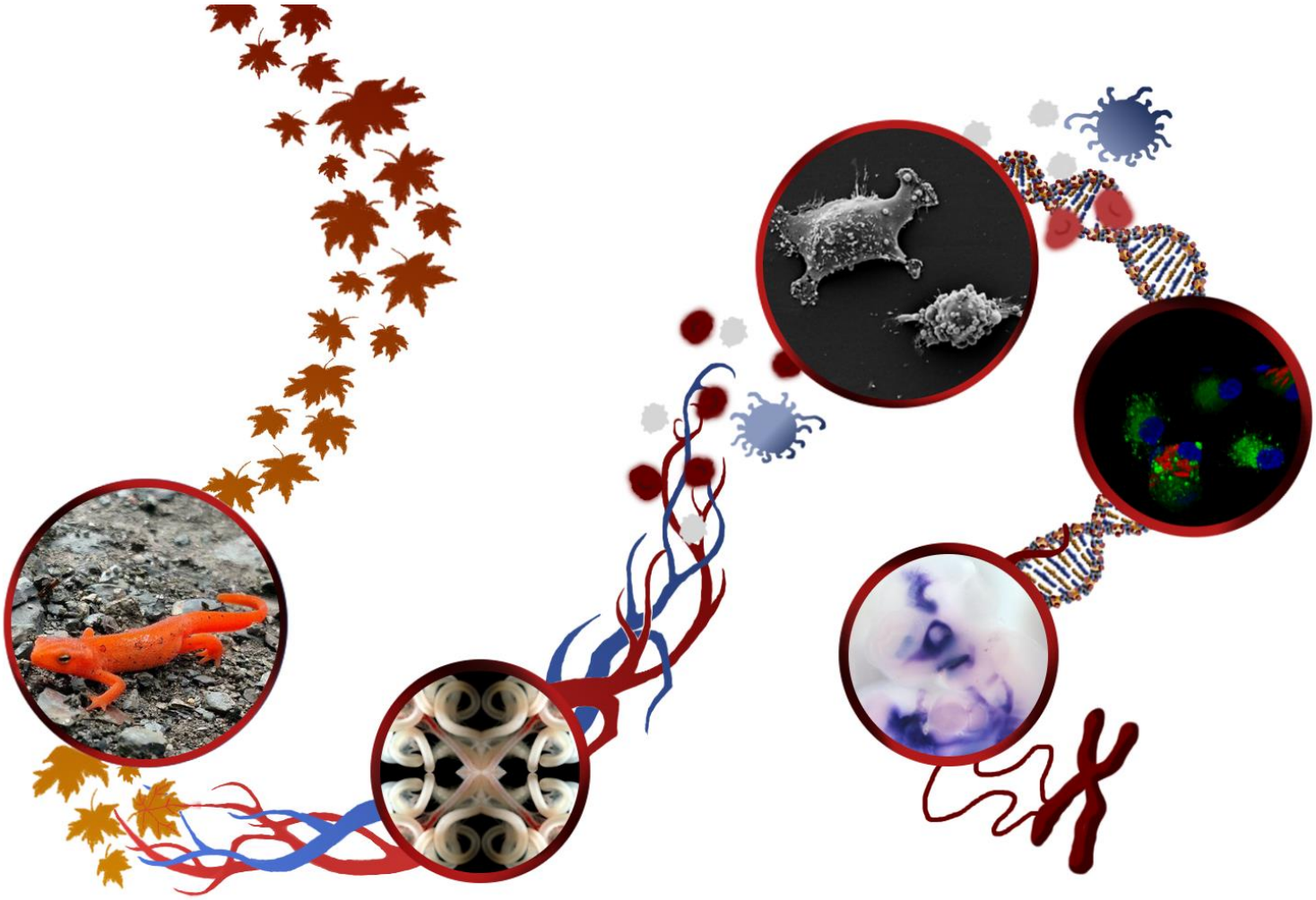
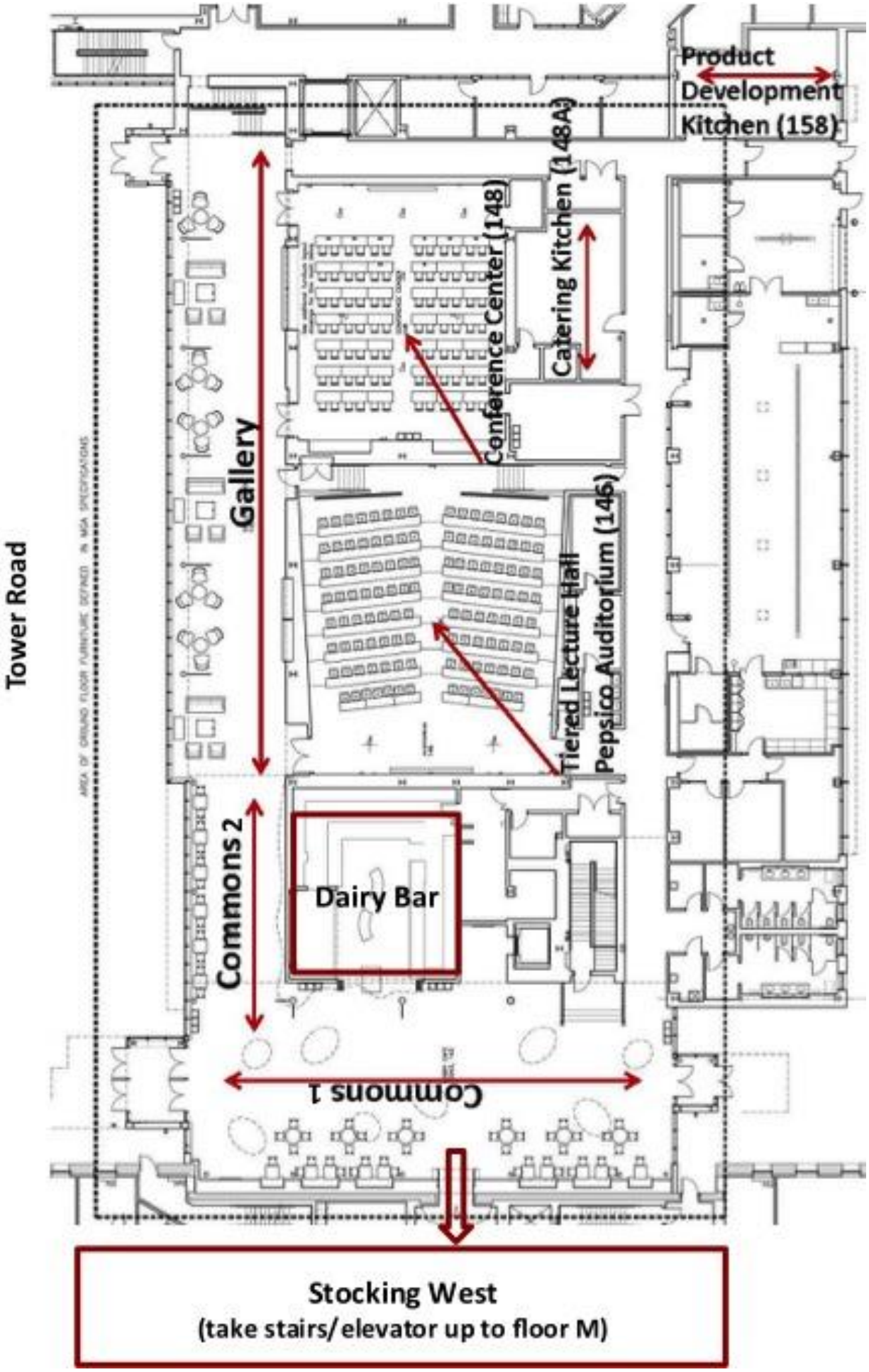
2016 Biomedical & Biological Sciences & Beyond Symposium

15th Annual



Connect. Collaborate. Create.


Product Development Kitchen (158)
Catering Kitchen (148A)
Conference Center (148)
Gallery
Tiered Lecture Hall
Pepsico Auditorium (146)
Tower Road
Commons 2
Dairy Bar
Commons 1
Stocking West
(take stairs/elevator up to floor M)

Program of Events

Time	Event	Location
8:00 – 9:00	**Registration and Introduction**	Gallery
9:00 –9:50	**Breakfast Breakout Sessions** *(supported by ZEISS Microscopy)* **All sessions are by RSVP** A. Think-Learn-Do: Approaches and Resources for your Career B. Strengthen skills with your graduate student community: Graduate student clubs & organizations C. Pioneer with your PhD: Opportunities in Entrepreneurship D. Navigating Your First Year of Grad School E. Bringing new technologies to the community: Light Sheet Microscopy (expert panel)	 M24 Conference Center M01 M26 Pepsico Aud.
10:00 – 11:15	**Faculty Talks** *Pamela Chang, Microbiology and Immunology* "Regulation of macrophages by gut microbial metabolites" *Gunther Hollopeter, Molecular Medicine* *"Molecular Mechanisms of Endocytosis Revealed by Genetics"* *Rick Cerione, Molecular Medicine* *"How Rho GTPases led us to new areas of biology and disease"*	Pepsico Aud.
11:15 – 12:30	**Poster Session I**	Gallery
12:30 – 1:00	**Lunch & DVM Student Poster Session**	Conference Center
1:00 – 2:15	**Poster Session II**	Gallery
2:15 – 2:30	**Ice Cream** *(supported by Laboratory Product Sales, Inc)* **and Coffee Break** *(supported by VWR)*	
2:30 – 3:30	**Student Presentations** *Adam Bisogni* "Combinatorial Adhesive Codes in Single Neurons" *Ezen Choo* "Maternal exposure to high-fat diet increases sweet taste response and sweet taste receptor mRNA expression in adult offspring" *Erika Gruber* "Extracellular substrate stiffness regulates macrophage response to foreign DNA" *Matthew Pennington* "Raltegravir exhibits antiviral, anti-inflammatory, and anti-angiogenic effects during feline herpesvirus (FHV1) infection"	Pepsico Aud.
3:30 – 4:30	**Douglas D. McGregor Research Lecture: Keynote Address** *Tobias Meyer, Stanford University* "Molecular choreography of the sequential events that drive cell cycle entry and exit"	Pepsico Aud.
4:30 – 4:45	**Award Presentation & Concluding Remarks by Dean Lorin D. Warnick**	Pepsico Aud.
4:45 – 6:00	**Reception** *(sponsored by Thermo Fisher Scientific and Life Technologies)*	Gallery

2016 Symposium Organizing Committee

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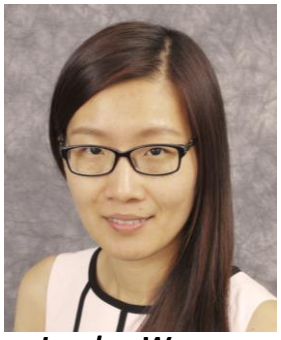

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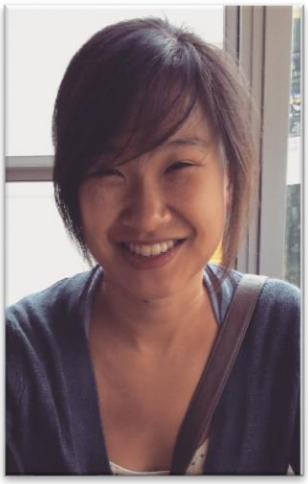

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Office of Graduate Education:

Arla Hourigan

Cindy Grey

Faculty Advisors:

Gunther Hollopeter

Holger Sondermann

Keynote speaker:
Dr. Tobias Meyer, PhD

This year's *Dr. Douglas D. McGregor Keynote Speaker* is Dr. Tobias Meyer, Professor and Chair of Chemical and Systems Biology at Stanford University School of Medicine.

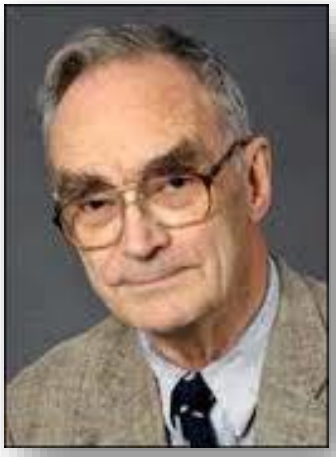
Dr. Meyer received his Masters in Experimental Physics at the University of Basel/CERN Geneva, Switzerland and his PhD in Biophysical Chemistry at the Biocenter of the University of Basel. He then did postdoctoral training with Professor Lubert Stryer at Stanford Medicine.

He was a Packard Fellow for Science and Engineering and an Associate Professor at the Departments of Cell Biology and of Pharmacology and Cancer Biology at Duke University Medical Center where he worked on developing new quantitative measurement approaches and analysis methods for single cell Ca^{2+} and lipid second messenger signals and on modeling signaling pathways and target activation. In 2000, he joined the Department of Molecular Pharmacology at Stanford Medicine. In 2009, he received the Endowed Winzer Professorship in Cell Biology at Stanford University.

Dr. Meyer has made several important contributions to the field of cell signaling, publishing over 140 scientific papers to date. His research seeks to understand how human cells sense hormones, growth factors and stress and how they integrate and transduce these signals to make decisions to polarize, move or divide. He investigates these cellular regulatory systems by identifying the key signaling components and measuring when and where signaling occurs as cells decide to move forward or enter the cell cycle. He has been intrigued by the near universal importance of locally acting Ca^{2+} and phosphoinositide lipid second messenger signals, Rho and Ras family small GTPases and protein kinases in controlling these decision processes. His projects are focused on understanding the general principles of how signal transduction systems work which often requires the development of new experimental and analysis tools involving fluorescent microscopy, small molecule and light perturbations, systematic siRNA screens, bioinformatics, genomics and quantitative modeling of signaling pathways.

The Douglas D. McGregor Research Lecture

The annual BBS Symposium's Keynote Address is named for the Baker Institute's own Dr. Douglas D. McGregor. Dr. McGregor served as Director of the Baker Institute from 1976-1991, and subsequently served as Associate Dean for Research and Graduate Education for ten years.



Dr. McGregor received his M.D. from the University of Western Ontario and earned his D. Phil. from the University of Oxford. He was awarded an honorary Doctor of Veterinary Science Degree from the University of Sydney in 2007, and in 2012 was named Honorary Associate of the Royal College of Veterinary Surgeons.

During an active research career that spanned four decades, Dr. McGregor conducted seminal research on lymphocyte function and the early immune response, making immeasurable contributions to the field of immunology.

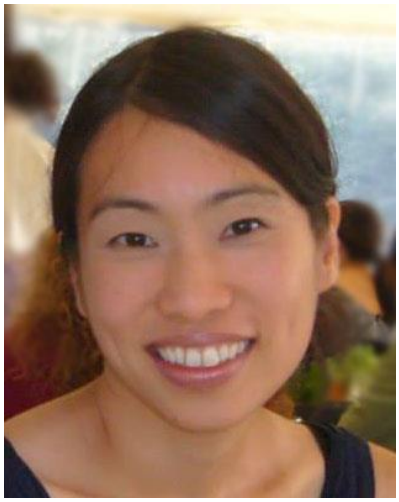
His substantial contributions to veterinary research and education at Cornell include securing funding for the NIH Comparative Medicine Training Grant and creating the Leadership Program for veterinary students, which provides an intensive, research-oriented summer experience for veterinary students who seek to broadly influence the veterinary profession through a science-based career.

Faculty Speakers:

Dr. Pamela Chang

Microbiology and Immunology

Regulation of macrophages by gut microbial metabolites



Dr. Gunther Hollopeter

Molecular Medicine

Molecular mechanisms of endocytosis revealed by genetics

Dr. Richard Cerione

Molecular Medicine

How Rho GTPases led us to new areas of biology and disease

Breakfast Breakout Sessions:

9:00 – 9:50 am (supported by ZEISS Microscopy)

A. Think-Learn-Do: Approaches and Resources For Your Career *(M24)*
Join ***Susi Varvayanis,*** Senior Director of the Cornell Broadening Experiences in Scientific Training (BEST) program, and ***Anne Poduska,*** Graduate & International Student Career Advisor in Cornell Career Services to learn about the different stages of career exploration and what transferable skills you might acquire during your time here at Cornell. You will set goals for how to use several of these transferable skills in today's symposium and learn about resources that will help you prepare for your future career.

B. Strengthen skills with your graduate student community: Graduate student clubs & organizations *(Conference Center)*
Ever wonder about how to acquire the skills away from the bench needed for your dream job? Interested in science communication or consulting?Join ***Johary Rivera*** from Cornell Graduate Consulting Club (CGCC) and ***Victor Aguilar*** from SciComm@Cornell to learn about the opportunities for skills and career development available on campus.

C. Pioneer with your PhD: Opportunities in Entrepreneurship *(M01)*
Interested in translating your science and your scientific mindset to the marketplace? Join PhD student ***Tiffany St. Bernard*** and recent graduate ***Wisler Charles*** who are already engaged in entrepreneurship on campus and learn how PhD training and skillsets are especially suitable in the startup environment.

D. Navigating Your First Year *(M26)*
This session was specifically designed for first year graduate students to connect with each other and their more experienced peers on how to make the most of their graduate experience. Join our interdisciplinary student panel: ***Maggie Gustafsson (BMCB), Roman Spektor* (GGD), *Alicia Brunson (BBS), and Julio Sanchez (BBS)*** for invaluable perspective for first year students.

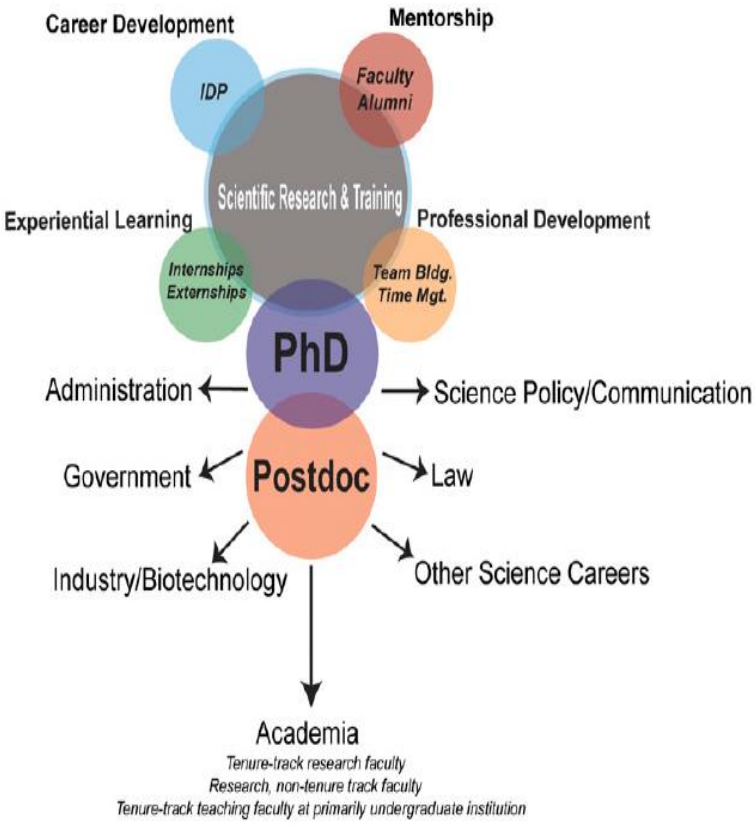
E. Bringing new technologies to the community: *Light Sheet Microscopy (expert panel) (Pepsico Auditorium)*
This special session is meant to drive discussion amongst key investigators on campus to initiate and accelerate the process of bringing light sheet microscopy platforms to Cornell. Join expert faculty, Biotechnology Resource Center (BRC) core staff ***Becky Williams*** and ZEISS representative, ***Nick George,*** in a discussion on how these technologies can best facilitate and enhance research workflows.

Broadening Experiences in Scientific Training

Cornell BEST Program:
Rethinking PhD Training

Trained scientists are needed in many sectors of our economy and in academia. The BEST (Broadening Experiences in Scientific Training) program provides accurate career information and career-oriented experiences to graduate students and post-docs. The goals are to help trainees make informed choices about their career and to help them find career matches that best fit their individual skills and strengths. With these goals in mind the nationwide consortium of 17 institutions awarded BEST grants funded by the National Institutes of Health are working together to redefine the paradigm for PhD education.

See http://www.fasebj.org/content/30/2/507.long



A new paradigm for PhD education FASEB 30(2016)

The Cornell BEST Program provides detailed information to STEM trainees about career options as well as active learning customized to specific career tracks in Science Policy; Industry, Entrepreneurship & Management; Science Communication; and Governance, Risk & Compliance. Getting involved in BEST requires a short application, but everyone is different and the BEST experience can include individual consultations with career mentors, participation in seminars, hands-on workshops, involvement in clubs, taking courses, site visits, and/or trainee initiated projects. Flexible "BESTernships" with advisor consent are also encouraged. Participation is voluntary according to individual time constraints, but the more you invest—in planning, exploring, and experiencing in order to make informed career decisions— the more you will benefit.

Numerous fruitful outcomes have emanated from trainees' participation in BEST, which have led to further successes in science policy, science communication, industry and entrepreneurship, including company formation and securing federal Small Business Innovation Research (SBIR) funding.

Cornell BEST Program
Senior Director: Susi Varvayanis

Several trainee-run clubs have been formed including the Cornell Graduate Consulting Club (CGCC), Advancing Science and Policy (ASAP); and others collaborate with the BEST Program, such as the Technology & Entrepreneurship Club (TEC) and Biotechnology Club. Together they provide training, interactions with practitioners in the field, case competitions, practice describing their expertise in the language of their future employer (or funder), and practical advice on how to find and land a job using the skills learned. Another successful outcome is that trainees might increase their resolve to become tenure track academics, but with increased skills and as a consequence of an informed decision.

Cornell BEST trainees have gone on to advocate for and practice what they learn. Through mentorship they have learned about the writing and editing process. They have authored articles in the BioMed Breakthroughs Industry Report, the Atlanta BEST Magazine; run and won business case competitions; interviewed for alumni magazines; and shared their opinions on energy generation, and even the future of the postdoctoral experience with *Science*. They have also given keynote talks, formal presentations at industry conferences, participated in live Science Cabarets and won prestigious policy fellowships.

As any 'BESTie' will tell you, the more you engage, the more you benefit. This and many other lessons, like 'opportunities aren't found, they're made'[2], underscore the importance of honing skills beyond your technical expertise for your future success.

[1]http://www.fasebj.org/content/30/2/507.long
[2]http://best.cornell.edu/index.cfm/news.details?newsID=1768
[3]http://best.cornell.edu/index.cfm/page/about/FAQ.htm

Listing of Student Abstracts:

The following pages contain the abstracts of student presentations, with oral presentations noted for the four students chosen by a panel of faculty judges.

ABSTRACT JUDGES

Carrie Adler
Doug Antczak
Margaret Bynoe
Scott Coonrod
Lisa Ann Fortier
Maurine Linder
Andrew White

ORAL PRESENTATION JUDGES

Gunther Hollopeter
David Holowka
Manfred Lindau
Brian Rudd

POSTER PRESENTATION JUDGES

Avery August
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Toshi Kawate
Natasza Kurpios
Dave Lin
Maurine Linder
Helene Marquis
Angela McCleary-Wheeler
Linda Nowak
Robert Oswald
John Parker
Kristy Richards
Carolyn Sevier
Holger Sondermann
Tracy Stokol
Rory Toddhunter
Brian VanderVen
Gregory Weiland

Oral Presentations

1 *Combinatorial Adhesive Codes In Single Neurons*

Adam Bisogni[1], Shila Ghazanfar[2], Jean Yang[2], and David Lin[1]
[1]Department of Biomedical Science, Cornell University, Ithaca, NY
[2]Department of Mathematics and Statistics, University of Sydney, Australia

The human brain consists of approximately 86 billion neurons, which form an estimated 1 x 10^16 synapses. A long standing model hypothesizes that neurons use combinatorial codes of axon guidance cues to generate a large amount of cellular diversity with only a small number of genes. Such diversity endows neurons with the ability to find and connect with their proper targets, while avoiding incorrect ones. To determine the combinatorial code used by neurons, we employ a novel single cell RNA analysis method to define the patterns of axon guidance cues expressed in individual neurons. We focus on the protocadherins, a cell adhesion gene family known to influence axon guidance. Using this approach, we show that in single neurons, the protocadherins are expressed in a wide range of combinations, confirming their ability to generate cell surface diversity. We then utilize functional adhesion assays to show that combinations of protocadherins can contribute critical roles in neuronal recognition. Together, these studies define combinatorial codes of protocadherins within single neurons, and demonstrate the functional significance of the combinatorial code.

2 *Maternal exposure to high-fat diet increases sweet taste response and sweet taste receptor mRNA expression in adult offspring*

Ezen Choo[1] and Robin Dando[2]
[1]Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, NY
[2]Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY

Based on epidemiological and animal studies, a maternal high fat (HF) diet predicts offspring preference for fatty foods. Additionally, maternal body mass index and gestational weight gain predict future over-weight/ obese status in children and adolescents. In rodent studies, maternal junk food consumption during pregnancy produces offspring that over consume and prefer junk food, suggesting an increased preference for foods rich in fat, sugar, and salt. Whether the underlying basis for this is due to changes in taste perception remain to be investigated. In this study, dams were fed a control or HF diet before and during pregnancy and then all offspring were maintained on control diet after weaning; thus, the only experience with HF food for the offspring was through maternal exposure during early development. Taste change was assessed in adult offspring using brief-access taste testing. Offspring of HF fed dams showed increased sensitivity to sucrose ($F_{2,255}=7.130$, $p=0.0010$). We hypothesize that this increase in sensitivity results from changes in the expression profile of taste buds for sweet taste receptors. We performed qRT-PCR to assay taste receptor expression, and found that both subunits composing the sweet receptor heterodimer to be increased in the offspring of HF fed dams (T1R2 $p=0.0001$, T1R3 $p=0.0033$). The results indicate that the taste behavior changes in the adult offspring induced by maternal HF diet exposure correlate with increased expression of sweet taste receptors in the taste buds. We hypothesize that sweet taste receptor expression is modulated in the offspring through epigenetic regulation prior to weaning.

3 *Extracellular substrate stiffness regulates macrophage response to foreign DNA*

Erika Gruber, Siddhartha Sinha, and Cynthia Leifer
Department of Microbiology & Immunology, Cornell University, Ithaca, NY

Macrophages are important in innate immunity, tissue repair, and tissue homeostasis, and can differentiate into a wide variety of functional phenotypes in response to biochemical stimuli, such as cytokines. Recent work has shown that biophysical stimuli, such as extracellular substrate stiffness, can mediate differentiation and function of multiple cell types through a process known as mechanotransduction. Less is known about how macrophages and other immune cells respond to biophysical cues and transmit mechanotransduction signals. To investigate this, we cultured murine macrophages on tunable polyacrylamide gels that approximate different tissue stiffnesses. Macrophages on rigid surfaces were more adherent and had a larger surface area and reduced circularity compared to macrophages on compliant gels. Interestingly, macrophages on rigid surfaces secreted less tumor necrosis factor, [TNF], a pro-inflammatory cytokine, in response to CpG DNA stimulation of Toll-like receptor 9 (TLR9). The attenuated response was not due to decreased uptake of CpG DNA, which implicates altered expression, trafficking, and/or signaling of TLR9 itself. Targeted inhibition of key mechanotransduction proteins has revealed a role for classic mechanotransduction signaling (e.g. integrin-mediated activation of focal adhesion kinase [FAK] and rho-associated coiled-coil forming kinase 1[ROCK1]) and a more recently described mechanosensor, transient receptor potential vanilloid 4 (TRPV4). These studies demonstrate that the mechanical properties of the extracellular environment and mechanotransduction have a direct impact on macrophage function. Understanding the roles of mechanotransduction in regulating macrophage function could have important implications in a variety of pathologic conditions that involve macrophages and altered tissue stiffness such as fibrosis, atherosclerosis, and neoplasia.

4 *Raltegravir exhibits antiviral, anti-inflammatory, and anti-angiogenic effects during feline herpesvirus (FHV1) infection.*

Matthew R. Pennington, Lauren Tofano, Jennifer Grenier, and Gerlinde R. Van de Walle
Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY

Feline herpesvirus type 1 (FHV1) is the most common viral cause of ocular surface disease in cats, frequently leading to blindness. In order to identify antivirals against FHV1, we recently showed that the retroviral integrase inhibitor raltegravir was capable of inhibiting FHV1 in cell culture as well as in a corneal explant model. We then adopted a dual RNA sequencing approach to study the effects of raltegravir therapy on both host and viral gene transcription. We initially used this data to investigate the antiviral mechanisms of raltegravir. Raltegravir therapy has been described to target either viral DNA replication (e.g. herpes simplex virus type 1) or DNA packaging (e.g. cytomegalovirus), depending on the herpesvirus. We found that with raltegravir treatment, 3 immediate early genes were upregulated, no change was observed in early gene expression, and 10 late genes were down regulated. This pattern of gene expression suggested that raltegravir inhibits FHV1 DNA replication. However, we observed (i) a more significant reduction in extracellular produced virus than in viral DNA genome copies following raltegravir treatment and (ii) a different gene expression pattern following phosphonoacetic acid treatment, a known inhibitor of DNA replication. These results collectively indicate that DNA replication might not be the major target of raltegravir, but that packaging might also be affected. Studies investigating whether raltegravir indeed inhibits DNA packaging are currently ongoing. Furthermore, we observed that raltegravir led to an upregulation of host anti-angiogenic factors during FHV1 infection. Finally, we observed that raltegravir led to an upregulation of a number of anti-inflammatory factors, including heme oxygenase 1, and downregulation of pro-inflammatory factors, such as hyaluronan synthase 2, in uninfected cells. Raltegravir-induced anti-angiogenic and anti-inflammatory effects could be beneficial during FHV1 ocular infection, as angiogenesis and inflammation are major contributors of corneal damage.

Poster Presentations

Immunology and Infectious Disease

5 *9-O-acetyl modified sialic acid and influenza A viruses*

Karen Barnard, Brian Wasik, and Colin Parrish
Department of Immunology and Infectious Disease, Cornell University, Ithaca, NY

Influenza A virus (IAV) utilizes sialic acid (Sia) as a receptor to mediate cell entry. Sia are a diverse family of carbohydrates that serve as terminal residues on cell surface glycans, including glycoproteins and gangliosides. They are widely distributed in animal tissues, including IAV hosts, with variations in modification seen between tissues and species. The different Sia forms and linkages found in various IAV hosts help determine host adaptation, as illustrated by the preference of IAV for α2,3- or α2,6-linked Sia. 9-O-acetyl Sia (9-O-Ac) is a modified form that is expressed in IAV hosts to varying degrees. Previous research has shown 9-O-Ac to be inhibitory to neuraminidase (NA) and may influence the ability of some IAV hemagglutinin (HA) to bind Sia; however, there have been few studies of how the presence of 9-O-Ac effects IAV infection and adaptation. To this end, we have utilized two new tools. First, we have developed specific probes consisting of virus hemagglutinin-esterase proteins fused to human IgG1 Fc (HE-Fc) that bind to 9-O-Ac Sia on cells. These probes have two forms: an esterase(+) form that can remove the acetyl modification and an esterase(−) form that binds the modification without removing it. Second, we have utilized CRISPR-Cas9 and the recently identified 9-O-acetyltransferase gene, CasD1, seeking to create knock-out lines of IAV host cells that lack 9-O-Ac expression. These tools can broaden our understanding of IAV interactions with modified Sia and can also be utilized for other Sia binding viruses such as polyomaviruses, parainfluenza viruses, and reoviruses.

6 *Parvovirus capsid structures required for infection: mutations controlling receptor recognition*

Heather Callaway and Collin Parrish
Baker Institute for Animal Health, Department of Microbiology and Immunology, Cornell University, Ithaca, NY

Parvoviruses are very small, single-stranded DNA viruses that can cause severe disease, but have also undergone development as gene therapy vectors and cancer therapeutics. Canine parvovirus (CPV) is unique among the parvoviruses because it only recently emerged as a pathogen of dogs. CPV is very closely related to the cat pathogen feline panleukopenia virus (FPV), and there are only 10 amino acid differences between the CPV and FPV capsid proteins, which control binding to the host cell transferrin receptor (TfR) and uptake into cells. Some of these mutations allow canine parvovirus to accommodate a bulky glycan on the canine TfR, which prevents FPV from infecting dogs.

7 *The human lung is a site of HIV replication during long term ART treatment: novel tools to study tissue HIV reservoirs*

David Gludish and David Russell
Field of Comparative Biomedical Sciences, Cornell University, Ithaca, NY

Background: We have developed novel tools to probe HIV biology in potential tissue reservoirs that persist during ART (anti-retroviral treatment). Our previous work found HIV mRNA in alveolar macrophages (AMs) of HIV patients. However, current methods to demonstrate such reservoirs require expensive tools such as the quantitative viral outgrowth assay (QVOA) and rely on bulk population PCR assays that lack cellular resolution. To determine if AMs produce infectious HIV, we sought robust, rapid readouts amenable to flow cytometry at point of care.

Methods: We have built novel tools to identify infected HIV cells in vivo. We generated reporter cells that express GFP dependent on HIV Tat and Rev protein (TZM-GFP). To identify HIV within single cells, we developed a fluorescent in situ hybridization assay that is quantified by flow cytometry. We sorted fixed HIV-positive cells from experimental cultures and from the blood and bronchoalveolar lavage of Malawian HIV-infected adults and perform RNAseq profiling in vivo, an experiment not previously possible. Finally to purify live HIV-infected cells from patient samples, we developed a synthetic reporter RNA that expresses mCherry in HIV-infected cells using straightforward and scalable methods.

Results: Clear GFP induction in TZM-GFP is observed on co-culture with experimentally infected macrophages. GFP-positive syncytium formation even by rare infected macrophages facilitates direct observation of infection in primary cells by electron microscopy. We show by limiting dilution that TZM-GFP cells can report a single infected macrophage, allowing quantification of cells that harbor infectious provirus. Co-cultured TZM-GFP with bronchoalveolar lavage (BAL) cells from asymptomatic HIV-positive Malawian adults reveals the induction of GFP and transfer of HIV from BAL cells of ART-treated and ART–naïve patients. Similarly using FISH:FACS, we find HIV mRNA predominantly in AMs at HIV diagnosis and in aviremic individuals on ART, suggesting the persistence of HIV in AMs despite successful therapy.

Conclusions: Using FISH:FACS to build RNAseq profiles of purified HIV-infected cells and novel viral outgrowth assays, we are generating a complete toolkit to identify *bona fide* ART-durable reservoirs in the deep tissues of human patients. These novel tools can facilitate the direct demonstration of HIV infection even from rare cells within human biopsy samples. We conclude that (1) ART, though successful in clearing viremia, spares HIV-infected AMs. (2) BAL cells from ART-treated patients generate infectious virus, showing the lung is a site of HIV maintenance. (3) HIV in the lung is mainly found in AMs, a potential anatomical reservoir that remains understudied. Our future work will apply synthetic reporter RNA to enable live sorting of HIV-infected cells from bulk human tissue samples, enriching these cells for more accessible assays of viral outgrowth. This study reports on the lung as a site of viral persistence and productive infection in the face of effective ART, and will bring significant changes to the way we approach clearance of HIV tissue reservoirs with novel therapies and vaccines.

8 *Beta-Cell GLP-1R Contributes to Improved Glucose Tolerance After Vertical Sleeve Gastrectomy in Mice*

Garibay D, McGavigan AK, Lee SA, Showalter AD, Michael MD, Sloop KW, Cummings BP

Vertical sleeve gastrectomy (VSG) produces high rates of type 2 diabetes remission; however, the mechanisms responsible for this remain undefined. Post-operative increases in postprandial glucagon-like peptide-1 (GLP-1) secretion may contribute; however, previous work has been equivocal. To test the contributions of β-cell GLP-1 receptor (GLP-1R) signaling we used a β-cell specific tamoxifen-inducible GLP-1R knockout mouse. At 8wks of age, Glp-1rβ-cell+/+ (WT) and Glp-1rβ-cell-/- (KO) male mice were placed on a high fat diet (HFD) for 6wks and then switched to HFD supplemented with 400mg tamoxifen/kg diet for the rest of the study. Mice underwent sham or VSG surgery at 16wks of age and were then fed ad libitum (n=7-9). Mice underwent oral glucose tolerance testing (OGTT) at 3wks and were euthanized at 6wks after surgery. Body weight and food intake was reduced after VSG compared to the respective sham-operated control ($p<0.05$). Body weight and food intake did not differ between genotype for either treatment. OGTT data revealed an improvement in glucose tolerance in VSG WT, but not VSG KO. Furthermore, the glucose AUC was significantly elevated in VSG KO versus VSG WT (Glucose AUC0-120:Sham WT=26788±1296, Sham KO = 30273 ± 4521, VSG WT=22312±2439, VSG KO=30766±2799 mg·min/dl; $p<0.05$). The augmentation in glucose-stimulated insulin secretion during the OGTT was blunted in VSG KO versus VSG WT (OGTT percent increase in insulin from baseline to peak values: Sham WT=148±48, Sham KO=96±43, VSG WT=294±79, VSG KO=138±31%; $p<0.05$). Overall, our data suggests β-cell GLP-1R signaling contributes to improved glucose regulation and insulin secretion after VSG.

9 *Anti-microbial properties of equine mesenchymal stromal cells*

Rebecca M. Harman and Gerlinde R. Van de Walle
Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca NY

Antibiotics (Abx) are commonly used in veterinary medicine to treat infectious diseases caused by bacteria and other microorganisms. The extensive use of Abx in recent years has led to the emergence and spread of Abx-resistant microorganisms. When resistance occurs, previously successful drugs are no longer effective, creating a need for alternative approaches to fight bacterial infections and/or to boost the host's antimicrobial defense system. Mesenchymal stromal cells (MSC) are adult multipotent progenitor cells, that can be isolated, expanded in culture, and used therapeutically. MSC actively contribute to healing processes by promoting tissue regeneration, modulating immune responses, regulating inflammation and preventing pathological scar formation. Our lab has carried out in vitro experiments designed to study the therapeutic effects of equine MSC on healthy and dysregulated cell types present in lower limb wounds of horses presenting with exuberant granulation tissue (EGT). Our results suggest that MSC can act on resident skin cells to alter the wound environment, improve healing and reduce EGT. Since bacterial infection is known to promote the formation of EGT, we have started to examine the anti-microbial properties of equine MSC as well. Our findings show that MSC secreted products can prevent bacterial growth and the formation of biofilms in vitro, and MSC can kill internalized bacteria. MSC express genes coding for anti-microbial peptides (AMP), and expression of these genes can be upregulated in the presence of bacteria. Several AMP are detected in conditioned medium from MSC cultures. In addition to these direct anti-microbial effects, MSC protect skin cells from bacterial toxin-induced cell death, and stimulate resident skin cells to increase AMP production. Based on these results, we believe MSC will be a useful therapy for horse EGT, in part due to their direct and indirect anti-microbial properties.

10 *Identification of full-length feline immunoglobulin sequences that can be used for developing expression vectors to produce 'felinized' monoclonal antibodies*

Zhengchun Lu[1], Rebecca L. Tallmadge[2], M. Julia. B. Felippe[2] and John S.L. Parker[1]
[1]Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY
[2]Equine Immunology Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY

B cells generate a repertoire of antigen-specific antibodies in response to immunization. Antibody-based immunotherapies hold great promise for treating cancer and other diseases in humans. However, for cats we need to identify species-specific B cell clones that produce antibody specific to the antigen of interest. From such clones, the mRNAs encoding the cognate heavy and light immunoglobulin (Ig) can be isolated, cloned and sequenced. Finally, with this information vectors that can express these antibodies in exogenous systems can be developed and the antibodies can be tested for therapeutic efficacy. Such approaches have been developed in human medicine. However, we lack information about feline Ig sequences and the feline IgG response to antigen. The goal of this study was to fully sequence Ig heavy and light chains from feline B cells and then to adapt human Ig expression vectors to allow ectopic expression of feline Ig molecules. Such vectors can then be used to insert variable heavy and light chain sequences that can confer different antigen specificities. We recovered mRNA from feline B cells from two cats and cloned and sequenced the variable and constant regions of the feline IgG1a, Igλ and Igκ sequences. The full-length feline IgG1a, Igλ and Igκ sequences were assembled and representative sequences were cloned into a bi-cistronic mammalian expression vector designed by Dodev et al (Scientific Reports, 4:5885, 2014). Here we report novel feline Ig sequences and on a technique to grow and clone feline Ig-secreting cells to allow identification of antigen-specific feline mAbs.

11 *Rv3723 coordinates uptake of fatty acids and cholesterol by Mycobacterium tuberculosis through modulation of the mce transport complexes.*

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Mycobacterium tuberculosis (Mtb) – causative agent of tuberculosis – relies heavily on fatty acids (FA) and cholesterol as carbon sources during infection. Here we performed a genetic screen to identify genes required for Mtb to utilize cholesterol. The screen identified Rv3723. Using radioactive labeling we showed significant decrease in uptake of cholesterol by Δrv3723 in broth, comparable to the limited uptake in a cholesterol import system mutant Δmce4.

Transcriptionally Δrv3723 responded to macrophage infection by downregulating cholesterol breakdown genes. Surprisingly, genes indicative of FA metabolism were also downregulated. Finally, genes of the related mce1 transporter complex were significantly upregulated.

We confirmed that Δrv3723 mutant is impaired in the import of FA, through analysis of FA uptake in media and during macrophage infection. The Δmce1 mutant had a similar defect in FA uptake.

Using an unbiased two-hybrid screen, we identified that Rv3492c and Rv0177 (proteins associated with mce4 and mce1, respectively) interact with Rv3723. These proteins are believed to stabilize mce complexes. We found that, in the absence of Rv3723, the mce1 complex was degraded.

Thus, Rv3723 is required for import of both cholesterol and FA through mce4 and mce1. Rv3723 stabilizes the mce4 and mce1 complexes through its interaction with Rv3492c and Rv0177. Cholesterol and FA transport is essential for Mtb infection, as a Δrv3723 mutant has impaired survival in both macrophage and in mouse infection models. Rv3723 appears to play a critical role in the integration of cholesterol and FA transport required to sustain Mtb in its host.

12 *HAP2-mediated cellular fusion in a sexual ciliate: what to look for in a gamete fusogen and what this means for eukaryotic sex*

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While the mechanisms underlying gamete cell fusion during sexual reproduction remain obscure, recent studies have implicated the conserved transmembrane protein, HAP2/GCS1, as an ancestral gamete fusogen. Despite the absence of sequence similarities with other known fusogens, genetic disruption of the HAP2 locus in a variety of species ranging from protists to flowering plants leads to a decisive block to fertilization due to an inability of gametes to catalyze membrane fusion events. To better understand the role of HAP2 in cell-cell fusion we developed a flow cytometry-based assay to assess membrane pore formation during conjugation in the model ciliate, Tetrahymena thermophila. This assay provided a streamlined method to investigate the functional significance of HAP2 domains. In the course of this work, we found the ectodomain of Tetrahymena HAP2 shares a high degree of predicted structural homology with the dengue virus E glycoprotein (a class II viral fusogen). This region contains a predicted peptide loop analogous to the viral fusion loop used to perturb target membranes at the initiation of virus entry into host cells. We found that deletion of the fusion loop or the entire dengue virus E glycoprotein homology domain from T. thermophila HAP2 completely eliminated fusion between mating Tetrahymena cells. In contrast, the HAP2 endodomain appeared largely dispensable for membrane fusion, as did ZFR1, a protein that localizes to the mating junction and is required for fertilization much like HAP2. Finally, biophysical studies with the predicted fusion loop suggested direct interactions of this peptide domain with artificial lipid bilayers. These findings raise interesting questions regarding the evolutionary origin of HAP2, and may lend important insights into the mechanistic function of this key developmental protein.

13 *Two viral nonstructural proteins are sufficient to compartmentalize the host cell translational machinery in a virus-derived matrix*

Dania Villarnovo and Kristy Richards
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Reoviruses compartmentalize translation within virus-induced inclusions called viral factories. Viral factories are the sites of viral replication, transcription, and assembly. Here we report that ectopic expression of two viral nonstructural proteins are sufficient to compartmentalize translation within viral factory-like structures that form within transfected cells.

14 *The independent emergences of H3N8 and H3N2 canine influenza viruses and their evolutionary dynamics in the USA.*

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Influenza A viruses (IAVs) are notorious for the ability to "spillover" from their reservoir hosts, waterfowl and shorebirds, to cause disease in other animals. While these cross-species transmission events are fairly common, they rarely result in sustained epidemics. Until recently, dogs were considered refractory to IAV infection. However, within the last 20 years, IAVs have successfully jumped into dogs twice independently, producing sustained canine-to-canine transmissions in both instances. H3N8 canine influenza virus (CIV) emerged in Florida from an equine IAV in 1999, while H3N2 CIV emerged directly from an avian IAV in China in 2006 and spread to domestic dogs in the USA in 2015. The emergences of the CIVs provide an opportunity to compare the evolutionary events that have enabled two recently emerged, distinct IAVs of different origins to convergently adapt to the same mammalian host. To this end, whole-genome amplification, sequencing, and phylogenetic analysis of the CIVs were conducted, enabling high-resolution reconstructions of the viruses' evolutionary histories. Results indicate that, in both subtypes, viruses are maintained in areas of dense dog populations and high contact frequencies after their initial emergence. Additionally, extensive host contact heterogeneity and rapid evolutionary rate typical of IAVs have produced geographically distinct viral clades with potentially unique characteristics. The CIVs provide an excellent model in which to answer basic questions in viral evolution. Furthermore, given their global abundance and synanthropicity, canine hosts are ideally suited to act as evolutionary and ecological stepping-stones for IAV transfer to humans – a threat that should not be overlooked.

15 *Defining Mechanisms of Lipid Utilization in Mycobacterium tuberculosis Using a Fluorescent Reporter of Cholesterol and Fatty Acid Metabolism*

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Department of Microbiology and Immunology, Cornell University, Ithaca, NY

B cells generate a repertoire of antigen-specific antibodies in response to immunization. Antibody-based immunotherapies hold great promise for treating cancer and other diseases in humans. However, for cats we need to identify species-specific B cell clones that produce antibody specific to the antigen of interest. From such clones, the mRNAs encoding the cognate heavy and light immunoglobulin (Ig) can be isolated, cloned and sequenced. Finally, with this information vectors that can express these antibodies in exogenous systems can be developed and the antibodies can be tested for therapeutic efficacy. Such approaches have been developed in human medicine. However, we lack information about feline Ig sequences and the feline IgG response to antigen. The goal of this study was to fully sequence Ig heavy and light chains from feline B cells and then to adapt human Ig expression vectors to allow ectopic expression of feline Ig molecules. Such vectors can then be used to insert variable heavy and light chain sequences that can confer different antigen specificities. We recovered mRNA from feline B cells from two cats and cloned and sequenced the variable and constant regions of the feline IgG1a, Igλ and Igκ sequences. The full-length feline IgG1a, Igλ and Igκ sequences were assembled and representative sequences were cloned into a bi-cistronic mammalian expression vector designed by Dodev et al (Scientific Reports, 4:5885, 2014). Here we report novel feline Ig sequences and on a technique to grow and clone feline Ig-secreting cells to allow identification of antigen-specific feline mAbs.

16 *Modified sialic acids and their expression in influenza hosts*

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Sialic acids (Sias) are displayed on the surfaces of vertebrate cells, and in large amounts at mucosal surfaces. Sias naturally occur in many different modified forms, and are attached through various linkages. Influenza viruses use Sias as primary receptors for cell entry. While the linkages (α2-3 vs α2-6) are well-known tropism determinants, the effects of modified Sias on influenza tropism and the function and evolution of hemagglutinin (HA) and neuraminidase (NA) are largely unknown. Some modified Sias are infection inhibitors (horse or guinea pig serum, Neu4,5Ac) or as negative regulators on NA efficiency (Neu5Gc, Neu5,9Ac). We are now analyzing the distribution and abundance of modified Sias in influenza hosts, and also seeking to directly assess their effects on the influenza infectious cycle. We have developed a toolkit of recombinant sialolectin probes (viral glycoprotein fused to the Fc of human IgG1) for detection of a variety of Sia forms. These include Nidovirus HEs (9-O- or 4-O-acetylated Sia), as well as influenza HAs that detect Neu5Ac or Neu5Gc in particular linkages. We have performed a histological survey of those various modified Sias in respiratory tissues of multiple influenza natural and laboratory hosts, and confirmed the presence of O-acetyl modified Sias with a wide variety and patterns of tissue distribution. The ubiquity of 9-O-Ac Sia in many species may be of particular interest in light of its effects on NA, and its role as a receptor for Influenza C and (Bovine) Influenza D. We are also screening common cell lines used in influenza research, as well as primary cells. While 4-O-Ac Sias appear limited to horse and guinea pig cells in culture, 9-O-Ac variants are expressed in many common cell lines (e.g. MDCK, A549). These probes can be used for various assays of a wide variety of biological samples. We are currently developing 'glyco-engineered' cell lines through CRISPR knockouts or plasmid over-expressions of Sia modifying enzymes to directly study the roles of modified Sias (and linkages) on infection of various influenza virus strains.

17 *Inhibition of metalloprotenase synergizes with frontline antibiotics against Mycobacteria tuberculosis*

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Mycobacteria tuberculosis (Mtb) is still a grave threat to world health with emerging drug resistant strains. One prominent feature about Mtb is its capability of reprogramming host cells to aid its survival and drug resistance. Here we report that inhibition of matrix metalloproteinases (MMP) secreted by infected host cells interferes with tissue remodeling and enhances the Mtb-killing effect of frontline antibotics. Part of this synergistic effect results from blocking MMP cleavage of mannose binding lectin (MBL), which makes the bacteria more prone to antibiotic killing. Our findings suggest that targeting host tissue reprogramming mechanism may shorten frontline TB therapies.

18 *Adenosine's role in glioblastoma multiforme pathogenesis*

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Glioblastoma multiform (GBM) is considered the most aggressive and most lethal central nervous system tumor. GBM patients often have to go through multimodal therapies including surgical resection, radiotherapy, and chemotherapy with temozolomide, which is the only chemotherapy drug shown to have some effect. Despite the treatments, the median survival for GBM patients is approximately 1 year. With GBM being highly infiltrative and invading normal brain tissue, surgical resection is often not possible. This inescapably leads to recurrence. To better treat GBM, we need a multi-angle approach that is non-invasive, targets chemoresistance and molecules/receptors located directly on GBM. Extracellular adenosine is a purine nucleoside that regulates many cell functions through its four receptors (A1, A2a, A2b, and A3). It is believed that extracellular adenosine is involved in the proliferation and pathogenesis of GBM. To understand the role of adenosine in GBM pathogenesis, we use in vivo and in vitro GBM models to monitor its development in adenosine-rich and poor environments. We target adenosine receptors using pharmacological inhibitors and activators and hinder extracellular enzymes that produce adenosine. We hypothesize that GBM usurps the host extracellular adenosine to promote its growth, proliferation, and survival. Further, we hypothesize that GBM uses the adenosine machinery to circumvent host anti-tumor mechanisms.

19 *Mir29-mir130 Axis Influences CD8+ T cell Memory Formation At Different Stages of Life*

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There remains a high incidence of infectious diseases and high mortality rates in infants worldwide owing to the fact that neonates are highly susceptible to pathogen infection due to significantly underdeveloped memory formation abilities. Immunological memory, which protects organisms from re-infection, is a hallmark of the mammalian adaptive immune system and the underlying principle of vaccination. In early life, however, mice and other mammals are deficient at generating memory CD8+ T cells, which protect organisms from intracellular pathogens. The molecular basis that differentiates adult and neonatal CD8+ T cells into a long lived memory pool is unknown. MicroRNAs (miRNAs) have recently been well established as a critical regulator of gene expression and function like transcription factors and. They are both developmentally regulated and required for normal CD8+ T cell functions. We used next-generation sequencing to identify mouse miRNAs that are differentially regulated in adult and neonatal CD8+ T cells, which may contribute to the impaired development of neonatal memory cells. The miRNA profiles of adult and neonatal cells were inerestingly similar during the effector phase of infection; however, large differences in expression were observed prior to infection as a naïve T cell, particularly with respect to miR-29 and miR-130. Importantly, using RNA-Seq, we detected reciprocal changes in expression of mRNA targets for both miR-29 and miR-130. Moreover, target analysis validated key genes that regulate the formation of memory CD8+ T cells which include Eomes and Tbx21 and IL6st. Notably, age-dependent changes in miR-29 and miR-130 are conserved in human CD8+ T cells, further suggesting that these developmental differences are biologically relevant. Interestingly, using adult wild type and mir-29 het KO mice infected with VACV-gB, the phenotype of gB specific CD8+ T cells at day 7 (peak of infection) showed that CD8+ T cells from mir-29ab het mice preferentially developed into SLECs (short lived effector cells) (KLRG1+ CD127-) while the wild type developed moreso into MPEC (memory precursor effector cells) (KLRG1- CD127+). Together, these results demonstrate that miR-29 and miR-130 are likely important regulators of memory CD8+ T cell formation, and suggest that neonatal cells are committed to a short-lived effector cell fate prior to infection by miRNA preprogramming of naïve CD8+ T cells. Mir-29 and mir-130 may be acting as a rheostat for adjusting the activation threshold of CD8+ T cells during different stages of life by targeting tbet, eomes and CD130 with high efficiency. This holds important implications in understanding unknown aspects of memory formation in neonates and for designing better T cell specific vaccines to aid in neonate survival.

Genetics, Genomics and Development

20 *Investigating Operative DNA Damage Response Pathways in Mouse Primordial Germ Cells*

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The ability of organisms to pass their genetic information onwards to subsequent generations is crucial for survival and propagation of a species. In mice, primordial germ cells (PGCs) are set aside very early in development to become the germline lineage. Because DNA replication associated with rapid PGC proliferation is subject to spontaneous errors, and because PGCs carry the genetic information that will be passed down to the next generation, mechanisms exist to avoid the propagation of these mutations. In accordance with the desire to maintain genomic integrity in the germline, studies have revealed that PGCs are, to a greater extent than somatic cells, highly sensitive to genetic defects and environmental perturbations affecting DNA. While studies of cultured somatic cells and single-celled eukaryotes such as yeast have elucidated DNA damage response pathways, cell cycle checkpoints, and DNA repair mechanisms, our understanding of these processes in the mammalian germline is much less clear. To investigate how genomic integrity is maintained in the mammalian germline, we are characterizing DNA damage response mechanisms in primordial germ cells. We found that mutations affecting the Fanconi Anemia DNA damage response pathway that responds to errors in DNA replication trigger cell cycle slowdown rather than apoptosis. To further explore this findings, we are conducting studies to examine mutational burden in mice defective for certain checkpoint pathways, as well as using CRISPR/Cas9 genome editing to develop in vivo DNA damage pathway sensors.

21 *DNA Mechanics Mediates Nucleosome Transfer*

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The spatial organization of epigenetic chromatin modifications is maintained throughout multiple rounds of cell division by the inheritance of parental nucleosomes and the ensuing duplication of covalent modifications to nascent nucleosomes. At the replication fork, a complex interplay of proteins and DNA mediate the faithful duplication of DNA sequence and the subsequent packaging of nascent DNA into chromatin. Parental nucleosomes are rapidly moved from ahead of the progressing replisome to the newly replicated DNA behind it, without release of the H3/H4 tetramers into solution. The transfer of parental nucleosomes is stringently regulated and intrinsically linked to replication fork dynamics, however the mechanism underlying this process has yet to be extracted from the many biochemical reactions simultaneously occurring at the replication fork. We therefore utilized a minimal experimental system to track the fate of a single nucleosome following its displacement, and examined whether DNA mechanics itself, in the absence of any chaperones or assembly factors, may serve as a platform for the transfer process. We found that the nucleosome was passively transferred to available dsDNA as predicted by a simple physical model of DNA loop formation. These results demonstrate a fundamental role for DNA mechanics in mediating nucleosome transfer and preserving epigenetic integrity during replication.

22 *CRISPR/Cas9 genome editing reveals a potential role for the long noncoding RNA Playrr in transcriptional regulation of organogenesis at the Pitx2 locus*

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Precise spatiotemporal expression and gene dosage of Pitx2 is essential for regulating (LR) asymmetric organ morphogenesis and development of craniofacial structures. Mutations in human PITX2 are causal for Axenfeld-Rieger Syndrome (ARS), characterized by mental retardation, craniofacial birth defects, and umbilical hernias. Screening of ARS patients has identified individuals who possess no mutations in Pitx2 coding sequences but harbor large deletions that encompass an adjacent gene desert devoid of coding genes, suggesting a cis-regulatory role in regulating Pitx2 expression. Despite key roles of *Pitx2* in the patterning and morphogenesis of multiple organs, the genomic cis-regulatory mechanisms mediating organ specific *Pitx2* expression remain unclear. The Kurpios lab has established the LR binary gut dorsal mesentery (DM) as a tractable in vivo model in which asymmetric *Pitx2* gene expression gives rise to asymmetric cellular behavior and conserved intestinal looping morphogenesis. Transcriptional profiling in the L vs R DM revealed the presence of an asymmetrically expressed long noncoding RNA (lncRNA), *Playrr*, at the *Pitx2* locus whose transcriptional start site overlaps a highly conserved DNA cis-regulatory element responsive to Pitx2, e926. To investigate the putative role for this noncoding regulatory module in regulating precise spatiotemporal Pitx2 gene expression critical for proper visceral organ morphogenesis, I generated deletion alleles (*PlayrrΔe926)* via CRISPR/Cas9 genome editing in mice. In order to parse out the putative regulatory function of the lncRNA transcript from its associated cis-element, we simulataneously used CRISPR/Cas9 to mutate the first exon-intron splice junction in *Playrr*, generating a *Playrr*-specific mutant allele (*Playrr-Ex1sj*). Using qRT-PCR and *in situ* hybridization techniques in heart, lungs, and intestines of *Playrr* mutant mice, I show here that *Pitx2* isoform expression is differentially upregulated in the absence of *Playrr*, suggesting that *Playrr* may modulate transcription of overall *Pitx2* dosage and/or relative isoform specificity and thus provide a cis-regulatory mechanism for organ-specific, dosage-dependent organogenesis.

23 *DNA Mechanics Mediates Nucleosome Transfer*

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The embryogenesis and cancer related Wnt modulator Glypican3 (Gpc3) is a key molecule orchestrates animal physiology, yet the mechanistic detail of how Gpc3 is regulated in intestinal development remains unclear. In chicken, Gpc3 and its downstream Wnt5a expression are highly restricted to the left dorsal mesentery (DM) under Pitx2—the paramount left determining transcription factor. Interestingly, though Gpc3 expression restricts to the left DM in chicken, Gpc3 expression in mouse DM is bilateral. I propose that it is the asymmetric posttranslational modification of Gpc3, but not the asymmetric expression of Gpc3 per se, that contributes to the left restricted Wnt pathway activation in both chicken and mouse DM. We have identified a proprotein convertase (PC) 5 as a candidate that displays this asymmetric Gpc3 modification. However, instead of an obvious left restricted diffused expression as Pitx2 (upstream) and Wnt5a (downstream of Gpc3), PC5 RNA expression matches arterial distribution in DM. This implies a vascular origin of PC5 in DM—either secreted by endothelial cells, or transported via blood vessels from remote origins. Intriguingly, the Kurpios lab has revealed that intestinal vasculogenesis in DM is first bilateral then left restricted, leaving only the left DM vascularized in this specific stage. I therefore hypothesize that the asymmetric Gpc3 activation is due to a vascular-related asymmetric distribution of PC5. In my study, I aim to clarify (1) the relationship between Pitx2, PC5, and Gpc3 (2) the specific PC5 isoform involved in gut development and (3) the origin of functional DM PC5 shaping gut development.

24 *The role of Arid1a as a suppressor of spontaneous mammary tumors in mice*

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Human cancer genome studies have identified the SWI/SNF chromatin remodeling complex member ARID1A as one of the most frequently altered genes in several tumor types. Its role as an ovarian tumor suppressor has been functionally demonstrated in compound knockout mice. In my primary thesis work, I have found genetic and functional evidence that Arid1a is a bona fide breast cancer tumor suppressor, using the Chaos3 mouse model of sporadic mammary carcinogenesis. Nearly all mammary tumors that form in these mice contain a deletion removing one Arid1a allele, while the remaining intact allele is silenced or downregulated in many cases. The epigenetically silenced allele could be reactivated by treatment with the cytosine methyltransferase inhibitor 5-azacytidine. Restoration of Arid1a expression in a Chaos3 mammary tumor line greatly impaired its ability to form tumors following injection into cleared mammary glands, indicating that ARID1A insufficiency is crucial for maintenance of these tumors. Transcriptome analysis of tumor cells before and after re-introduction of Arid1a expression revealed alterations in growth signaling and cell-cycle checkpoint pathways, suggestively in a TRP53-dependent manner. These preliminary results provide in vivo evidence for a tumor suppressive and/or maintenance role in breast cancer. My current thesis work involves the generation of a mammary-specific conditional knock-out mouse model of Arid1a, which will help determine whether loss of ARID1A is an initiating driver of mammary tumors. I am also attempting to induce expression of the remaining Arid1a allele present in multiple Chaos3 mammary tumor cell lines in a locus-specific manner, using CRISPR-activation (CRISPRa) technology. If successful, this would indicate a potential opportunity for therapeutic intervention in ARID1A-deficient human breast cancer subtypes.

25 *Female-Biased Embryonic Death from DNA Replication Stress-Induced Inflammation*

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Replication stress (RS) can trigger genomic instability, checkpoint activation, senescence, and apoptosis. Though extensively studied in cell culture and cancer paradigms, little is known about the impact of RS during embryonic development, a period of rapid cellular proliferation. We have found that female mouse embryos with a high level of genetically-encoded RS are dramatically more susceptible than males to embryonic lethality. This bias was not attributable to sex differences in DNA replication competence or X-inactivation defects, but rather differential sensitivity to RS-induced inflammation. XX embryos with high intrinsic RS could be rescued by testosterone (abundant in male fetuses) or NSAID (ibuprofen) treatment during gestation, transgenic sex reversal, and maternal genotype. The results are relevant for pregnancies in which the female fetus and maternal environment have high RS and/or inflammation.

26 *A novel role for the Fanconi Anemia pathway in bile acid homeostasis*

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Fanconi anemia (FA) is a genomic instability syndrome characterized by developmental defects, bone marrow failure, cancer predisposition, and metabolic disorders. The FA pathway becomes activated by DNA replication stresses and plays a major role in responding to interstrand DNA crosslinks (ICLs). FA patients are hypersensitive to exogenous genotoxins; however, the endogenous sources of damage repaired by the pathway remain poorly characterized. The link between a defective FA pathway and the development of endocrine and metabolic dysfunction is also unclear. We tested the hypothesis that the FA pathway protects against DNA damage caused by lipid metabolism using a Fancd2-/- mouse model. Fancd2-/- and wildtype (WT) mice were continued on standard diet (SD) or challenged with a high fat, high cholesterol, cholic acid enriched Paigen diet (PD). Fancd2-/- mice exhibited decreased survival compared to WT mice in a long term PD trial ($p=0.0137$). In male Fancd2-/- mice, PD feeding resulted in increased hepatic pathology relative to WT controls, including increased ALT, hepatic parenchymal inflammation, and hepatocellular apoptosis, suggesting that the FA pathway plays a role in protecting against PD induced damage. Additionally, male Fancd2-/- mice fed PD developed more severe hepatomegaly, hepatocellular swelling, and biliary hyperplasia relative to WT controls, suggesting the FA pathway may have roles in hepatic lipid, cholesterol, and/or bile acid metabolism. The hypersensitivity in Fancd2-/- mice to the high fat, high cholesterol, cholic acid enriched diet described here provides a powerful opportunity to define the roles of the FA pathway in maintenance of genomic stability and metabolic homeostasis.

27 *Tumor promoting functions for the DNA damage response protein HUS1*

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DNA damage checkpoint pathways have distinct, stage-specific roles in tumorigenesis. Checkpoints protect against cancer-initiating mutations in normal cells, but in some cases support tumor growth at later stages, allowing cancers to tolerate elevated stress levels associated with malignant transformation. Here, we tested the role of the DNA damage response protein HUS1 in malignant transformation. As a 9-1-1 complex component, HUS1 participates in DNA repair and promotes ATR activation. Hus1 impairment increased oncogene-induced chromosomal aberrations and checkpoint activation, resulting in reduced proliferation and cell transformation. Moreover, Hus1 inactivation decreased tumorigenesis in mouse models of lung and skin cancer. HUS1 levels were elevated in multiple cancer cell lines through a mechanism involving alternative polyadenylation. Unlike their normal counterparts, which express Hus1 as a long isoform with an extensive UTR, cancer cells utilized a proximal polyadenylation site, yielding a short isoform with a truncated UTR lacking sequences for negative regulation of Hus1 expression. These data suggest that cancer cells can have an elevated dependency on HUS1 that they satisfy in part by upregulating HUS1 expression through alternative polyadenylation

28 *How DNA-damage machinery protects the genomes of mouse oocytes*

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During meiosis hundreds of endogenously induced DNA double stranded breaks (DSB) are made by the SPO11 endonuclease in order to assure homologous chromosome pairing (synapsis) and their proper segregation. Because DSBs are potentially mutagenic, quality control mechanisms are in place to guarantee that they are all repaired, preferentially by interhomolog recombination, otherwise apoptosis is induced. It is currently accepted that this pachytene checkpoint senses DSB repair and synapsis by independent mechanisms. However, we have experimental evidence that oocytes defective for either DNA repair or synapsis are both eliminated by a canonical DNA damage response checkpoint pathway dependent upon signaling of the CHK2 kinase to downstream effectors p53 and p63). During the first meiotic division, mechanisms are in place to inhibit the DNA-repair machinery from using the sister chromatid as recombination repair donors, making the homologous chromosome the substrate of choice. Whereas this block to sister chromatid repair of DSBs assures interhomolog (IH) recombination, it also prevents the chromosomes that fail to undergo IH repair from repairing residual DSBs. Here, we present evidence consistent with a model whereby asynaptic oocytes accumulate SPO11-independent DSBs, and are eliminated via CHK2 activation.

29 *Members of the ORRM family affect organelle RNA editing and impact plant development*

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Post-transcriptional C-to-U RNA editing occurs at specific sites in plant chloroplasts and mitochondrial transcripts. This process is regarded as a correction mechanism for defective T-to-C mutations in plant organelles that would otherwise impair the proper function of gene products, even leading to lethality. Editing is carried out by small RNA/protein complexes called editosomes, whose compositions are beginning to be unraveled. Recognition of the proper C target for editing is mediated by pentatricopeptide repeat (PPR) motif-containing proteins that specifically recognize cis-elements. Members of the Arabidopsis RNA-editing factor Interacting Protein (RIP, also characterized as MORF) family and Organelle RNA Recognition Motif-containing protein 1 (ORRM1) have been recently identified as essential components of the RNA editing apparatus. ORRM1, a chloroplast editing factor, belongs to a distinct clade of RNA Recognition Motif (RRM)-containing proteins. By subjecting additional members of the ORRM clade to insertional mutagenesis and virus-induced gene silencing, we identified several ORRM proteins as mitochondrial RNA editing factors. Altered expression level of ORRM editing factors not only affect the editing efficiency, but also impact plant development and flowering. Protein-protein interaction assays with these editing factors demonstrated their interaction with other known components of the RNA editosome. This study reveals a previously unknown role of plant RRM proteins as components of the mitochondrial editing apparatus. The establishment of a new family as mitochondrial RNA editing factors further expands our knowledge of the composition of the editosome. Thus, it paves the way for future genetic engineering of plants through deliberate post-transcriptional modifications of nucleotide sequence.

30 *CDK2-Tyr15 phosphorylation is required for spermatogonial stem cell niche maintenance*

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Spermatogonial stem cells (SSCs) support spermatogenesis throughout entire life span; however, the molecular mechanisms that regulate the balance between self-renewal, proliferation and differentiation of SSC niches are poorly understood. To address the role of CDK2-Tyr15 during SSC maintenance, we generated mice mimicking a human non-sysnonymous SNP in cyclin dependent kinase 2 (CDK2). Mice bearing this Cdk2Y15S allele exhibit a SCO-like phenotype. Abolition of this phosphorylation site is predicted to render CDK2 constitutively active, and causes misregulation of self-renewal and differentiation events of SSCs after birth. Our results thus suggest that phosphorylation of this site has two roles in SSC maintenance: 1) in the first wave of spermatogenesis, to restrain proliferation of Tra98+PH3+ germ cells, and 2) maintaining a steady-state of spermatogenesis balancing proliferation and differentiation. In mutants, the SSCs assume a quiescent state. We will report on how this gain-of-function point mutation results in failure of SSCs to propagate and initiate waves of spermatogenesis.

31 *Investigating the endonucleolytic role of MLH3 during prophase I of mammalian meiosis*

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During meiotic prophase I, homologous chromosomes pair, synapse, and undergo recombination, the latter of which results in crossover formation. All these events ensure that homologs will segregate equally into daughter cells during the first meiotic division. In mouse, crossover formation is initiated by ~250 double strand breaks (DSB) of which 90% are resolved as non-crossovers, while 10% are resolved as a crossover. MLH1/MLH3 (MutLÎ³) has been identified to play a role specifically in processing of crossovers. MLH3 contains a potential endonuclease domain thought to have enzymatic activity in crossover formation and/or double Holliday junction processing. To investigate the endonucleolytic role of MLH3 in vivo, we generated an Mlh3D1185N (Mlh3DN) mutant mouse harboring a point mutation at a conserved site within the endonuclease domain. Mlh3DN/DN males show significantly lower testes weights, no spermatozoa in the testes nor in the epididymis, and no offspring when mated with wild-type females. Chromosome immunofluorescence studies show that Mlh3DN/DN spermatocytes undergo normal DSB processing events during prophase I, as demonstrated by normal dynamics of Î³H2AX and RAD51 appearance and disappearance from autosomes. Mlh3DN/DN spermatocytes also undergo complete synapsis, as shown by SYCP3 (lateral element) and SYCP1 (central element). In addition, Mlh3DN/DN spermatocytes display normal localization of both MLH3 and MLH1 to the synaptonemal complex. However, disruption of the endonucleolytic function of MLH3 results in less than 22% of crossovers when compared to wild-type, but more when compared to Mlh3-/- diakinesis-staged spermatocytes, suggesting that the endonuclease activity may be substituted by other proteins at a small subset of crossover sites. In conclusion, while the endonuclease domain of MLH3 appears to not play a significant role in the processing of DSBs, it is essential for the processing of the majority of DNA events that are ultimately destined to become crossovers.

32 *Enhancers that regulate spermatogenesis*

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Enhancers are non-coding DNA that recruit transcriptional machinery to promoter sites. They range from 50 basepairs to 20 kilobases in length, consisting of repetitive transcription factor binding and promoter interaction sites. Currently, enhancers are poorly understood in the context of spermatogenesis. This is due to several reasons, including an absence of in vitro systems for studying meiosis and lack of technology for genome-wide enhancer screens.

Only recently has the surge of new sequencing technology made enhancer screens feasible, and this year, was spermatogenesis recapitulated in culture for the first time (Zhou et al., 2016). With the advent of these techniques, we propose to identify enhancers of genes involved in spermatogenesis, specifically at the stages of leptotene, zygotene, and pachytene of Prophase I. We will utilize genome-wide sequencing techniques, ATAC-seq and PRO-seq, on mouse spermatocytes specific to these stages and create genome-wide maps of their chromatin structure. In combination with previous histone modification data, these findings will provide clues on the loci of enhancers.

Not only will these findings provide insight on spermatogenesis regulation and enhancer-promoter relationships, but this will let us better understand meiosis and how haploid gene expression works. We will study the consequences of disrupted enhancers on gene expression by introducing single nucleotide polymorphisms into candidate enhancer sequences. We hypothesize that mutations to enhancer sequences play a role in causing infertility.

33 *Genetic mapping of novel loci affecting canine blood phenotypes*

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Since the publication of the dog genome and the construction of high-quality genome- wide SNP arrays, thousands of dogs have been genotyped for disease studies. For many of these dogs, additional clinical phenotypes are available, such as hematological and clinical chemistry results collected during routine veterinary care. Little is known about the genetic basis of variation in blood phenotypes, but this variation may play an important role in the etiology and progression of many diseases. From a cohort of dogs that had been previously genotyped on a semi-custom Illumina CanineHD array for various genome-wide association studies (GWAS) at Cornell University Hospital for Animals, we chose 353 clinically healthy, adult dogs for our analysis of clinical pathologic test results (14 hematological tests and 25 clinical chemistry tests). After correcting for age, body weight and sex, genetic associations were identified for amylase, segmented neutrophils, urea nitrogen, glucose, and mean corpuscular hemoglobin. Additionally, a strong genetic association ($P = 8.1\times10\text{-}13$) was evident between a region of canine chromosome 13 (CFA13) and alanine aminotransferase (ALT), explaining 23% of the variation in ALT levels. This region of CFA13 encompasses the GPT gene that encodes the transferase. Dogs homozygous for the derived allele exhibit lower ALT activity, making increased ALT activity a less useful marker of hepatic injury in these individuals. Overall, these associations provide a roadmap for identifying causal variants that could improve interpretation of clinical blood tests and understanding of genetic risk factors associated with diseases such as canine diabetes and anemia, and they demonstrate the utility of holistic phenotyping of dogs genotyped for disease mapping studies.

34 *Functional Analysis of Conserved miRNAs in Spermatogenesis:*

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Micro RNAs (miRNAs) play a major role in regulating gene expression, and thus impact organismal biology. However, there is a dearth of functional miRNA studies in mammalian gametogenesis due to the physiological intricacy of gametogenesis and lack of appropriate in-vitro systems. There are many conserved miRNAs that are expressed during mouse and human spermatogenesis that have not yet been explored. Here, we use a CRISPR-mediated mutagenesis strategy to simultaneously knockout multiple miRNAs, which are expressed and conserved between mouse and humans, in mouse embryos. Here we show that disruption of multiple miRNAs result in aberrant spermatogenesis. Our goal is to identify and validate these mutations by correlating the phenotype to the genotype using both PCR and sequencing data and ultimately to further identify the mRNA targets of these miRNAs.

35 *Drivers of Ovarian Carcinoma*

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Ovarian cancer is a complex disease consisting of several genetically distinct subtypes. High grade serous ovarian carcinoma (HGSOC) is the most lethal subtype, largely due to late diagnoses, imprecise screening methods, and frequent metastasis. While no single mutation explains HGSOC initiation, common pathway mutations and alterations have recently been identified through the Cancer Genome Atlas Research Network. These include retinoblastoma (RB) (67% of cases), PI3K/Ras (45%), and Notch (22%) signaling pathways, along with genes involved in homologous recombination DNA repair (51%). Though these genes and pathways are associated with cancer incidence, correlations are unable to differentiate initiating (driver) mutations from downstream (passenger) mutations. The HGSOC cell of origin also remains uncertain, with candidate populations including ovarian surface epithelium, fallopian tubal epithelium, and stem cell niches. To address these issues, we will perform random, combinatorial mutagenesis of 20 HGSOC-associated genes on putative cells of origin using a minilibrary of lentiviral CRISPR/Cas9 (LentiCRISPRv2) constructs. Cells will be isolated, randomly edited, and injected into the ovarian fat pad of mice, allowing for in vivo transformation of cells with initiating mutations. Tumors will be characterized via identification of initiating LentiCRISPRs by qPCR, next generation sequencing, histology, and protein analyses. Cell migration assays will also be performed to identify mutations associated with increased cell mobility. We hope that our results will provide significant insight into the origin of HGSOC and contribute to early detection and diagnosis.

Population Medicine

36 *Modeling the Transmission Dynamics of Johne's Disease: An Individual Based Approach*

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Johne's disease (JD), an enteric infectious disease, is caused by Mycobacterium avium subsp. paratuberculosis (MAP) in a dairy herd. To date, there is no optimal remedy for JD. But researchers have suggested several control strategies, such as test based culling strategies, separate calf rearing management, hygiene improvement, and vaccinations. However, these control strategies do not perform optimally due to the slow progressing nature of MAP and low sensitivity and specificity of the diagnostic tests. Also, traditional mathematical/epidemiological MAP models are insufficient to provide the details of individual infected animals on a daily basis, and information about parity, lactation status, days in milk, and true identification of low and high shedders is needed for adopting control decisions along with their economic justification. Here, we introduce an individual based model (IBM) where we consider sufficient daily life details of a dairy cow along with MAP infection dynamics. It includes key dynamic processes of the dairy cow such as lactation cycle, calving, voluntary waiting period, insemination, pregnancy, dry-off period and calf and heifer rearing. After validating the uninfected herd model with empirical data, we integrate MAP infection structure on top of that. Adult animals are divided into four categories (susceptible, latent, low shedding and high shedding), while calves and heifers are considered in separate loops of management. The model considers the five routes of transmission throughout the three loops of animals (adult, calves and heifers): adult-to-adult, adult-to-calf, calf-to-calf, heifer-to-heifer, and vertical transmission (in utero). The simulated results confirm the suitability of the IBM for MAP infection and provide access to the individual information needed to determine which control strategies to choose in an endemic situation. In conclusion, this model can serve as a novel and unique tool which can not only investigate each transmission path separately through the individual animal level, but also provide a flexible model structure where more detailed dairy herd problems can be integrated.

37 *Community Engagement in Biomedical Sciences and Engineering*

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Do you want to make a difference in the lives of people who are affected by cancer? Research is not the only way to have a positive impact. Our partnership with the Cancer Resource Center provides opportunities to interact with and learn from cancer patients and survivors. Through dialogue with patients, we extend our understanding of cancer and enrich our disciplinary perspectives. One participant noted, "With this experience, the disease has evolved from a biological problem in the lab to something much more personal." In addition, the partnership facilitates the dissemination of cutting-edge research with our local community. Through the Engaged Cornell initiative, we now offer a certificate program in public engagement for cancer researchers, comprising four courses on science communication and public involvement in science. The Cancer Brainstorming Club also sponsors a variety of informal events. Overall, the partnership reminds us that people with cancer are more than cells or molecular pathways. They are friends, neighbors, relatives, and colleagues. They are integral partners in the fight against cancer. For more information, visit cancer.cornell.edu/education.html.

38 *Livestock ownership is not associated with improved dietary quality or growth among children in the Luangwa Valley, Zambia*

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The two principal causes of undernutrition among children are poor dietary quality and exposure to pathogens. Because livestock can influence both pathways, the net impact of household livestock ownership on children's growth in low-income countries may be positive, negative, or neutral. Recent research throughout sub-Saharan Africa have had mixed findings, suggesting the link between livestock ownership and child nutrition is complex and context specific. We investigated the association between household livestock ownership and children's diets and linear growth in 40 villages surrounding Zambia's South Luangwa National Park. We collected dietary and anthropometric information from 838 children aged 6-36 months in January 2015, along with information on household and maternal characteristics, as part of baseline data collection for a nutrition-sensitive agricultural intervention. Overall, 63.1% of households owned livestock, though average livestock holdings were very small. In multilevel mixed-effects models, livestock ownership had mixed impacts on child dietary diversity and animal source food consumption depending on the measure of livestock ownership used. There was no association between livestock ownership and child height-for-age z-score or stunting odds. Our findings suggest that livestock may not contribute meaningfully to child diets in this population, perhaps due to small livestock holdings and the availability of wild fish and meat. Although livestock may positively affect child nutrition through other unmeasured pathways (e.g. income), these benefits were either 1) too small to affect growth in the face of other factors (e.g. poor feeding practices) or 2) negated by negative consequences of livestock ownership (e.g. pathogen exposure).

39 *Biphasic Induction of Invasion Genes Enables Salmonella's Success in the Gut*

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In mammalian hosts, ingested Salmonellae can cause acute gastroenteritis. This inflammation is induced in response to bacterial invasion of the intestinal epithelium, a process carried out by genes encoded within the Salmonella Pathogenicity Island 1 (SPI-1). SPI-1 encodes the regulatory, structural, and effector proteins of a type three secretion system that, when expressed, induces epithelial membrane ruffling and promotes bacterial uptake. For Salmonella, the implications of SPI-1 expression are complex; while the host inflammatory response to bacterial invasion provides a proliferative advantage to luminal Salmonella, the expression of SPI-1 inflicts a considerable metabolic burden. In light of the conflicting benefits and burdens of SPI-1 expression, Salmonella employs biphasic regulation of invasion genes. This tactic permits a subpopulation of bacteria to undertake invasion gene expression without imposing undue burden on the entire population, leaving non-expressing bacteria to benefit from the resulting inflammation.

Here we investigate the genetic regulatory components that establish biphasic control of SPI-1. We demonstrate that the transcriptional activator HilD plays an essential role in establishing all-or-none SPI-1 expression. This function requires both the hilD promoter and gene product, which participate in a positive-feedback loop. We further demonstrate that, in vitro, this autoinduction relies on additional transcription factors to augment the feedback loop. Finally, we demonstrate that structurally disparate inhibitors of invasion gene expression target these transcriptional activators to achieve SPI-1 repression. That the actions of many inhibitors converge to control biphasic induction underscores the importance of this regulatory mechanism in balancing the costs and benefits of SPI-1 expression.

40 *Platelet-Mediated Equid Herpesvirus Type 1- Infection Of Endothelial Cells Cultured Within An In Vitro Microfluidic Model*

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Equid herpesvirus type 1 (EHV-1) causes rhinopneumonitis, abortion and myeloencephalopathy in horses. Abortion and myeloencephalopathy are a consequence of endothelial cell infection, resulting in vasculitis and ischemia. We have found that platelets are activated by EHV-1 as a consequence of virus-associated thrombin generation. Platelets can carry and transfer viruses, such Dengue virus, to other cells. We hypothesized that platelets are “Trojan horses”acting as a source of EHV-1 infection for endothelial cells. To test this hypothesis, we developed a microfluidic model to study the interaction of platelets, virus and endothelial cells. Equine carotid endothelial cells were seeded inside polydimethylsiloxane microchannels at a shear stress equivalent to a venule (2 dynes/cm2). Endothelial cell confluency was achieved after 48 hours. Platelet-rich plasma was derived from citrate-anticoagulated blood of healthy horses by low speed centrifugation. Platelets (1 x 107 cells/mL) were incubated with the RacL11 strain of EHV-1 at 1plaque forming unit/cell for 10 minutes at 37°C. Endothelial cells were exposed to virus only or platelets previously exposed to virus for 2 h at low sheer stress (0.7 dynes/cm2). Cells were observed for cytopathic effect at 24, 48 and 72 hours post-exposure. Virus-exposed platelets, but not virus alone, induced cytopathic effect in the first 72 h post infection. Our data suggests that platelets may play an important role in endothelial infection by EHV-1. Our microfluidic model will allow further investigation of platelet- and leukocyte-endothelial cell interactions with EHV-1 infection.

Biochemistry, Molecular and Cell Biology

41 *Assessing species-specific determinants of McrB-DNA binding*

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Modification-dependent restriction systems (MDRs) recognize and cleave methylated – and in some cases glucosylated – DNA. These proteins are thought to play a role in establishing the epigenetic landscape of bacterial genomes and are especially important in protecting against predatory bacteriophages, which often incorporate modified bases into their DNA to evade detection by the host machinery. One such example is McrBC, a two-component MDR that targets and cleaves DNA containing 4-, 5-, or 5-hydroxy-methylcytosines. McrB is a two domain protein with an N-terminal domain that binds fully or hemi-methylate R^MC sites and a C-terminal AAA+ domain that binds/hydrolyzes GTP and mediates nucleotide-dependent oligomerization. Recent crystallographic data shows that *E. coli* McrB flips the methylated cytosine out of the duplex and stabilizes the resulting gap by inserting a tyrosine residue. However, this tyrosine is not conserved, suggesting that other mechanisms may exist for binding modified DNA. To examine the species-specific determinants of McrB DNA binding, I have purified the putative DNA binding domains of different bacterial (*B.cereus*) and archaeal (*T.gammatolerans*) McrB homologs and compared their ability to bind modified DNA with the prototypical *E. coli* McrB binding domain. Furthermore, I have also crystallized the putative *B. cereus* and *T.gammatolerans* DNA binding domain (Bc36-308 and TgD185 respectively), and solved the structure of the *T.gammatolerans* DNA binding domain. The structure shows structural homology to the YTH protein domain, which in eukaryotes, bind RNAs containing methylated adenosine bases. Structural superposition of the *T.gammatolerans* McrB identifies a conserved aromatic cage involved in binding the modification. Together, these data provide insights into the molecular details and species-specific differences in McrB-DNA binding and have the potential to aid in the design of novel species-specific therapeutics for treating multi-drug resistant bacterial infections.

42 *Defining the Functions of Extracellular Vesicles in Renal Cancer Progression*

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Cancer cells release two distinct populations of extracellular vesicles (EVs) that appear to be generated via distinct mechanisms: exosomes, which are less than 0.2 µm in diameter, and microvesicles (MVs), which typically are 0.2-2 Î¼m. EVs shed from cancer cells induce cancer-like phenotypes in normal cells. Thus, EVs may potentially play important roles in cancer progression. Here, we set out to characterize the different classes of EVs generated by renal cancer cells and determine how they contribute to disease progression. Renal cancer is one of the most common cancers and is a diagnostic challenge because patients are not typically diagnosed until the later stages of the disease. Therefore, understanding disease progression may aid in diagnosis and treatment. To this end, we discovered that renal cancer cells shed both exosomes and MVs. MVs but not exosomes isolated from aggressive renal cancer cells enhance anchorage-independent growth in less malignant renal cancer cells. To determine the MV cargo responsible for this effect, we tested the effect of inhibitors on MV- and exosome-mediated enhancement anchorage-independent growth. Although anchorage-independent growth in recipient cells did not depend on EGFR signaling, an EGFR inhibitor suppressed the ability of the MVs to enhance anchorage-independent growth in recipient cells. Conversely, RGD (a peptide that blocks fibronectin-integrin interactions) had no effect on the the MV-mediated enhancement of anchorage-independent growth. These studies demonstrate that EVs may play an important role in renal cancer progression and that EGFR signaling is necessary for the MV-mediated enhancement of anchorage-independent growth in recipient cells.

43 *CD44 regulation by stem cell niche-derived growth factors may play a critical role in the carcinogenesis at the gastric transitional zone*

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Gastric cancer is the third leading cause of cancer-related death worldwide. The incidence of the cancer arising from the squamo-columnar transitional zone (TZ) between the esophagus and the stomach has been increasing over the past few decades. Unfortunately, the carcinogenesis of TZ gastric cancer remains poorly understood. Recent studies have shown that epithelial TZs may contain stem cell niches, which are composed of adult stem cells, their committed progenies, fibroblasts, endothelial and perivascular cells, and extracellular matrix, controlling stem cell fate by providing signals through cell-cell contacts and diffusible factors. The adult stem cells at the gastric TZ express a member of WNT-signaling pathway, leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5). We have found that conditional knockout of the common tumor suppressor genes Trp53 and Rb1 in LGR5-expressing (LGR5+) TZ stem cells leads to gastric cancer in all mice. At the contrary, only few benign neoplasms develop in the gastric pyloric regions, which also contain LGR5+ stem cells. The normal gastric TZ, but not pyloric zone, contains significant population of CD44+Lgr5- progenitor cells characterized by high proliferation. Consistent with the known roles of CD44 in the facilitation of carcinogenesis, inactivation of this gene reduces gastric organoid formation. CD44 expression is regulated by niche growth factors, such as stromal-derived factor (SDF), hepatocyte growth factor (HGF) and osteopontin (OPN). All of these factors promote the growth of gastric TZ organoids. Taken together, our results suggest that the niche growth factors may promote gastric TZ carcinogenesis by regulating CD44 expression.

44 *Small companion animals as models to evaluate epigenetic drugs for breast cancer treatment*

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Murine models are indispensible for the study of human breast cancer, but they have some limitations: tumors arise spontaneously in humans, but routinely must be induced in mice, and long-term follow up is limited by the short life span of rodents. In contrast, dogs and cats do develop mammary tumors spontaneously and are relatively long-lived. Also, canine and feline mammary tumors share many features with human breast tumors including initiation, progression and response to therapy. The similarities observed between these three species suggest that dogs and cats can be useful models for the study of human breast cancer and the pre-clinical evaluation of novel therapeutics. This study examined the in vitro effects of the DNA methyltransferase (DNMT) inhibitor 5-Azacytidine (5-AzaC) and the protein arginine deiminase (PAD) inhibitor BB-Cl-amidine on normal and tumoral mammary cell lines derived from dogs and cats. Our findings show that treatment with both epigenetic drugs reduced in vitro tumorigenicity based on growth and invasion assays, mitochondrial activity and susceptibility to apoptosis. Moreover, we found that treatment with a high dose of these drugs was toxic to tumoral, but not healthy, canine and feline mammary cell lines, suggesting their therapeutic potential. These encouraging in vitro data now provide the basis for future in vivo experiments in mouse xenograft models of canine and feline mammary cancer, and ultimately, clinical trials in dogs and cats.

45 *Molecular mechanism underlying ATP-gated ion channel antagonism*

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P2X7 receptors are ATP-gated ion channels that play important roles in immune response and neurotransmission. Pharmacological inhibition of these receptors attenuates neuropathic and inflammatory pain, a devastating condition that affects more than 1.5 billion people worldwide. As a result, many P2X7 receptor-specific compounds have been developed by pharmaceutical companies for alleviating chronic pain associated with rheumatoid arthritis (RA). Unfortunately, the results in clinical trials for RA have been disappointing, largely due to the lack of mechanistic understanding of how these drugs antagonize the P2X7 receptor. Here we present the first crystal structures of the P2X7 receptor that uncover the novel antagonist binding site. Structure-based functional studies demonstrate that the P2X7 specific drugs allosterically prevent the upper vestibule from expanding upon ATP binding.

46 *Understanding the mechanism and cellular function of atlastins*

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In order to carry out the diverse processes required for cellular homeostasis, the cell has evolved membrane-bound organelles to create microenvironments that are favorable for specific processes. One vital mode of organelle regulation can occur through fusion and fission of membranes. Since it is not energetically favorable to disrupt membranes, the cell has several enzyme families that catalyze these reactions, including SNARES and dynamin-related proteins. Our lab is interested in studying a subfamily of the dynamins, called atlastins (ATL), which fuse smooth ER tubules creating and maintaining a peripheral, reticular network. Humans have three ATL isoforms (ATL1-3) that display variations in cellular localization and tissue-specific expression patterns. The atl gene was first identified as a mutational hotspot in the neurodegenerative disease, hereditary spastic paraplegia (HSP), which causes loss of control of lower limbs. Previous work in the field has focused on solving the structure of the catalytic domains, attempting to define catalytic mechanisms, and characterizing ATL behavior in the cell and model organisms. Currently our lab is working on problems including: better understating the catalytic mechanism; determining functional and cellular differences among ATL isoforms; and structurally characterizing the full-length ATL.

47 *The Wnt pathway and Its role in lymphoma migration*

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The Wnt signaling pathway has many important roles in embryogenesis and is activated in a range of solid cancer types. This leads to malignant transformation and metastasis in many cancers such as colon, breast and prostate cancer. Its role in lymphoma is poorly defined and this study aims to investigate the Wnt signaling pathway in diffuse large B cell lymphoma. Canonical Wnt pathway activation results in nuclear translocation of the key transcriptional activator b-catenin. This leads to upregulation of genes involved with invasion, metastasis and migration primarily through the process of epithelial to mesenchymal transition. This study uses Boyden chamber migration assays, hydrogel organoids as well as the identification of key genes involved in migration to investigate the potential role of the Wnt pathway in promoting disease progression and spread to distant anatomical sites. Furthermore a range of Wnt pathway inhibitors are tested in these in vitro assays, with the hopes of transitioning into clinical trials for promising candidates.

48 *Assessing Oligomerization of DHHC Proteins by TIRF Microscopy*

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DHHC enzymes are a family of integral membrane proteins characterized by a canonical DHHC (Asp-His-His-Cys) motif within a highly conserved cysteine-rich domain. Functioning as protein acyltransferases, these enzymes catalyze post-translational palmitoylation, thereby regulating substrate localization, trafficking, and membrane signaling. Several DHHC proteins have been shown to have important roles in human pathophysiology and disease. Yet, little information is known about their mechanism and regulation. Previous work in our lab has shown that some members of DHHC oligomerize in cells. In detergent solution, DHHC3 appears to exist in a monomer–dimer equilibrium, with the monomeric state having higher activity. However, the stoichiometry of DHHC oligomers in cells is unresolved. In addressing this question, we have used Total Internal Reflection Fluorescence (TIRF) Microscopy to count the oligomeric state of individual GFP-tagged DHHC molecule. We have found that in HeLa cells, DHHC2 exists predominantly as monomers, whereas DHHC20 has a higher proportion of the dimer.

49 *Use of CRISPR-dCas9 to Investigate the Requirement of "Escape Pathway" Signaling Factors for Tamoxifen Resistance in Breast Cancer*

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About 70% of breast cancers are estrogen receptor alpha (ERα) positive and tamoxifen, an ER antagonist, is frequently used to treat these tumors. Unfortunately, some ERα positive breast cancer is, or becomes, resistant to tamoxifen. While the mechanism(s) driving resistance are not well-defined, several "escape pathway" signaling cascades are known to become activated in these resistant tumors. To identify possible mediators of tamoxifen resistance, we documented changes in gene expression and enhancer activity between tamoxifen sensitive (B7 and C11) and de novo resistant (G11 and H9) ER+ MCF-7 breast cancer cell lines using PRO-Seq. One of the most highly upregulated transcripts in the resistant lines was glial-cell derived neurotropic factor (GDNF), and our preliminary studies suggest that this growth/survival factor appears to be required for tamoxifen resistance in our model system. The goal of my project is to use the CRISPR-dCas9 system to modulate GDNF levels in our cell lines to further test the requirement of GDNF for tamoxifen resistance and investigate the mechanisms by which GDNF confers resistance. To date, I have successfully cloned four GDNF gRNA target sequences into the pGL3 plasmid and infected G11 and B7 cells with dCas9-KRAB and dCas9-VP64 lentivirus, respectively, to make stable cell lines. I will transfect the gRNAs into the stably infected G11 and B7 cells to target the GDNF promoter. I will then test tamoxifen sensitivity and perform qPCR to look at expression of GDNF, ER downstream targets, dCas9, and each gRNA.

50 *Carbenoxolone inhibits Pannexin1 channels through interactions in the first extracellular loop*

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Pannexin channels are involved in inflammation and immune response signaling, and are known to permeate large molecules. Carbenoxolone (CBX) is one of the most potent inhibitors of pannexin1, but its mechanism of action is unclear. Our aim is to understand how CBX inhibits pannexin1, which could identify mechanisms involved in channel gating.

51 *Potential Roles for Electrostatic Charges within the Epidermal Growth Factor Receptor's Juxtamembrane Domain in Receptor Dimerization*

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Constitutive activation of the epidermal growth factor receptor (EGFR) signaling pathway via activating mutations is frequently observed in cancer. However, the exact nature in which these mutations and their corresponding structural regions lead to constitutive activation is poorly understood. Here, we present evidence that activating electrostatic mutations of the juxtamembrane domain of the EGFR, the EGFR R1-6 and endoplasmic reticulum-retained EGFR R1-6/L393H, signal as constitutively active dimers. Our findings contribute to a growing body of evidence demonstrating the importance of dimeric signaling in constitutively active receptors.the novel antagonist binding site. Structure-based functional studies demonstrate that the P2X7 specific drugs allosterically prevent the upper vestibule from expanding upon ATP binding.

52 *Defining the mechanism of melanoma initiation from adult stem cells*

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Melanoma is considered the deadliest skin cancer with a gradually increasing annual mortality rate. In recent years, multiple genetic etiologies in melanoma have been identified, and great progress has been made in development of targeted therapies especially for aberrant signaling pathways. Unfortunately, most cases, however, eventually recur and are resistant to current therapies. While the vast majority of current studies focus on developing new therapeutic targets in primary and metastatic tumors, identification of potential preventative strategies is significantly underdeveloped. This study aims to determine potential means of prevention by identifying the environmental causes and cellular conditions responsible for tumor initiation and progression from cancer cells of origin. Adult stem cells and their transit amplifying progenies are now thought to be compelling candidates of tumor initiation, but melanocyte stem cells (McSCs) have not been studied in the context of melanoma initiation. McSCs found within hair follicles can contribute to hair pigmentation but also skin re-pigmentation. In our study, we aim to determine whether McSC activation is necessary for melanoma initiation and to identify the role of environmental extrinsic factors in initiating melanoma from adult stem cells. To achieve our aims, we used previously established genetically engineered mouse models of melanoma in combination with a lineage tracing model to identify the cellular dynamics and molecular mechanisms inherit to melanoma initiation. Interestingly, our studies found a significant contribution of extrinsic factors in melanoma initiation through McSC activation. Our studies will provide a new framework for the initiation of melanoma, with the ultimate goal to find preventative therapeutic applications for melanoma initiation and targeted therapy for melanoma.

53 *SIRT6 is a Potential Therapeutic Target for Neurodegenerative Disease*

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The pathological hallmark of neurodegenerative diseases is the loss of vital neuron populations. The prognosis for such diseases is bleak with few interventions available. In Parkinson's disease the progressive loss of dopaminergic neurons leads to loss of motor control and eventually death. Increasing the survival of these critical neurons is paramount to improving patient outcome. Sirtuin 6 (SIRT6) is a member of the Sirtuin family which are NAD+ dependent enzymes implicated in numerous aspects of metabolism and cellular survival. Recently the overexpression of SIRT6 has been shown to render cells more susceptible to apoptosis. Here we demonstrate that the suppression of SIRT6 increases the survival of cells under stress conditions. We also show that mice lacking neuronal SIRT6 are resistant to MPTP induced Parkinson's disease. These mice exhibit resistance to both behavioral and pathological changes. In corroboration with these results mice overexpressing SIRT6 display the opposite outcome with more severe pathology. In addition, we are exploring interactions between SIRT6 and components of tobacco smoke because fascinatingly tobacco users have a decreased risk for the development of Parkinson's. Our data suggests there is a functional relationship between nicotine and SIRT6 that may underlie tobacco user's resistance to neurodegenerative disease.

54 *Role of the Hsp70 nucleotide exchange factor Fes1 in the cellular oxidative stress response*

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Elevated levels of reactive oxygen species (ROS) are associated with many degenerative disorders (like aging, Alzheimer's, atherosclerosis, and diabetes). Increased cellular ROS levels correlate with accumulation of irreversibly oxidized proteins, which are prone to misfolding and aggregation. 70-kDa heat shock proteins (Hsp70s) are a conserved family molecular chaperones that refold misfolded and aggregated proteins, such as those damaged by ROS. As such, Hsp70s likely play a critical role in protein quality control under oxidative stress.

To examine the role of cytosolic Hsp70s in coping with oxidative stress, I screened S. cerevisiae strains mutated for each of the chaperones and their cofactors for peroxide sensitivity. Notably, I found that the nucleotide exchange factor Fes1 is required for cell survival during oxidative stress. My preliminary data suggest that when cells are grown in medium containing peroxide, Fes1 is post-translationally modified directly by ROS, which alters Fes1 activity. We speculate that this altered activity confers a protective role during stress conditions, allowing cells to sense and respond to elevated ROS levels. I am currently focused on characterizing this response of Fes1 to peroxide, including what residue(s) in Fes1 is (are) modified and how oxidation affects Fes1's protein quality control activities in the cell.

55 *T-REX™ on-demand redox targeting: A toolset for functional discoveries and validations*

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Uncontrolled generation of reactive oxygen and electrophilic species (RES/ROS) has long been associated with diseases such as cancer and Alzheimer's. However, recent research has identified the signaling roles of RES/ROS under physiological concentrations to be important in the maintenance of cellular health. Traditionally, redox signaling has been studied by swamping a model system (cell/organism) with reactive signals leading to the generation of mixed responses from multiple simultaneous events. Such approach mimics oxidative stress and is less amenable to the study of redox signaling. To side-step such problems, our lab has developed Targetable Reactive Electrophile and Oxidants (T-REXTM), a toolset that enables (1) on-demand redox targeting to a protein-of-interest (POI) in a complex model system, (2) interrogate the consequences of specific redox events (3) and allows unbiased screening for novel redox sensors. T-REXTM uses an inert photocaged precursor of a specific reactive signal that can selectively tag any HaloTag fused POI in a model system. The reactive signal can be released on-demand with low energy light consequently leading to covalent target modification owing to the reactivities of the signal and the amino acid residues on the target protein. The downstream response of the target-specific modification can be analyzed by in-gel fluorescence, proteomics, dual-luciferase reporter assays, qRT-PCR or flow-cytometry.

56 *Investigating the interaction between PGRN and NAGA as a pathological mechanism of the neurodegenerative diseases, FTLD and NCL*

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Progranulin (PGRN) is a secreted glycoprotein that has been implicated in a wide array of processes, including inflammation and tumorigenesis. Recently, it has been discovered that clinical patients with heterozygous mutations in the gene encoding PGRN develop the neurodegenerative disease, frontotemporal lobar degeneration (FTLD), a progressive, incurable, and terminal condition resulting in atrophy of the frontal and temporal lobes of the brain. Despite a multitude of studies, the exact function of PGRN and its mechanistic relationship to FTLD remains unclear. However, increasing evidence suggests a role for PGRN in lysosomal physiology - most strikingly the fact that homozygous mutations in the GRN gene result in neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease.In an attempt to better understand the lysosomal function of PGRN by identifying its lysosomal protein binding partners, we completed a mass spectrometry-based screen which successfully identified the lysosomal enzyme, α-N-acetylgalactosaminidase (NAGA). NAGA is responsible for assisting in the degradation of glycoconjugates in the lysosome, and deficiency results in Schindler disease, a distinct lysosomal storage disease with neurological sequelae. Based on the observed interaction between these two proteins and the association of both with lysosomal storage diseases and neurodegeneration, I hypothesize that PGRN regulates and augments the enzymatic activity of NAGA in the lysosome and that loss of PGRN results in NAGA dysfunction, which leads, at least in part, to the observed phenotypes in FTLD and NCL with PGRN mutations. Additionally, I will examine the possible reciprocal roles PGRN and NAGA play in one another's lysosomal trafficking.

57 *Cancer Derived Microvesicles and their Potential Role on Metabolic Changes Within Non-Cancerous Recipient Cells*

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In 1996, when B-cells were shown to secrete functional extracellular vesicles (EVs) which facilitated activation of the adaptive immune response, scientists began taking EVs seriously as a means of cell-cell communication. This led to a surge in research involving extracellular vesicles and has since differentiated into two categories; exosomes and microvesicles (MVs). MVs have been shown to allow a horizontal transfer of proteins and nucleic acids specific to their cell of origin. Although secreted by many cell types, it is in the context of cancer where MVs draw much attention. Various labs have shown that cancer derived MVs induce drastic changes within recipient cells in vivo and in vitro such as cancer-like transformation, better survival, increased angiogenesis, ECM degradation and more. These phenomena make MVs an important aspect to study in cancer biology. With many cancer cells undergoing aberrant metabolic strategies like aerobic glycolysis (Warburg effect) and glutamine dependence, it begs the question if these hallmark metabolic behaviors can be transferred to non-cancerous recipient cells. To test this hypothesis, MDA-MB-231 breast cancer cell line was used for their aggressive behavior, glutamine dependence and aerobic glycolysis strategies; MVs from their conditioned media were isolated. Protein analyses of these lysed MVs revealed several metabolic proteins present. Additionally, incubating these cancer derived MVs with a non-cancerous cell line (NIH 3T3 cells) resulted in an increase in extracellular ammonia and lactate concentrations, notable byproducts of a shifted metabolic pathway. Although much more thorough studies need to be performed, these data suggest a correlation in the ability to change metabolism of a non-cancerous cell by cancer derived MVs, potentially enriching for a pre-metastatic niche.

58 *The Role of Sil1 in the Endoplasmic Reticulum Oxidative Stress Response*

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Oxidative protein folding within the endoplasmic reticulum (ER) generates reactive oxygen species (ROS) that can aberrantly oxidize proteins. These damaged proteins aggregate in misfolded states and disrupt ER function. Furthermore, these aggregates are associated with a number of diseases. As such, mechanisms exist to maintain ER protein homeostasis amidst fluctuating ROS levels. Our lab has shown the ER Hsp70 chaperone (BiP) is oxidized in response to increasing concentrations of ROS. Oxidation converts BiP from an ATPase-driven protein foldase to a nucleotide-independent protein holdase. This enables BiP to bind misfolded proteins with higher affinity, minimizing aggregation. While beneficial during stress, prolonged BiP oxidation negatively impacts cell fitness necessitating a means for reducing the protein once ROS levels have subsided. Unexpectedly, we have found that Sil1, a BiP nucleotide exchange factor, possesses BiP-reductase activity. After reduction, BiP's ATPase activity is restored, allowing it to resume its role in protein folding. Currently, we are interested in the regulation of Sil1's reducing activity. To this end, we have developed Sil1 mutants that trap covalently linked reaction intermediates. Using these mutants, we aim to identify regulators of Sil1's redox state that in turn modify BiP's activity in response to ROS levels in the ER.

59 *The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway*

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Hexanucleotide repeat expansion in the C9orf72 gene is a leading cause of frontotemporal lobar degeneration (FTLD) with amyotrophic lateral sclerosis (ALS). Reduced expression of C9orf72 has been proposed as a possible disease mechanism. However, the cellular function of C9orf72 remains to be characterized. Here we report the identification of two binding partners of C9orf72: SMCR8 and WDR41. We show that WDR41 interacts with the C9orf72/SMCR8 heterodimer and WDR41 is tightly associated with the Golgi complex. We further demonstrate that C9orf72/SMCR8/WDR41 associates with the FIP200/Ulk1 complex, which is essential for autophagy initiation. C9orf72 deficient mice, generated using the CRISPR/Cas9 system, show severe inflammation in multiple organs, including lymph node, spleen and liver. Lymph node enlargement and severe splenomegaly are accompanied with macrophage infiltration. Increased levels of autophagy and lysosomal proteins and autophagy defects were detected in both the spleen and liver of C9orf72 deficient mice, supporting an in vivo role of C9orf72 in regulating the autophagy/lysosome pathway. In summary, our study elucidates potential physiological functions of C9orf72 and disease mechanisms of ALS/FTLD.

60 *Arf1 sets a TRAPP for Ypt31/32 at the trans-Golgi network*

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A major challenge for eukaryotic cells is the need to transport cargo between membrane-bound organelles in a regulated manner. Virtually every step of trafficking requires coordination by Rab GTPases, which recruit effectors that facilitate vesicle budding, transport, tethering, and fusion. Rab GTPases are regulated through activation by guanine nucleotide exchange factors (GEFs).

The Rab Ypt31/32 mediates secretory vesicle formation at the trans-Golgi network (TGN), and subsequently facilitates vesicle transport and fusion at the plasma membrane. Data from our lab and others indicate that the GEF that activates Ypt31/32 is the TRAPPII complex. As secretory vesicle formation is a highly orchestrated process, the question becomes: what signals direct TRAPPII to the Golgi to activate Ypt31/32 at the correct time?

We have found that Arf1, another key regulator of vesicle formation, cooperates with anionic lipids enriched at TGN membranes to recruit TRAPPII and activate Ypt31/32. In support of this model, loss of Arf1 function in cells reduces localization of TRAPPII at Golgi compartments. Additionally, active Arf1 recruits TRAPPII to membranes in an anionic lipid-dependent manner to activate Ypt31/32 in vitro. Moreover, a mislocalized TRAPPII mutant is rescued by increased Arf1 expression. These findings reveal an important layer of regulation in secretory vesicle trafficking, in which Arf1 recruits TRAPPII to the late Golgi to activate Ypt31/32 on nascent vesicles.

61 *Two viral nonstructural proteins are sufficient to compartmentalize the host cell translational machinery in a virus-derived matrix*

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Reoviruses compartmentalize translation within virus-induced inclusions called viral factories. Viral factories are the sites of viral replication, transcription, and assembly. Here we report that ectopic expression of two viral nonstructural proteins are sufficient to compartmentalize translation within viral factory-like structures that form within transfected cells.

62 *Mechanism of action of RNR inhibition by halogenated nucleotide anticancer agents*

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Ribonucleotide reductase (RNR) plays an indispensable role in the regulation of dNTP pools in all organisms because it is the only catalyst available for the de novo synthesis of dNTPs required for DNA replication and repair. Human (h)RNR activity is positively correlated with cancer cell proliferation. Nucleoside analogs that can inhibit hRNR activity such as gemcitabine and clofarabine (ClF) are proven effective in chemotherapy. However, the precise mechanisms by which these drugs in clinical use inhibit hRNR are unknown in most cases. The active hRNR is minimally an $\alpha_2\beta_2$ holocomplex in which α and β are the two distinct subunits. α subunit is a larger subunit harboring three distinct nucleotide binding sites; catalytic site (C-site), substrate specificity site (S-site), and allosteric activity site (A-site). β subunit houses a di-iron-tyrosyl-radical, an important component in C-site activation process. Our previous studies have shown that nucleotides of the leukemia drug ClF inhibit hRNR through persistent hexamerization of α without requiring β. This finding uncovers oligomeric regulation as a powerful small-molecule approach to inhibit hRNR activity. However, the oligomeric regulation of hRNR is, up to now, limited to ClF. Hence, its generality is still unknown. My recent studies of the modes of action of two halogenated adenosine analogs, cladribine (CLA) and fludarabine (FLU), have allowed us to evaluate the extent of the generality and clinical relevance of ligand-induced α hexamerization. This presentation describes my findings about the active forms of CLA and FLU, their inhibition kinetics, binding site specificities and oligomerization capabilities. The role of hexamerization of α in drug toxicity resistance is also described.

DVM students

63 *The Effect of High Density Lipoproteins on Bovine Neutrophil Activation Within Non-Cancerous Recipient Cells*

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High density lipoproteins (HDL) are responsible for the transport of cholesterol from peripheral tissues to the liver. In addition to HDLs role in lipid transport, this lipoprotein has been shown to have anti-oxidative and anti-inflammatory properties in mice and humans. Previous studies performed by our research team have shown that there is a marked decrease in HDL during the transition period in cows. This is important because the transition period is the time when dairy cows are at their highest risk for the development of costly inflammatory diseases. Therefore, we hypothesized that the decrease in HDL we documented during the transition period contributes to immune dysregulation and the high incidence of inflammatory disease seen in cows during the transition period. To begin to test this hypothesis, we first sought to determine if bovine HDL has an effect on neutrophil activation. Whole blood from dry cows was treated with either lipopolysaccharide (LPS) as a positive control, HDL, or HDL and LPS in combination. Using flow cytometry, changes in the expression of the adhesion molecule CD11b, a marker for neutrophil activation, were quantified. Lipopolysaccharide caused a robust increase in CD11b expression whereas HDL decreased the effect of LPS. Assays measuring reactive oxygen species (ROS) formation are being validated and will be used to determine if HDL has an anti-oxidative effect on bovine neutrophils. We are also evaluating HDL isolated from cows during different life stages to determine if bovine HDL function changes with the physiologic and metabolic state of the cow.

64 *Lu/BCAM Mediates Red Blood Cell Adhesion and Cortical Capillary Stalling in Mouse Models of Polycythemia Vera*

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Polycythemia vera (PV) is a myeloproliferative disorder characterized by red blood cell (RBC) overproliferation and microcirculatory thrombosis. A mutation in Jak2 kinase causing it to be constitutively active is associated with the development of PV and increased RBC production. Mutant Jak2 also leads to increased expression and phosphorylation of Lutheran/Basal Cell Adhesion Molecule (Lu/BCAM), which binds the α5 chain of laminin when phosphorylated and helps reticulocytes remain adhered to the extracellular matrix as they mature. Lu/BCAM expression is markedly decreased in mature RBCs, but it is known to be expressed and phosphorylated in mature RBCs of PV patients. To further understand the molecular mechanisms for microcirculation problems associated with PV, we investigated mouse RBC adhesion in vitro to laminin-coated surfaces and endothelial cells using a parallel flow plate chamber. We also investigated microcirculatory dysfunction in vivo in a mouse model of PV using two-photon microscopy. We found a six-fold increase in adhesion of PV RBCs to laminin compared to control RBCs ($p<0.05$). Treatment with anti-Lu/BCAM antibodies reduced the number of RBCs bound to laminin to control levels ($p<0.0033$). PV RBCs were also found to adhere to endothelial cells in vitro and treatment with anti-Lu/BCAM antibodies reduced adhesion. Two-photon imaging of mouse models of PV revealed that on average 30.7% of cerebral capillaries have stalled blood flow, with the majority of stalls due to RBCs adhering to the endothelium, compared to 5% of stalled capillaries in wild type controls. Our findings provide a molecular basis for vascular disturbances in PV and invite further exploration of Lu/BCAM targeted therapies for PV patients. (Characters 1750/1750).

65 *The role of Hus1 impairment in non-small cell lung cancer tumorigenesis*

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Lung cancer is the leading cause of cancer-related deaths in the United States. Non-small cell lung cancer (NSCLC) is the most common form of lung cancer. As *K-RAS* is a gene implicated in NSCLC, mouse models with the activating mutation *K-ras*^{G12D} are frequently used to study this cancer. Our research explores impairment of the DNA damage response gene *Hus1* as a mechanism to halt lung cancer. HUS1 is part of a heterotrimeric Rad9-Rad1-Hus1 complex that plays key roles in checkpoint signaling and DNA repair. Previous studies have suggested that there is a requirement for *Hus1* following *K-ras* oncogene activation as tumorigenesis progresses. Therefore, targeting *Hus1* may offer insights for treatment of NSCLC, though partial inactivation in mouse models is necessary as complete inactivation leads to early embryonic death. Due to its role in the DNA damage response, we hypothesize that mice with partial *Hus1* impairment will be more sensitive to DNA damage, which may affect tumor growth. To achieve this, we use mice with partial *Hus1* impairment (*Hus1*$^{neo/\Delta 1n}$) that are afflicted with NSCLC via oncogenic *K-ras*. Mice receive doxycycline to activate tumor formation. After 6 months of oncogene activation, tumor size is measured. This study may reveal the potential of impairing *Hus1* as a treatment strategy for NSCLC.

66 *Use of an individual based model to determine the contribution of environmental transmission in Mycobacterium avium subsp. paratuberculosis.*

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Understanding how animals become infected with *Mycobacterium avium subsp. paratuberculosis* (MAP), the causative agent of Jonne's disease is essential in evaluating control strategies to reduce its prevalence on dairy farms. It is well documented that animals become infected with MAP from ingesting contaminated material in their environment, but there are very few models that describe the role of the environment in transmission. Our model is the first individual based model (IBM) that describes the contribution of environmental transmission of MAP in a dairy herd.

We developed an individual based model of a closed dairy herd with typical dairy herd dynamics using Netlogo software. We then converted an existing MAP transmission model to the IBM to include a new infection structure where animals could become infected from an environment contaminated with MAP in addition to becoming infected in utero and from colostrum and milk. Using this model we explored three levels of hygiene to simulate the effect of different hygiene strategies on MAP prevalence.

Our model more accurately described transmission pathways than previous models because it considered the environment explicitly as a major source of infection and includes herd and infection dynamics at the individual level. We also showed that better hygiene leads to decreased MAP prevalence. Our model can be a useful tool for farmers to control MAP prevalence, and can be used as a framework for future research.

67 *Risk factors for canine dystocia and stillbirths*

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Studies that describe risk factors for canine dystocia and stillbirths typically do not have appropriate non-dystocia controls. This study aimed to 1) evaluate risk factors for canine dystocia; and 2) assess risk factors for stillbirth in puppies, by examining all whelpings and puppies born in a breeding population of guide dogs for a defined period of time. We hypothesize that dystocia risk is affected by maternal factors, and stillbirth risk is influenced by whelping and puppy parameters. We performed two retrospective observational studies. In the first study, we evaluated the risk factors for dystocia using a repeated measures model on 696 litters from 265 bitches. The overall dystocia rate was 24.9%. Risk factors identified for dystocia were: first-parity litters were more likely to have dystocia than subsequent parities, and maternal age at whelping where bitches younger than two years had a dystocia rate of 19% which increased to 31% in two-year-old and then decreased before increasing again to 50% in bitches > six years. Surprisingly, litter size was not associated with dystocia risk. In the second study, we evaluated the risk factors for stillbirth using a mixed-effect model with litter included as a random variable. Risk factors identified for stillbirths were: large (OR = 2.3) and small (OR = 9.2) puppies in the top/bottom 2.5% of birth weights, assisted deliveries (OR = 3.9), C-section intervention (OR = 2.1), unusual birth position (OR = 4.8), and oxytocin use (OR = 1.5). Of note, the use of calcium in managing dystocia was not associated with increased stillbirths while the use of oxytocin was associated with increased stillbirths. In conclusion, dystocia risk generally decreases with increasing maternal age and parity. Stillbirth risk increased as expected in large and small puppies, abnormal position and obstetrical interventions such as C-section and oxytocin use.

68 *Roles for the 9-1-1 DNA Damage Response Complex in Meiosis*

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Due to the volume of endogenous and exogenous stresses encountered by our cells every day, a process known as the DNA damage response (DDR) plays a critical role to help maintain proper genomic integrity. The DDR has been shown to allow germ cells to successfully advance through meiosis, particularly Prophase I, where double-stranded breaks (DSBs) occur to facilitate pairing of homologous chromosomes and crossing over. A central part of the DDR pathway is the RAD9A-RAD1-HUS1 (9-1-1) complex, a heterotrimeric clamp that acts as a molecular scaffold for DNA repair and checkpoint signaling activation, through interaction with several DNA damage response proteins. It has been previously established that testis-specific inactivation of Hus1 or Rad9a leads to; germ cell depletion, and severe meiotic defects. Recently paralogs of 9-1-1 subunits, RAD9A and HUS1, termed RAD9B and HUS1B, have been proposed to form alternative, non-canonical meiotic 9-1-1 complexes (RAD9B-RAD1-HUS1B and RAD9B-RAD1-HUS1), which are still poorly understood. We used Hus1b knockout mice (Hus1b-/-), in conjunction with Hus1 inactivation (hypomorphic Hus1 allele or conditional knockout targeted to the testis) to better understand the roles of these alternative 9-1-1 complexes. We hypothesize that disruption of multiple 9-1-1 complexes with loss of both Hus1b and Hus1 will disrupt proper progression through meiotic Prophase I. Supporting our hypothesis, preliminary results show germ cell loss in both males (increased lumen space) and females (decreased primordial follicle counts). These studies will help elucidate the diverse roles of the 9-1-1 complex on fertility and overall DNA stability.

69 *Parvovirus detection by PCR and ISH is associated with myocarditis and cardiomyopathy in young dogs*

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Perinatal parvoviral (PV) infection of pups born to nave dams causes necrotizing myocarditis resulting in cardiac failure, sudden death, and high mortality at 3-4 weeks of age. Acute infections are characterized by the presence of viral inclusions, necrosis, and colocalization of PV antigen to cardiomyocytes. Viral inclusions and detection of antigen are rapidly lost in pups surviving acute infection. Given the heart's stereotypic response to injury, we hypothesized myocarditis or cardiomyopathy in dogs <2years was frequently initiated by perinatal PV infection. For our study, DNA was extracted from archived formalin-fixed paraffin-embedded (FFPE) tissues from 41 such cases and age-matched controls from the NYS Animal Health Diagnostic Center archives. Samples were examined histologically and scored for myocardial necrosis, inflammation, and fibrosis by a blinded pathologist. Conventional PCR and sequencing was performed on FFPE tissue to detect the VP2 region of CPV-2. PV DNA was detected in 12/41 myocarditis/fibrosis cases and 2/41 controls. We found that myocardial PV DNA was significantly associated with myocarditis and/or fibrosis (p=0.007). PCR results were confirmed by in situ hybridization (ISH) which identified PV DNA in cardiomyocytes of juvenile and young adult dogs (median age 61 days) and in juveniles with PV enteritis, indicating a longer window of cardiac susceptibility to PV myocarditis than previously reported. PV DNA was also detected in cardiomyocyte nuclei of dogs with moderate to severe myocardial fibrosis (age range 126-243 days) suggesting earlier myocardial damage by PV. Given the temporal limitations of antigen-based testing, ISH offers expanded diagnostic utility for the identification of PV.

70 *Principal Component Analysis Describes Stifle Shape and its Impact onCranial Cruciate Ligament Disease*

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Cranial cruciate ligament disease (CCLD) is a widespread canine orthopedic condition. Rupture of the cranial cruciate ligament generates instability in the stifle joint during ambulation, leading to chronic pain, lameness and osteoarthritis. The evident heritability of CCLD has inspired efforts to characterize genomic regions where susceptibility or resistance may be encoded. In this study we seek to identify genetic influences on stifle morphology which may affect expression of CCLD using a genome-wide association study (GWAS). 174 dogs representing 60 breeds had stifle radiographs taken at the Cornell University Hospital for Animals and were genotyped on the Illumina version 2 High Density mapping array by the Cornell BioBank. Clinical diagnosis of CCLD for cases (n=161) and controls (n=13) was confirmed by clinical evaluation and radiographic analysis. We performed 10 quantitative measurements on the stifle radiographs for each dog. Principal component analysis (PCA) of these measurements enabled us to describe the size and shape of stifle joint features. PC1 concerned average stifle size. PC2 described an inverse relationship between tibial plateau angle (TPA), and the size of the infrapatellar fat pad. As a small, displaced fat pad is a standard indicator of CCLD, this relationship suggests that increased TPA indicates susceptibility to CCLD. Going forward we will conduct a GWAS on both the raw measurements and the PCs to identify genetic loci associated with stifle shape which may lead to loci associated with CCLD. If we find any genomic regions of interest, in-depth analysis of potential candidate genes will be warranted. While the power of the study is limited by population size and the clinical nature of the data, we hope to provide some insight into the genetic factors influencing stifle morphology and CCLD.

71 *Diagnostic accuracy of Keto-Test milk strips for cowside detection of elevated milk Î²-hydroxybutyrate*

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Hyperketonemia is a common disease of early postpartum dairy cattle characterized by an excess of circulating blood ketone bodies without clinical signs. This disorder leads to economic losses from decreased milk production, reduced reproductive performance, and increased risk of displaced abomasum and removal from the herd. Ketones can be detected in blood, urine, and milk, with the gold standard being laboratory measurement of blood α-hydroxybutyrate (BHB). While cowside blood tests provide an accurate means of diagnosis, this method is time-consuming and invasive; milk collection does not pose these same challenges. Keto-Test milk dip-strips provide an easy, non-invasive means of testing milk BHB concentration cowside, but more research is needed to determine the diagnostic accuracy of these strips and establish definitive cut-points for elevated milk BHB based on the color change of the strip. Our objective is to determine the diagnostic accuracy and precision of Keto-Test dip-strips in measuring milk BHB compared to the gold standard mid-infrared (IR) spectroscopy method. Milk was collected on 2 herds from 50 dairy cows between 2 and 16 days in milk using proportional samplers. Samples were tested immediately with the Keto-Test dip-strip according to label directions and then transported on ice to the laboratory for testing via IR methodology. The two diagnostic methods will be compared using a kappa calculation for inter-method agreement. Precision of the Keto-Test dip-strips will be determined by repeatedly measuring 1 milk sample in each of the 6 colored BHB concentration categories using 10 different test strips. Results are pending.

72 *α- cell glucagon-like peptide-1 receptor improves islet morphology after vertical sleeve gastrectomy in mice*

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Vertical sleeve gastrectomy (VSG), a surgical obesity treatment, results in type 2 diabetes (T2DM) remission days after surgery prior to significant weight loss. However, the mechanisms by which this occurs have not been established. VSG increases postprandial glucagon-like peptide-1 (GLP-1) secretion. This incretin hormone stimulates insulin production, insulin secretion, and α -cell protection. Surprisingly, studies using whole body GLP-1R knockout mouse models or GLP-1R antagonists suggest that GLP-1R signaling does not contribute to glucose regulation after VSG. Recently, our lab confirmed that GLP-1R signaling contributes to improved glucose regulation after VSG using a α -cell specific tamoxifen-inducible knockout mouse model, eliminating potential compensatory pathway development that may have confounded previous studies. We hypothesized that α -cell GLP-1R does this by improving islet morphology and insulin production. Male 2 month old inducible α -cell specific Glp-1r+/+ (WT) and Glp-1r -/- (KO) littermates were placed on a high fat diet (HFD) for 1.5 months, then switched to tamoxifen supplemented HFD for the rest of the study. Four month old mice underwent VSG or sham surgery. One and a half months after surgery, tissues were collected. Paraffin embedded sections (n=3) were fluorescently stained for insulin and quantified. VSG WT mice had decreased Î²-cell area per islet compared with WT sham-operated (S WT) mice. However, α -cell area per islet did not differ between S KO and VSG KO (avg. Î²-cell area per islet: S WT = 5648Â±190, VSG WT = 3578Â±501, S KO = 4965Â±1194, VSG KO = 5895Â±1927 Â μm^2; P<0.05 S WT vs VSG WT). This demonstrates for the first time that VSG improves islet morphology through α -cell GLP-1R signaling.

73 *Investigation Of Reproductive Setbacks In A Livestock Guarding Dog Breeding Colony*

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The Livestock Guarding Dog Program is a crucial part of The Cheetah Conservation Fund's multifaceted approach to conservation. The program has been extremely successful in reducing Human-Wildlife Conflict between humans and predators in Namibia. In the past twenty years, over 500 Anatolian Shepherd/Kangal dogs have been placed and retaliatory killings have been reduced by >90% in these areas. In the past two years the program has faced setbacks with its breeding colony, including failures to conceive, pseudopgregnancies, and neonatal mortalities. My objective was to evaluate the history, health, and management of the breeding colony; to identify the causes of the reproductive problems; and to work with the Livestock Guarding Dog staff to improve reproductive management of the colony. The project spanned a total of 8 weeks. I worked with the males and females of the breeding colony and performed thorough physical exams, evaluated their records for relevant reproductive history, and monitored the breeding pairs for any abnormalities during estrus and breeding. This study concludes that two of the three stud males are infertile, and that previous breeding attempts may have failed due to infertility and mismanagement of breeding pairing. I collected vaginal swabs from the breeding bitches throughout their estrus cycle for cytological estrus monitoring, and banked the samples for future reference. I evaluated male fertility via manual collection of semen, collection of semen using a teaser mount, and post-copulation vaginal cytology. I also created reproductive protocols, reproductive evaluation forms, and pregnancy tracking forms to assist the Livestock Guarding Dog management staff with estrus cycle analysis, male fertility evaluation, pregnancy and whelping management, and colony management. I then worked with the Livestock Guarding Dog staff to train them using these protocols and created visual guides to assist them in the future. Utilization of these results and implementation of these protocols will improve breeding colony management of the Livestock Guarding Dog Program, thereby further decreasing Human-Wildlife Conflict and benefitting both humans and animals alike.

Undergraduate students

74 *To Define the Role of A1 and A2A Adenosine Receptors in Oligodendrocyte Function*

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Multiple Sclerosis is an autoimmune disease of the central nervous system (CNS) in which the inflammatory immune cells migrate to the CNS and mount an inflammatory response against the myelin sheath, resulting in demyelination and pathological interference. Cuprizone is a copper chelator that removes copper form the brain. It induces neurotoxicity, that results in the death of oligodendrocytes, and the acute demyelination of axons mainly in the corpus callosum. In this study, we fed a 0.2% cuprizone diet to various strains of mice lacking or expressing different adenosine receptors (ARs) to determine the role of this toxin in A1 or A2AAR signaling in demyelination/remyelination and oligodendrocyte maturation. Our preliminary studies show that mice lacking the A1AR seem less affected by cuprizone neurotoxicity as they did not display significant sign of weight loss. This is in contrast to wild type mice and mice lacking the A2AAR that exhibit significant weigh loss by four weeks post cuprizone diet. This indicates that oligodendrocytes lacking A2 and A2AAR are differently affected by cupriozne neurotoxicity. Upon conclusion of these studies we will demonstrate the different impact of cuprizone neurotoxicity on oligodendrocytes lacking or expressing A1 or A2A AR.

75 *Precrystallization Screening of a plant Gibberellic Acid Transporter*

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Gibberellins (GA) are hormones that promote many important functions in plant development including seed germination, root and shoot elongation, flowering, and fruit patterning.[1] Accordingly the levels of Gas are tightly regulated by multiple mechanisms such as synthesis or degradation. Recently a class of Nitrate Transporter1/ Peptide Transporter family (NPF) was demonstrated to possess GA transport activity, postulating an exciting possibility that Gas might be actively delivered to the site of plant growth.[2] However, the mechanism underlying GA transport is essentially unknown, mainly due to the lack of an atomic resolution structure. Here I present a precrystallization screening of NPF proteins for structural studies. Thus far, I have screened six different subclasses of putative GA transporters using fluorescence detection size exclusion chromatography (FSEC). I found that NPF3.1 behaved well, as it was stable and monodisperse in detergents commonly used in crystallization. Importantly, purified NPF3.1 remained stable and monodisperse, suggesting that this construct is a promising candidate for crystallization.

76 *Characterizing the Multipotency of Bovine MaSCs In-Vitro*

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Mammary stem/progenitor cells (MaSC) are responsible for the repeated cycles of proliferation, growth, and physiological functions of the mammary gland. In our lab, we isolate and enrich for MaSCs by propagating primary mammary epithelial cells, freshly isolated from the animal's tissue, in non-adherent culture conditions. This method selectively allows the growth of anoikis-resistant cells, a property attributed to stem cells. MaSCs then form floating colonies termed mammospheres, which are then transferred to adherent culture and propagated as mono-layered cells. Our lab has shown that Bovine MaSCs secrete anti-microbial factors as well as support epithelial regeneration, and they may therefore prove useful as an alternative to conventional antibiotics in treating bovine mastitis. This would address current public health issues with the use of cephalosporin drugs in cows as well as antibiotics' inability to fully restore damaged tissue to its healthy previous levels of milk production. It is also believed that MaSCs play a significant role in the origination and development of mammary gland tumors. In conjunction with the current knowledge that cows only rarely develop mammary gland tumors, studying bovine MaSC may be of great interest to understanding their role in tumorigenesis. However, current knowledge on MaSCs in mammals other than humans and mice is limited.

The goal of this study is to characterize the multipotency of MaSC isolated from bovine mammary gland tissue, by analyzing their ability to differentiate into the two main lineages comprising the mammary gland: myoepithelial and luminal epithelial. This is achieved by culturing the bovine cells in a medium containing prolactin, hydrocortisone, and insulin, and analyzing the expression of lineage markers by immuno-fluorescence staining. Lineage markers included Vimentin, Smooth Muscle Actin, and Cytokeratin 14 for myoepithelial cells, and Cytokeratin 18 and Estrogen Receptor Alpha for luminal cells.

Characterizing the multipotency property of bovine MaSCs will support their study for the long term goals of: i) improving milk yield and animal health by treating bacterial infections of the gland, and ii) understanding the underlying properties behind the low incidence of mammary tumors in cows.

77 Molecular Features of an Epigenetic Regulatory Element at Rasgrf1 in Mouse

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The term "Epigenetics" refers to chemical changes in traits that regulate gene expression without changing the DNA sequence. Non-coding RNAs (ncRNA) have been shown to influence the regulation of the epigenome by regulating transcriptional activity, transposon silencing, and chromatin state. However, little is known about how factors locally (in *cis*) contribute to the establishment of epigenetic marks such as DNA methylation. It is critical to investigate epigenetic state as the epigenome influences several biological mechanisms such as cell identity, gene expression, cell growth and fertility. In the Soloway Laboratory, we study the Rasgrf1 imprinting control region, a *cis*-acting regulatory region that is methylated on the paternal allele. Previous research in the Soloway Lab has shown that a ncRNA, pit-RNA, is necessary for the establishment of DNA methylation in *cis* via the piRNA pathway at the differentially methylated domain of Rasgrf1 in mouse germ cells. Ptbp1 has been identified by mass spectrometry to interact with the pitRNA and it is hypothesized to be important for placing methylation at the locus. CRISPR/Cas9 was used to knock out Ptbp1 in germ cell derived cells (RST7Ar19) to examine its effects on the expression of pit-RNA and the establishment and maintenance of DNA methylation at Rasgrf1. Neomycin-selectable Cas9 constructs targeting the RNA binding domain of Ptbp1 (Exon 5-7) were lipofected into RST7Ar19 cells. Cells with the CRISPR/Cas9 construct were selected for with G418 and screened for a homozygous deletion at Ptbp1. Future work will include investigating the influence of Ptbp1 on the expression of pit-RNA and retrotransposons and DNA methylation at Rasgrf1.

78 *Purification of Recombinant Human Sil1 from Bacteria*

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Protein purification is the process of isolating one specific protein from others within a cell lysate, which will allow us to study the protein of interest isolated away from other cellular proteins. By purifying proteins, we can determine the biological activity that these proteins possess. We are interested in purifying and studying the activities associated with a protein found in human cells called Sil1. Our lab has discovered that the human Sil1 homolog in yeast has a (previously) unknown activity as a cellular reductase. Our main goal is to discover if the human Sil1 protein is also a reductase. A thorough characterization of human Sil1 will not only confirm a new reductase activity for these proteins across species but also will provide important information relating to human health, as mutations within the Sil1 protein are associated with the disease Marinesco Sjogren Syndrome (MSS).

Our goal is to express and purify human Sil1 from bacteria cells. Bacteria provide an inexpensive and rapid means to express large amounts of protein for purification. In order to purify human Sil1 from bacteria and to see if the human Sil1 is a reductase, the protein needs to be expressed and correctly folded to enable purification. A simple assay for correctly folded protein is whether the protein of interest is "soluble" after cell lysis. Proteins that are unable to adopt the correct fold most often pellet with the cell debris when centrifuged. The proteins that are found in the pellet are not suitable for experiments whereas proteins that are properly folded are usually found in the soluble fraction (supernatant).

Unlike yeast Sil1, when human Sil1 is expressed in bacteria it is found in the pellet of the bacteria lysate. Currently, we have been trying different approaches to increase the solubility of human Sil1 expressed in bacteria, so we can purify it. Some approaches we are currently trying are: using an autoinduction system, expression at lower temperature to increase the fidelity of protein folding, expressing different segments of the protein, and expressing the protein with different affinity tags.

Clinical Fellow

79 *Effect of macrophage phenotype on the regeneration of peripheral nerves*

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Current treatments for peripheral nerve injury produce moderate results that seldom reach full functional recovery. Emerging evidence suggests macrophage phenotype plays an important role in nerve regeneration. We hypothesized that Il4ra$^{-/-}$ mice would have lower expression of M2 associated genes and reduced nerve regeneration. Peripheral nerve transection and repair with an inert silicone conduit was performed in Il4ra$^{-/-}$ mice and their background wild-type strain (BALB/cJ). Macrophages were isolated by FACS from transected sciatic nerve at 5, 14, or 28 days after injury. Clustering and principal components analysis of RNA sequencing data from those macrophages was used to describe macrophage phenotype in injured nerve over time. Pathway analysis identified a panel of 90 genes used for Nanostring gene expression analysis to confirm findings from RNA sequencing. Nerve regeneration across the injury was investigated by retrograde labeled motor neuron counts and cranial tibial muscle weights 4 weeks after common peroneal nerve transection and repair. Our data provides the first comprehensive description of macrophage phenotype in injured nerve over time. We demonstrated an early pro-inflammatory component that declines by day 28. M2 associated gene expression was more variable. Il4ra$^{-/-}$ had a much smaller effect on macrophage gene expression than time after injury, however we demonstrated reduced expression of many M2 markers and increased expression of some M1 markers in Il4ra$^{-/-}$ macrophages compared to BALB/cJ. Despite confirming the expected effect on macrophage phenotype in Il4ra$^{-/-}$ mice, there was no effect on regenerated motor neuron counts or cranial tibial muscle weights.

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