids share a common structure and play important roles as signaling molecules and chromophores in all kingdoms of nature. Modifications of their ionone rings and variations in chain length contribute to the large diversity of these compounds. Apocarotenoids have been implicated in modulating cellular signaling pathways across different species. However, the mechanism that controls the activity of these dietary compounds remain undefined. We here show that the enzyme β-carotene oxygenase-2 (BCO2) plays a critical role in this process. Recombinant mouse BCO2 cleaves the alcohol, aldehyde, and carboxylic acid of an apocarotenoid substrate. Chain length variation (C20 to C40) and ionone ring site modifications of the apocarotenoid substrate did not impede the catalytic turnover by BCO2.

Furthermore, structural modeling identified Asn132 and Phe525, two highly conserved amino acids in the substrate tunnel of BCO2, as critical for the binding of the carbon backbone of an apocarotenoid and the positioning of the scissile C9, C10 double bond to the ferrous iron in the active center. Site-directed mutagenesis, Asn132Leu, and Phe525Leu impeded catalytic turnover by a mutant BCO2 variant. Mice deficient for BCO2 displayed impaired metabolism and altered signaling responses when supplemented with apocarotenoids. Thus, our analysis established BCO2 as an apocarotenoid scavenger with broad substrate specificity that prevents inadvertent side reactions of these dietary compounds with endogenous signaling pathways.

Mini Oral Presentations, Wednesday October 28

Molecular Therapeutics and Cancer

Presenter Name: Leslie Cueller Vite – Graduate Student (Keri Lab)
Title: Targeting the MUVB complex potentiates the efficacy of CDK4/6 inhibitors in breast cancer

Uncontrolled progression through the cell cycle sustains the growth and progression of cancer. The cell cycle is composed of 4 phases that are tightly regulated by transcriptional modulators. Two such cell cycle regulators are the RB-E2F and MUVB complexes. Both complexes are known to regulate transcription of genes during the G1 to S and G2 to M cell cycle phases. The regulated transcriptomes of both complexes overlap, suggesting that in order to inhibit cell cycle progression, both complexes should be targeted. Inhibitors that regulated the RB-E2F signaling axis have been FDA approved for treatment of a subset of breast cancers. These drugs specifically inhibit two kinases involved in the cell cycle known as CDK4 and CDK6. Palbociclib and abemaciclib, CDK4/CDK6 inhibitors (CDK4/6i), decrease cell proliferation by inducing a G1 cell cycle arrest. We previously reported that the activity of MUVB complex can be repressed using inhibitors of BET proteins, including the tool compound, JQ1. We hypothesized that dual targeting of MUVB and CDK4/CDK6 will synergize in breast cancer models to further decrease growth. To test this hypothesis, we assessed the impact on growth of combining JQ-1 and CDK4/6i in the intrinsically resistant cell lines, MDA-MB-231 and SUM149. We determined that adding JQ-1 reduced the IC_{50} of either CDK4/6i by 50-75% and the combination of JQ-1 and CDK4/6i displayed synergistic growth suppression. Mechanistically, analysis of mRNA and protein expression revealed that the combined drugs (CDK4/6i + JQ1) had a greater ability to suppress the expression of cell cycle genes compared to single agents. A preclinical, xenograft mouse model of breast cancer confirmed that the combination of MuvB and palbociclib can synergistically suppress tumor growth, *in vivo*. These results provide a rationale for clinically targeting the two major cell cycle regulators to block breast cancer growth.

Presenter: Xinran Geng – Graduate Student (Zhang Lab)
Title: CRISPR/Cas screen of factors regulating heterochromatin liquid phase separation

Heterochromatin is characterized by compact DNA structure, which not only protects DNA from damage, but also represses genetic/epigenetic transcription. Hence, it is not surprising that some cancers are associated with abnormalities of proteins involved in the formation or maintenance of heterochromatin. For example, breast cancers express decreased levels of the heterochromatin protein 1 alpha (HP1α). Recently, a novel concept called liquid-liquid phase separation (LLPS) has been presented to be critical for heterochromatin function, in which heterochromatin factors including HP1α form liquid droplets to promote gene silencing. However, how exactly LLPS regulates heterochromatin and what are other critical factors required for this process are poorly understood.

53BP1 is a multiple-domain protein that is best known for DNA double strand break (DSB) repair. However, whether it is involved in other biological processes is unclear. Our unpublished results show that 53BP1 forms a unique nuclear punctate at heterochromatin. Build upon this observation, I revealed that the 53BP1 punctate depends on HP1α and bears the same LLPS properties as HP1α. Importantly, I found that 53BP1 and HP1α depend on each other to undergo LLPS. These findings unveil an innovative role for 53BP1 in heterochromatin LLPS.

The goal of this project is to utilize the CRISPR/Cas technique to perform genome-wide screen to identify genes that regulate 53BP1 LLPS at heterochromatin. A lentivirus sgRNA library pool targeting the entire human genome will be used and the CRISRP-Cas9 screen will be performed on the Cytation 5 Cell Imaging Multi-Mode Reader System at the CWRU Genetics Core. To automate the identification of hits, I will first develop a machine-learning model trained to identify 53BP1 or HP1α LLPS. Subsequently, the genome-wide screen will be performed and top candidates will be identified by DNA sequencing. Eventually, their roles in 53BP1 LLPS and heterochromatin function will be explored.

Presenter: Parth Majmudar – Graduate Student (Keri Lab)
Title: Lis1 is necessary for TNBC cell viability and proper cell cycle progression

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, exhibiting faster growth and higher metastatic spread than other subtypes. TNBC lacks expression of estrogen receptor, progesterone receptor, and HER2, thus limiting the use of targeted therapies that are available for treating other subtypes of breast cancer. These factors underlie the poor prognosis observed in TNBC patients and highlight the need for identifying novel druggable targets. The aggressive growth seen in TNBC cells makes mitosis a lucrative target; however, current antimitotic therapies have a number of limitations, such as high incidence of acquired resistance, toxicity, or limited efficacy. One understudied class of antimitotic therapies includes targeting the motor protein dynein and associated proteins. Lis1, a gene identified to cause lissencephaly upon loss or mutation, was identified in an Achilles shRNA screen as a gene potentially essential for breast cancer growth, and it associates with and regulates the function of dynein, important for chromosomal movement and mitotic spindle orientation. Loss of Lis1 in TNBC cells reduces viability by interfering with proliferation. FACS analysis using propidium iodide shows that Lis1-KD cells have altered cell cycle dynamics, with an accumulation in the G2/M phase. Current work is aimed at generating H2B-GFP expressing TNBC cell lines to visualize chromosomes at high resolution without disrupting mitosis or nuclear structure in order to elucidate the mitotic defects that occur following loss of Lis1. Additionally, live cell imaging with markers for cell cycle transitions is being explored to track individual cells through the cell cycle in order to assess mitotic duration as well as cell fates. As the structure of Lis1 is conducive for a potential inhibitor to bind, uncovering the dependence of TNBC cells on Lis1 for proper mitosis may represent the first step in the development of a novel treatment for TNBC.

Presenter: Xu Han – Graduate Student (Nieman Lab)
Title: Protease activated receptor 4 is a novel target associated with venous thromboembolism

Venous thromboembolism (VTE) is the leading cause of mortality. Although many risk factors of VTE have been identified, the molecular mechanisms are largely unknown. Hypercoagulation is one of the main drivers of the VTE and growing evidence indicate platelets have a key initiating role. Given that protease activated receptor 4 (PAR4) mediates sustained thrombin signaling leading to procoagulant platelets, we hypothesized that PAR4 has a central unrecognized role in VTE development. Our recent studies demonstrated the rigidity of extracellular loop 3 (ECL3) is critical for PAR4 activation. Further, an uncharacterized single nucleotide polymorphism (PAR4-310P/L (rs2227376)) located in ECL3 affects the loop rigidity. We examined the association of rs2227376 with VTE using the International Network Against Venous Thrombosis (INVENT) Consortium, which is a multi-ancestry GWAS meta-analysis to identify novel genetic markers for VTE. The PAR4 allele encoding for Leu^{310} had a 15% relative risk reduction for VTE compared to the Pro^{310}, which suggested that Leu^{310} leads to a hyporeactive receptor.

A mouse model is needed to further examine the mechanism of this association between PAR4-310L and VTE. PAR4 is highly conserved between human and mouse, especially in the ECL3 region. Using calcium assay, we confirmed mutating Pro^{322} in mouse PAR4, which is the homologous site of the Pro^{310} in human PAR4, to Leu also reduced Ca^{2+} signaling in response to the PAR4-AP. We generated a knock-in mPAR4-P322L mouse using CRISPR. We will compare PAR4 reactivity in platelets from WT (P/P), PAR4-P322L (L/L), or heterozygous (P/L) mice.

In conclusion, we have identified that the PAR4-310L sequence variant resulting from rs2227376 leads to a hyporeactive receptor compared to PAR4-310P. Further, PAR4-310L was associated with a reduced risk for VTE. Future studies need to examine the mechanism of this association and it broader impact on platelet function.

Name: Katrina Piemonte – Graduate Student (Keri Lab)
Title: GAS2L3, a novel regulator of chromosomal instability in Triple Negative Breast Cancer

The distinct molecular profile and increased chromosomal instability (CIN) of Triple Negative Breast Cancer (TNBC) make it difficult to treat. CIN is both a mechanism of tumorigenesis and a point of therapeutic vulnerability. Moderate CIN can lead to unstable aneuploidy, promoting cellular evolution

and the ability to accommodate distinct microenvironments, induce chemoresistance, and promote metastasis. Alternatively, excessive CIN is detrimental to cancers and is the basis for efficacy of cytotoxic therapies. Understanding the mechanisms by which CIN develops provides an opportunity to identify novel druggable targets in TNBC. We identified Growth Arrest Specific 2 Like 3 (GAS2L3), a regulator of cytokinesis, as a potential driver of CIN that is overexpressed in TNBC and associated with decreased disease-free survival. Previous studies have shown that alterations in *GAS2L3* expression result in CIN and aneuploidy due to failed cytokinesis in normal tissues, but none have explored its role in cancer. We hypothesize that TNBC is dependent on elevated *GAS2L3* expression for mitotic fidelity and that elucidating the transcriptional and post-translational regulation of this protein would uncover novel druggable targets. We have found that *GAS2L3* suppression causes decreased cell proliferation, changes in cell cycle dynamics, and increased CIN in TNBC, indicating a reliance of these cancer cells on sustained *GAS2L3* expression. We have also shown that *GAS2L3* expression is regulated by the mitotic transcription factor, LIN9, suggesting that its aberrant transcriptional regulation may cause CIN in TNBC. More recently we have discovered that post-translational modifications may alter the activity *GAS2L3* to promote CIN. The ability of GAS2L3 to regulate CIN in TNBC makes it a promising candidate as a driver of disease and a druggable target. Future studies focus on discovering the mechanistic role of GAS2L3 in CIN development in TNBC and how to target it by modulating its gene expression or post-translational regulation.

Presenter: Yuli Buckley – Graduate Student (Mears Lab)
Title: Elucidating the role of Fis1 in mediating mitochondrial fission within the context of glioblastoma multiforme

Mitochondria are dynamic organelles, undergoing fusion and fission to maintain cell energy and health. These opposing processes are mediated by dynamin superfamily proteins, and dynamin-related protein 1 (Drp1) acts as the sole mediator of fission. Dysfunctional mitochondrial dynamics contribute to many pathologies, and elevated levels of mitochondrial fission have been observed in glioblastoma multiforme (GBM). An aggressive brain cancer, GBM has a poor prognosis and less than 5% of patients survive five years. Inhibitors of Drp1 offer a therapeutic strategy, but mitochondrial fission is an essential process, and inhibiting the key protein in this process may be too aggressive. Therefore, Drp1 partner proteins that recruit and regulate its function offer a novel therapeutic approach. The focus of my project is to elucidate the role of mitochondrial fission 1 protein (Fis1), an integral outer membrane partner protein, in mitochondrial fission within GBM cells. Previous studies have shown that disrupting the interaction between Drp1 and Fis1 with a neuropeptide can reduce excessive fission in a Parkinson's Disease model, but the underlying mechanism of inhibition is unknown. My initial studies have determined that the neuropeptide binds to Fis1, rather than Drp1, and *in vitro* studies will measure the affinity of this interaction and use structural methods to characterize neuropeptide interactions with Fis1. Additionally, I am assessing Fis1 levels in various GBM cell lines to identify potential changes in GBM. Genetic approaches will be used to interrupt the Drp1-Fis1 interaction, and different GBM cell lines will be treated with the neuropeptide to disrupt mitochondrial fission. Changes in mitochondrial morphology and physiology will be measured, and I anticipate that inhibiting Drp1-Fis1 interactions will limit mitochondrial fission in GBM cells. This work will demonstrate the roles of these proteins in tumor cell growth and proliferation.

Presenter: Wilnelly Hernandez-Sanchez – Postdoc (Taylor Lab)
Title: Mechanistic framework for telomerase kinetics identifies regulation of telomerase by dNTP substrate inhibition

Telomeres are repetitive DNA sequences located at the ends of all linear chromosomes. Since replicative polymerases are unable to extend the extreme ends of the lagging strand of DNA, telomeres become shorter during each cell division. To compensate for this loss, an enzyme called telomerase synthesizes telomere DNA, thus becoming essential for complete genome replication. Telomerase is a unique RT that uses its own RNA template in a dynamic way, allowing it to translocate for template regeneration and synthesize telomeres in a processive manner. In this work, we explored how telomerase nucleotide addition activity and processivity are modified in the presence of different nucleotide concentrations. We were able to show that increasing the concentration of dATP, dTTP, and dGTP affects telomerase nucleotide addition activity during different steps. We also calculated the processivity associated with the addition of each nucleotide in the presence of different nucleotide concentrations and observed that

increased dATP levels could decrease telomerase processivity. Our advanced kinetic approach reveals new and unexpected details about how telomerase activity is regulated by deoxynucleotide (dNTP) concentrations. These new insights into telomerase kinetics provide a basis for the development of new strategies for telomerase inhibition by targeting cellular dNTP levels.

Presenter: Franklin Mayca Pozo
Title: b-TrCP ubiquitin ligase recognizes a DEGRON motif of Myosin-X and modulates the genomic instability in cancer cells

Myosin-X is an unconventional myosin and is expressed in many tissue types. This protein is commonly found in the tips of filopodia and is linked to the normal biological process of cell migration. Overexpression of myosin-X has been detected in different cancer (breast cancer, prostatic cancer, acute lymphoblastic leukemia, etc.) and is associated with metastasis.

The ubiquitin proteasome pathway drives the degradation of the majority of eukaryotic proteins. β-TrCP is a member of SCF (Skp1-Cullin 1-F-box protein) Ub-E3 ligase protein complex. β-TrCP recognizes the substrates in a phosphorylation-dependent manner.

Here, we reported a significant increase in the frequency of micronucleus, abnormal mitosis and chromosome abnormalities in Myosin-X overexpressed cells. Additionally, depletion of Myosin-X in aggressive cancer cells decreases the genomic instability. The regulation of Myosin X is the key to modulating the aggressiveness of the cancer. We identified a phospho-degron domain (DSGxxS) in Myosin-X between D1059 and S1066 nucleotides. We observed interaction between Myosin-X and b-TrCP through this phospho-degron domain. We found that Myosin-X is rapidly degraded via the ubiquitin proteasome system using the SCFβ-TRCP complex. A S1066A point mutation in Myosin-X avoided the interaction with b-TrCP, consequently delayed the degradation, and then stabilized the Myosin X protein. We identified cancer patient's carrier mutations in the Degron domain of myosin-X (S1062R, L1063P). These mutations prevent the interaction between Myosin-X and b-TrCP, resulting in the delay of Myosin-X degradation and increasing the genomic instability.

We propose that the stability and ubiquitination of Myosin-X is regulated by the SCF-β-TrCP ubiquitin enzyme. Furthermore, Myosin-X acts as a tumor-promoting gene by causing genomic instability. These data suggest that Myosin-X could be a target for cancer therapy in the future.

Full Oral Presentations – Thursday, October 29

Cancer and Translational Therapeutics

Presenter: Elizabeth Knauss – Graduate Student (Nieman Lab)
Title: The crosstalk between venous thromboembolism and pancreatic cancer
Pancreatic ductal adenocarcinoma (PDAC) is a particularly aggressive cancer with a clear link to inflammation as a promoter of disease onset and progression. A critical complication in cancer patients is the development of a venous thromboembolism (VTE). The risk of cancer-associated VTE is well established. Clinical observations also suggest that VTE leads to a poorer cancer prognosis, however the impact of VTE on cancer progression has yet to be investigated mechanistically. The goal of this project is to determine how VTE promotes cancer progression by identifying the pathways involved. Due to the high level of inflammation and established role of neutrophils in both VTE and cancer, I hypothesize that VTE modifies circulating neutrophils to drive pancreatic cancer progression. To replicate clinical observations in a mouse model, I orthotopically administered the syngeneic pancreatic tumor cell line PAN02 into the pancreas of C57Bl6 mice. Half of the mice were induced to develop VTE by ligating the inferior vena cava (IVC). Mice that developed a VTE had larger tumors after 16 days of growth. These results agreed with the clinical observations that VTE increases tumor burden, and the model can be used to further investigate the mechanism behind VTE-related tumor growth. PAN02 tumors were also grown on the flanks of mice with and without VTE, and immunohistological staining with Ly6G showed that tumors from mice with VTE had an increased number of neutrophils, although it was not statistically significant. To further establish how VTE alters neutrophils to promote tumor growth, peripheral blood

neutrophils from mice with pancreatic tumors, VTE, or both were harvested and subjected to RNA-sequencing. Results showed that the presence of VTE induced unique changes in the transcriptome. Together, this suggests a major and undetermined link between VTE and pancreatic cancer, as well as a crucial role of neutrophils.

Presenter: Peng Zeng – Graduate Student (Schmaier Lab)
Title: Vascular inflammation and platelet potentiation contribute to the CML T315I targeting Ponatinib caused thrombosis

Ponatinib (Iclusig) is an essential tyrosine kinase inhibitor that targets the T315I polymorphism in Bcr-Abl1 kinase in 15% of CML and ALL patients. However, its use is associated with vascular occlusive events in 31% of patients. Previous studies from our laboratory show that ponatinib-treated mice at therapeutic concentrations in man are prothrombotic on carotid artery assays. Our group also found increased reactive oxygen species as indicated by nitrotyrosine expression and apoptosis as shown by cleaved caspase 3 in vessel wall adventitia. Also, those animals also have hyperactive platelets. Bulk RNAseq on aorta from mice treated with ponatinib reveal that the top up-regulated gene sets are involved in immunologic activation, hemostasis, platelet activation and GPVI-mediated activation cascade. Investigations sought to corroborate genetic studies with phenotypic abnormalities of ponatinib-treated mice.
Cleaved caspase 3+ cells found to colocalize with those that express nitrotyrosine. Inspired by RNA-seq data, we stained aorta for Ly6G and CX3CR1 and found significant elevation. CX3CR1+ cells colocalize with nitrotyrosine and cleaved caspase 3. Ly6G does not. CD19+ cells colocalize with nitrotyrosine and cleaved caspase 3; CD8+ cells colocalize with cleaved caspase 3. Additional studies show significantly more FVIII or FV positive cells in aortic adventitia of ponatinib-treated mice. Further, the tunica adventitia of aorta from ponatinib-treated mice has higher levels of tissue factor. Ponatinib treatment leads to immune cell vascular inflammation. Additional studies examined the GPVI pathway in platelets from ponatinib-treated mice. In ponatinib-treated platelets, *p*-LynY^{507}, but not *p*-LynY^{396}, is dephosphorylated in both murine and human platelets. These data suggest that with ponatinib treatment platelet Lyn changes from a closed to open conformation making it more susceptible to agonist activation. These investigations indicate that the pharmacologic effects of ponatinib *in vivo* make individuals more susceptible to thrombosis by combined vessel wall and platelet mechanisms.

Presenter: Christine Lee-Poturalski – Graduate Student (Eckhard Jankowsky Lab)
Title: The translational landscape of patient-derived glioblastoma stem cells

Glioblastoma stem cells (GSCs) are responsible for initiation and therapeutic resistance of adult glioblastoma (GBM). Therefore, targeting of GSCs is considered a promising avenue to treat GBM, but effective therapies are not yet available. To understand GSC biology and to identify new therapeutic targets, we investigate the regulation of translation in GSCs. Here, we performed ribosome profiling on GSCs and corresponding differentiated glioblastoma cells (DGCs) from patient-derived xenografts. We find marked discordance between mRNA abundance and levels of translation in GSCs and in DGCs, indicating that gene expression in GSCs and DGCs is largely regulated on the level of translation. We also observe significantly higher accumulation of ribosomes in 5'UTRs in GSCs, compared to DGCs, suggesting roles for uORFs in the translational regulation in GSCs. In addition, our data reveal GSC-specific pathways on affected the translational level, for which we utilized a pooled drop-out CRISPR screen to test specific genes. Our CRISPR screen results pinpoint HSPA8 (heat shock protein family A member 8) GSC-specific dependency on the translational level to promote stemness and growth in patient-derived GSCs. Collectively, our data show that systematic analyses of the translational landscape provides new insight into GSC biology and the potential to identify therapeutic targets specific for glioblastoma stem cells.

Presenter: Sarah Kohrt – Graduate Student (Grabowska Lab)
Title: MAP3K11 as a driver of enzalutamide resistance in castration-resistant prostate cancer

Prostate cancer is estimated to result in the deaths of 31,000 men each year, making it the second leading cause of cancer-related deaths in the US. A majority of these deaths are attributed to castration-resistant prostate cancer (CRPC), which emerges in response to androgen deprivation therapy. CRPC patient tumors can be treated with the androgen receptor (AR) inhibitor enzalutamide, and while enzalutamide is effective, tumors will inevitably become resistant. How resistance to enzalutamide occurs is still not

fully understood. Our lab has identified MAP3K11, also known as mixed-lineage kinase 3 (MLK3), as a putative driver of enzalutamide resistance. Using crystal violet cell survival assays, our lab shows MAP3K11 knockdown decreased cell survival in CRPC cell lines C4-2B and 22RV1, and in enzalutamide resistant cell lines CWR-R1-EnzR and C4-2B MDVR. Additionally, treatment with CEP-1347, a patient-safe MAP3K11 inhibitor, sensitized CRPC cells to enzalutamide treatment in proliferation assays. CRPC and enzalutamide-resistant cell lines show decreased cell survival when treated with CEP-1347 in combination with enzalutamide, compared to either treatment alone. MAP3K11 is a known component of the JNK, p38 MAPK, and ERK signaling pathways, however, these interactions have not been elucidated in CRPC. Furthermore, JNK has been implicated in PCa progression as a phosphorylator of AR serine 650 to induce nuclear shuttling. These studies show phopho-AR-Ser650 localizes to the cell nucleus while a decrease in AR-Ser650 phosphorylation is observed with MAP3K11 KD and CEP-1347 treatment. Overall, this data suggests MAP3K11 plays a role in driving resistance to enzalutamide in CRPC by affecting AR phosphorylation and can be targeted to increase efficacy of treatments. Our future studies include generating stable MAP3K11-altered and AR-Ser650 mutant cell lines to further determine the mechanism of resistance in CRPC and assess enzalutamide sensitivity using *in vitro* and *in vivo* models.

Presenter: Kimberly Parker – Graduate Student (Schiemann Lab)
Title: The IncRNA BORG mediates alternative splicing to enhance cancer stem cell properties in TNBC

Breast cancer is the second leading cause of cancer-related deaths among women in the United States. A subset of breast cancer called triple-negative breast cancer (TNBC) has the worst prognosis among breast cancer subtypes, due to both the frequency and location of the resulting metastases. In TNBC, the underlying mechanisms that mediate metastatic outgrowth after disseminated tumor cells implant into distal sites is unknown. However, our laboratory recently identified the long noncoding RNA, BORG (BMP/OP-Response Gene), as a driver of TNBC metastasis. BORG expression enhances neoplastic proliferation and chemoresistance, which are important cellular functions associated with metastatic phenotypes. Mechanistically, our group showed that BORG complexes with the E3 SUMO ligase TRIM28 to enhance neoplastic proliferation. The proliferative and cell survival pathways activated by BORG reflect breast cancer stem cell (BCSC) phenotypes, including self-renewal, tumor initiation, and cell differentiation. Therefore, I hypothesize that BORG enhances BCSC phenotypes in TNBC cells. In addressing this hypothesis, I demonstrate that BORG enhances BCSC mammosphere formation and tumor growth both *in vitro* and *in vivo*, and that this phenotype relies in part on the formation of BORG:TRIM28 complexes. Further, the mechanisms through which BORG and TRIM28 enhance BCSC plasticity are not yet understood. To this end, I find that BORG expression causes significant transcriptomic reprogramming, suggesting that BORG enhances cellular plasticity by mediating transcriptomic changes (*e.g.,* alternative splicing) which broadly influences BCSC-like phenotypes. Accordingly, I show that BORG expression plays a role in alternative splicing by dysregulating 144 alternative splicing events. Moreover, I determined that BORG downregulates the expression of 38 splicing factors, 16 of which rely upon BORG:TRIM28 complexes. These and other findings suggest that BORG mediates alternative splicing changes, which contribute to aggressive BCSC-like cells in TNBC.

Presenter: Tawna Mangosh – Graduate Student (Taylor Lab)
Title: SLX4IP promotes telomere maintenance in androgen receptor-independent castration-resistant prostate cancer through ALT-like telomeric PML localization

In advanced prostate cancer, resistance to androgen deprivation therapy is achieved through numerous mechanisms, including loss of the androgen receptor (AR) allowing for AR-independent growth. Therapeutic options are limited for AR-independent castration-resistant prostate cancer, and defining mechanisms critical for its survival is of utmost importance for targeting this lethal disease. Our studies have focused on defining the telomere maintenance mechanism (TMM) required for castration-resistant prostate cancer (CRPC) cell survival. TMMs are responsible for telomere elongation to instill replicative immortality and prevent senescence, with the two TMM pathways available being telomerase and alternative lengthening of telomeres (ALT). Here, we show that AR-independent CRPC exhibits ALT hallmarks and limited telomerase expression and activity, whereas AR-dependent models use telomerase for telomere maintenance. AR-independent CRPC exhibited elevated levels of SLX4IP, a protein implicated in TMM switching. SLX4IP overexpression in AR-dependent C4-2B cells promoted ALT hallmarks *in vitro*. SLX4IP knockdown in AR-independent CRPC cells (DU145 and PC-3) led to the loss of

ALT hallmarks, dramatic telomere shortening, induction of senescence, and reduced tumor volume. Using an *in vitro* model of CRPC progression, induction of neuroendocrine differentiation in AR-dependent CRPC cells promoted ALT hallmarks in an SLX4IP-dependent manner. Lack of sufficient SLX4IP expression prevented ALT hallmarks rendering a TMM deficient environment, thus inducing senescence. This study demonstrates a unique reliance of AR-independent CRPC on SLX4IP-mediated ALT. Furthermore, ALT hallmark inhibition via SLX4IP induces senescence, thereby abolishing the replicative immortality of AR-independent CRPC.

Mini Oral Presentations, Thursday October 29

Animal Models of Eye Diseases and Regulation of Visual Processes

Presenter: Elliot Choi – Graduate Student (Palczewski Lab)
Title: Retinal pigment epithelium-specific tamoxifen-inducible *Cre* mice

The retinal pigment epithelium (RPE) is a monolayer of cells that closely interacts with the photoreceptors of the retina providing metabolic support to maintain visual function. The RPE plays a critical role in the photochemistry of vision by supplying visual chromophore, 11-*cis*-retinal, for rod and cone opsins through the visual cycle pathway. The RPE also supports photoreceptors by forming the blood-retinal barrier allowing selective transport of nutrients and waste products between the photoreceptor cells and the systemic circulation and contributes to the maintenance of the photoreceptor outer segments. Due to these unique and essential roles, RPE dysfunction underlies a broad spectrum of retinal diseases including age-related macular degeneration, retinitis pigmentosa, Leber congenital amaurosis and fundus albipunctatus.
To understand the function of a gene expressed in the RPE, its spatiotemporal regulation is requisite. Since many genes thought to mediate critical RPE functions are expressed in other regions of the retina, global knockout mice are often inadequate to address gene function specifically in the RPE. The Cre-LoxP recombinase system is one of the most effective and versatile tools available for precise excision of a DNA sequence in a specific tissue. Consequently, there has been significant efforts to generate RPE-specific Cre mouse lines. However, there are several limitations with currently available RPE specific mouse lines. Recurring inadequacies associated with RPE-directed Cre lines are their mosaic expression of Cre within the RPE and consequent incomplete levels of recombination, Cre expression outside of the RPE, and/or Cre-mediated toxicity to the RPE. Notably, all RPE-directed Cre mouse lines reported to date have been generated using transgene approaches with random incorporation of the expression sequence into the genome. To overcome these shortcomings, we generated a new inducible RPE-specific Cre mouse line in which CreERT2 expression is driven by the native promoter of Rpe65.

Presenter: Anahita Daruwalla – Graduate Student (Kiser Lab)
Abstract: New large animal model for RDH5-associated retinopathies

RDH5, an 11-*cis*-retinol dehydrogenase present in the retinal pigment epithelium (RPE) of the eye retina catalyzes oxidation of 11-*cis*-retinol to 11-*cis*-retinaldehyde, thus regenerating the visual chromophore. In humans, mutations in RDH5 are predominantly associated with fundus albipunctatus, characterized by presence of white flecks in the retina and delayed rod adaptation impairing night vision. However, a subset of patients also develops macular atrophy or cone dystrophy. *Rdh5*$^{-/-}$ mice do not recapitulate human phenotype and exhibit delayed dark adaptation only after prolonged bleaching. In this study, we characterized a cat model homozygous for a G181V mutation in RDH5. Homology modeling predicted that this substitution could alter dimerization or disrupt the structure of nearby residues of catalytic importance. *RDH5*$^{G181V/G181V}$ cats were found to have delayed rod and cone recovery times and tomography and electron microscopy images of their retina show distorted photoreceptors and degeneration in their area centralis, resembling human macular degeneration. Moreover, *in vitro* expressed G181V mutant in insect cells has loss of dehydrogenase activity, indicating a null mutation.

Analyses of retinoids from *RDH5*$^{G181V/G181V}$ cat retina show elevated 13-*cis*-retinyl esters compared to *RDH5*$^{WT/G181V}$ and *RDH5*$^{WT/WT}$ cat retinas, indicating an abnormality in their visual cycle. Our results describe a new, large animal model to study RDH5-associated retinopathies seen in humans. To this end, we sought to characterize RDH5 mutations recently known to be associated with three human patients similarly showing central retina atrophy. We found the *in vitro* activity for two of these mutants, S201F and R209X to be abolished. However, activity of the third mutant M253R was conserved, albeit, at efficacy levels much lower to wild-type enzyme. Further establishing a relationship between type of mutation and etiology of macular lesions will be critical in studying disease pathophysiology. Additionally, we plan on identifying other putative modifying loci in the RPE that likely play a role in disease progression and pathogenesis.

Presenter: Jennings Luu – Graduate Student (Palczewski Lab)
Title: Epigenetic hallmarks of age-related macular degeneration are recapitulated in a photosensitive mouse model

Age-related macular degeneration (AMD) is a chronic, multifactorial disorder and a leading cause of blindness in the elderly. Characterized by progressive photoreceptor degeneration in the central retina, disease progression involves epigenetic changes in chromatin accessibility resulting from environmental exposures and chronic stress. Here, we report that a photosensitive mouse model of acute stress-induced photoreceptor degeneration recapitulates the epigenetic hallmarks of human AMD. Global epigenomic profiling was accomplished by employing an Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq), which revealed an association between decreased chromatin accessibility and stress-induced photoreceptor cell death in our mouse model. The epigenomic changes induced by light damage include reduced euchromatin and increased heterochromatin abundance, resulting in transcriptional and translational dysregulation that ultimately drives photoreceptor apoptosis and an inflammatory reactive gliosis in the retina. Of particular interest, pharmacological inhibition of *histone deacetylase 11 (HDAC11)* and *suppressor of variegation 3-9 homolog 2 (SUV39H2),* key histone-modifying enzymes involved in promoting reduced chromatin accessibility, ameliorated light damage in our mouse model, supporting a causal link between decreased chromatin accessibility and photoreceptor degeneration, thereby elucidating a potential new therapeutic strategy to combat AMD.

Presenter: Susie Suh – Graduate Student (Palczewski lab)
Title: Treatment of an inherited retinal disease by in vivo base editing in an adult mouse model

CRISPR-Cas9 genome editing has great potential for the treatment of numerous genetic diseases. However, the clinical translation of the CRISPR-Cas9 system is impeded by its low editing efficiency by homology-directed repair and substantial indel formation caused by double-stranded DNA breaks. Cytosine and adenine base editors (CBEs and ABEs) enable conversion of a point mutation in a predictable manner independent of Cas9-induced double-stranded DNA breaks and homology-directed repair. Here, we demonstrate in a mouse model of an inherited retinal disease that subretinal injection of a virus expressing the ABE and a single-guide RNA (sgRNA) can efficiently correct a pathogenic mutation in the *Rpe65* gene up to 29% with minimal indel and off-target mutations despite the absence of a canonical "NGG" protospacer-adjacent motif (PAM) sequence. The ABE-treated mice show restored RPE65 expression, retinoid isomerase activity, and retinal and visual function at near-normal levels. Our findings demonstrate the therapeutic potential of ABE for inherited retinal diseases and the expanded applicability of ABE to target pathological mutations with non-canonical PAM sequences.

Presenter: Linda Thomas – Graduate Student (von Lintig Lab)
Title: Human β-carotene-oxygenase 2 and carotenoid metabolism

Carotenoids (C_{40}) are yellow to red pigments whose light-absorbing property and physiological functions in organisms are attributed to their conjugated double-bond system. Centric or eccentric oxidative cleavage of carotenoids are catalyzed by carotenoid cleavage dioxygenases (CCDs) and result in apocarotenoid products. In animals, apocarotenoids function as vitamins, visual pigments, and signaling molecules. Humans have two CCDs that directly cleave carotenoids. These are β-carotene-15,15'-dioxygenase (BCO1) and β-carotene-9',10'-dioxygenase (BCO2). In humans, the aggregation of

zeaxanthin, mesozeaxanthin, and lutein, collectively referred to as the macula pigment, is found in the retina of the eye. However, given the expression of BCO2 in human retina, there remains to be elucidated the mechanisms underlying the formation and maintenance of the macula pigment. It remains controversial whether humans express an enzymatically inactive BCO2 variant and its physiological relevance.Therefore, the focus of this work involves understating BCO2's role in human carotenoid metabolism and its clinical relevance. Health professionals advocate for carotenoid supplementation for patients experiencing age-related macular degeneration. While such recommendations are based on the relationships between carotenoid intake, blood, and tissue concentrations, studies in animal models demonstrate that this relationship depends on BCO2 activity. Thus, excessive levels of carotenoids may cause detrimental effects.Here we expressed recombinant isoforms of human BCO2 as well as mouse BCO2 in order to determine the biochemical factors that influence its enzymatic activity. We performed both cell-based enzymatic assays and *in vitro* enzymatic activity assays to examine differences in enzymatic activity between various isoforms. High performance liquid chromatography (HPLC) was used to determine BCO2's capability to cleave these substrates and identify the products that were generated.

Presenter: Ramkumar Srinivasag – Research Associate (von Lintig Lab)
Title: LRAT is critical for the negative feedback control of vitamin A production in mammals

In mammals, dietary β-carotene is split into two molecules of retinaldehyde by the enzyme β-carotene oxygenase-1 (BCO1). Retinaldehyde can be enzymatically reduced to retinol and esterified to retinyl esters, the transport and storage mode of vitamin A. Some retinaldehyde is oxidized to retinoic acid which acts signal molecule in the negative feedback control of vitamin A production. Here we analyzed how the retinoic acid concentration are controlled in enterocytes of the intestine. Our analysis in mice revealed that retinol: lecithin acyl transferase (LRAT), a vertebrate-specific member of the NlpC/P60 thiol peptidase protein superfamily, is critical for this process. LRAT drives the pathway towards retinyl ester formation and vitamin A storage. In LRAT-deficiency, dietary β-carotene supplementation increased retinoic acid concentration and induced the expression of the intestine specific homeobox transcription factor ISX. ISX repressed *Bco1* gene expression and caused a premature shutoff of vitamin A production. The dysregulation of the pathway displayed in β-carotene accumulation and systemic vitamin A deficiency. Genetic deletion of *Isx* gene restored *Bco1* gene expression and vitamin A production in a LRAT- and ISX-deficient double mutant. Thus, our study revealed that the catalytic activity of LRAT dynamically adjusts retinoic acid concentration in the intestine to maintain homeostasis of the essential nutrient.

Presenter: Joseph Ortega – Research Associate (Jastrzebska Lab)
Title: Protective role of flavonoids in light-induced photoreceptors' degeneration

Retinal damage caused by excessive illumination, inherited mutations or aging are the principal pathophysiological factors related to blinding diseases. Targeting the visual receptor, rhodopsin, with compounds that increase its stability and modulate cellular pathways associated with the death of photoreceptors could be useful to prevent retinal damage. Interestingly, flavonoids can modulate the cellular processes such as oxidative stress, inflammatory responses, and apoptosis that are activated during retinal degeneration. We described previously that flavonoids bind directly to opsin, enhancing its folding, stability, and regeneration. Thus, we evaluated the effect of two main dietary flavonoids, quercetin, and myricetin in Abca4−/−Rdh8−/− and BALB/c mice susceptible to light-induced photoreceptors' degeneration. Using in vivo imaging and histological assessment of retinal morphology, we found that treatment with these flavonoids before light insult remarkably prevented retina damage and preserved its function. Using HPLC-MS analysis, we detected these flavonoids in the mouse eye upon their intraperitoneal administration. The molecular events associated with the protective effect of quercetin and myricetin were related to changes in the expression of photoreceptor-specific proteins, decreased expression of the specific inflammatory markers, and the shift in the equilibrium between BAX/BCL-2 towards an anti-apoptotic profile. Furthermore, these results were validated by using photoreceptor-derived 661W cells treated with either H2O2 or all-trans-retinal the main stressors implicated in retinal degeneration. Altogether, these results revealed the potential of flavonoids as lead compounds to develop new pharmacological agents to prevent or treat retinal degenerative diseases.

Full Oral Presentations – Friday, October 30

Molecular and Structural Biology and Pharmacology

Presenter: Shelby Dahlen – Graduate Student (Osei-Owusu Lab)
Title: Fine-tuning of Gi/o signaling by RGS2 and 5 is critical to ensuring normal ventricular rhythm

Cardiac arrhythmia is attributed partly to abnormal G protein signaling that may result from the loss of signaling regulation by regulators of G protein signaling (RGS) proteins which act as GTPase activating proteins to control the kinetics and amplitude of G protein signaling; thus a decrease in their expression and/or function could lead to loss of fine-tuning of G protein signaling. Because RGS2 and 5 proteins are prominently expressed in the myocardium, we hypothesized that the dual absence of RGS2 and 5 predisposes to decreased heart rate variability (HRV) leading to the development of cardiac dysfunction. We generated mice dually lacking RGS2 and 5 (dbKO). We used radiotelemetry to acquire diurnal blood pressure (BP) and ECG data from conscious mice to compute HRV in both time and frequency domains. Surgery-induced stress caused a marked increase in BP in dbKO mice (WT: 140±6 vs. dbKO: 170±2 mmHg; $p<0.01$) initially, and gradually declined but remained elevated several days later. Measures of HRV in time and frequency domains: standard deviation of cardiac cycles (SDNN) and total power (TP), were lower in male dbKO relative to WT mice. Interestingly, TP was markedly increased in female *Rgs2/5* dbKO compared to WT mice. We then assessed excitation-contraction coupling (ECC) in primary cardiomyocytes. Cells from male dbKO mice showed decreased fractional shortening (WT: 16.1±4.3 vs. dbKO: 7.4±1.1 %; $p<0.01$) but high Ca^{2+} transients (WT: 117±20 vs. dbKO: 198±50 au; $p=0.07$) at baseline. Application of low concentration of the non-selective β-adrenergic agonist, isoproterenol (ISO), triggered premature Ca^{2+} transients, tachyarrhythmia and death of cells from dbKO mice. We conclude that RGS2 and 5 are critical for proper control of cardiomyocyte ECC and normal cardiac rhythm.

Presenter: Allison Grenell - Graduate Student (Anand-Apte Lab)
Title: Mutations in TIMP3 leads to metabolic dysregulation in retinal pigment epithelial cells

Currently, age-related macular degeneration (AMD), a multifactorial disease that leads to vision loss, is the leading cause of blindness in adults over 50. Sorby's Fundus Dystrophy (SFD) is a rare blinding disease caused by mutations in a single gene called Metalloprotease inhibitor 3 (TIMP3). AMD and SFD share many clinical features but SFD is easier to replicate in the laboratory because it is a monogenetic disease. Using animal and cell culture models of SFD, I hope to understand underlying pathologies of both diseases. During AMD and SFD, retinal pigment epithelial (RPE) dysfunction precedes retinal degeneration, yet the mechanism of this is still unclear. Proteomics and transcriptomics were carried out on RPE of mice that express mutant $TIMP3^{S176C}$ which is known to cause SFD in humans. Both analyses revealed substantial changes in the abundance of proteins and transcripts related to metabolic processing. RPE metabolic change can impact retina because the retina has a uniquely high metabolic demand and all nutrients needed to power the retina must be transported across or generated by RPE. Proper RPE metabolism is required in order for the retina to receive adequate nutrients. Interestingly, TIMP3 has never before been found to directly modulate metabolism and is primarily known to regulate extracellular matrix (ECM) homeostasis through inhibition of metalloproteinases (MMPs). Gas Chromatography/ Mass spectrometry (GC/MS) confirmed metabolic changes in human immortalized RPE cells (ARPE19) which express mutant $TIMP3^{S176C}$. More specifically, $TIMP3^{S176C}$ ARPE-19 cells had significantly reduced levels of Tricarboxylic acid cycle (TCA) intermediates. It has been recently reported that dysfunctional RPE metabolism directly causes photoreceptor starvation in the retinal degenerative disease of retinitis pigmentosa. I hypothesize that aberrant RPE metabolism is similarly contributing to retinal degeneration in AMD and SFD.

Presenter: Jean Moon – Graduate Student (von Lintig Lab)
Title: Vitamin A homeostatis is controlled by retinoic acid-dependent feedback mechanisms

Vitamin A distribution is critical for retinoid function in peripheral tissues and for optimal vision. Impaired vitamin A homeostasis is associated with blinding, inflammatory, and viral diseases. Vertebrates devote a set of proteins to control vitamin A homeostasis. ISX (intestine-specific transcription factor) negatively regulates the conversion of dietary β-carotene to vitamin A. Dietary vitamin A is delivered in chylomicrons to peripheral tissues and stored in hepatic stellate cells. The liver secretes vitamin A bound to RBP which then binds to STRA6, a receptor facilitating cellular uptake of vitamin A. The mechanism underlying homeostatic control between dietary and stored vitamin A remains to be determined. To genetically dissect the vitamin delivery pathway, we exploited *Isx*$^{-/-}$/*Stra6*$^{-/-}$ mice. We measured retinoid concentrations and associated retinoic acid levels with the expression of genes controlled by retinoid signaling. Our studies revealed unliganded opsin and poor visual responses in *Stra6*$^{-/-}$ mice, suggesting impairment of ocular vitamin A homeostasis. *Isx*$^{-/-}$ mice displayed normal ocular vitamin A concentrations but highly elevated retinoid concentrations in the liver and peripheral tissues. Genetic deletion of ISX partially rescued ocular vitamin A deficiency in *Stra6*$^{-/-}$/*Isx*$^{-/-}$ mice. These mice also showed retinoid redistribution from pulmonary stores to adipose tissue. Vitamin A concentration in lymph nodes exclusively depend on the dietary supply pathway. Our study revealed that overlapping pathways for delivery of vitamin A to most peripheral tissues exist in mice. Hereby, retinoid homeostasis is regulated by negative feedback regulation via retinoic acid signaling. Our analysis suggests STRA6 is critical for maintaining ocular vitamin A homeostasis and buffering vitamin A concentration in tissues during times of excessive dietary supplies. This study provides evidence for crosstalk of the pathways for delivery of dietary and stored vitamin A.

Presenter: Christopher Sander – Graduate Student (Palczewski and Kiser Labs)
Title: Structural evidence for visual arrestin priming via phosphoinositol complexation.

Arrestins are responsible for terminating GPCR signaling by blocking further binding of G proteins to their respective GPCRs. Hundreds of GPCRs are all turned off by just four arrestin types, arrestins 1-4 (Arr1-4). While Arr2 and Arr3 are ubiquitously expressed, Arr1 and Arr4 are particular to the retina. Arr1 silences the phototransduction pathway by binding activated, phosphorylated rhodopsin (Rho*P). To accomplish this, visual arrestin (Arr1) translocates from the inner (IS) to the outer segment (OS) of photoreceptors upon light stimulation. To gain insight on potential conformational changes affecting translocation and Rho*P recognition, we have crystallized bovine Arr1 in basal and phosphoinositol-bound states (IP3 and IP6). Our basal structure allowed building a near-complete model for the C-terminus of the protein (C-tail) that spans the N-domain basic patch, revealing four novel salt bridges that stabilize the basal conformation, including one at the penultimate residue D403 (C-latch). We observed that inositides bind to the N-domain basic patch by displacing the C-tail, rendering the C-tail unresolved in the electron density map. The N-domain IP binding site is unique among other IP-bound arrestin structures, which show monomer-coordination of IP6 in the C-domain. Notably, the polar core is intact in both IP-bound structures, suggesting that the observed C-tail removal does not activate Arr1, but instead primes it for binding to active, phosphorylated rhodopsin. IP binding to Arr1 may trigger its dissociation from the inner segment, leading to its translocation to the OS.

Presenter: Weiyang Zhao – Graduate Student (Taylor and Dealwis Labs)
Tile: EM studies of multimers of human ribonucleotide reductase.

Ribonucleotide reductase catalyzes the rate-limiting step in dNTPs synthesis and is tightly regulated to maintain a balanced dNTP pool. Human RR is a major cancer target as it is essential for DNA replication and repair. Human RR (hRR) is a multi-subunit enzyme consisting of the catalytic subunit RR 1 and a free radical housing subunit RR 2 that initiates catalysis. RR 1 forms hexamers when the activator ATP or the inactivator dATP binds at the allosteric A – site while nucleotide triphosphate binding at the S – site selects for substrates that are catalyzed. The molecular basis for enzyme regulation is not fully understood. Current chemotherapies targeting hRR are toxic and against pancreatic cancer ineffective after one month. Hence, we have been searching for non-nucleoside, small molecule inhibitors against hRR. Our lab discovered the first small molecules that are competitive and noncompetitive inhibitors with

improved therapeutic indices for the treatment of cancer. The competitive inhibitor inhibits PC tumors by 65%. The molecular mechanism of activation and inactivation is unknown, prompting us to conduct cryo-electron microscopy of these holo- complexes to elucidate the mechanism. We will use knowledge – based drug design to improve inhibitor efficacy against PC by determining the cryo- EM structure of both the competitive and noncompetitive inhibitors. Here, we have determined the dATP-induced hRR1 hexamer to 2.8 Å resolution and the inactive holo-complex to 3.4 Å resolution, although the hRR2 subunit is only visible at 12 Å. Structures determined in this study reveal unique conformational changes important in allosteric regulation. Future work will include determining structures with competitive inhibitors and noncompetitive inhibitors bound that will help with inhibitor design.

Presenter: Matthew Pleshinger – Graduate Student (Adams Lab)
Title: Identifying novel targets to lead to 8,9 unsaturated sterol accumulation and enhancement of oligodendrocyte formation

Multiple Sclerosis (MS) is a debilitating disease that is characterized by loss of oligodendrocytes in the brain. A potential therapeutic angle for MS is through the increased differentiation of oligodendrocyte progenitor cells (OPCs) into oligodendrocytes. Increasing differentiation of OPCs is a viable therapeutic option because they are a stem cell population that is found in the adult brain with the ability to differentiate. Different labs have conducted high throughput screens to identify novel small molecules that lead to increased OPC differentiation. In work from our lab, we identified a unifying mechanism of the hits from these screens. Each small molecule inhibited one of three steps within the cholesterol biosynthesis pathway, which we identified the accumulation of the 8,9 unsaturated sterols from these steps as being the mechanism of action of these top small molecules. Our lab has evaluated the accumulation of different sterols and evaluating the cholesterol biosynthesis pathway for other novel targets. We have developed tools and small molecules to target previously overlooked enzymes within the cholesterol biosynthesis pathway, SC4MOL, NSDHL, and HSD17B7. These enzymes constitute the C4 Demethylation Complex and their inhibition would lead to 8,9 unsaturated sterol accumulation. Yet, due to lack of inhibitor development and availability of their substrates, targeting them to promote OPC differentiation had been unknown. However, we have recently been analyzing these enzymes as potential targets through CRISPR/Cas9 genetic targeting, substrate synthesis and evaluation, and development of novel inhibitors. With these data, new targets to promote OPC differentiation will be evaluated, which provides more potential remyelinating therapeutics.

Excellence in Research Award Presentation

Presenter: Wei Huang – Research Scientist (Taylor Lab)
Title: An odyssey of small molecule and biomacromolecule interactions

Small molecules, such as NTPs, metabolites and amino acids, are not only the fundamental building blocks in the central dogma, but are also critical effectors in gene regulation. The classical view of small molecule binding pocket is a rigid cavity residing within a single chain biomacromolecule, allowing the ligand to function in a lock-and-key model. Drugs are usually developed to interact with this same pocket to, therefore, interfere with ligand binding and prevent the biomolecule and associated ligand signaling processes from functioning properly. We have recently identified an atypical mechanism of a small molecule binding to a novel pocket of a multimeric enzyme complex. Our recent studies demonstrate that the small molecule binding pocket is formed by three individual subunits and that binding of the small molecule behaves as a molecular glue to hold the enzyme its fully assembled and substrate-specific state. The study showcases how cryo-electron microscopy has become an increasingly powerful tool in visualizing small molecule and biomacromolecule interactions, and providing innovative sources for understanding important biological questions and facilitating the drug discovery process. Together, the first in-class mode of action and ability to visualize molecular interactions has expedited a program to rapidly develop new analogs that are designed to selectively enhance the activity of tumor suppressor complexes.