SILS VII

Summer Institute for Life Sciences
Annual Undergraduate Research Symposium

August 9, 2017
Warren Hall
Room 175

Morning Session (AM)	
8:30-9:00	***Morning Session Registration, coffee and bagels in lounge outside Room 173. Registration will be self-serve after that. Presenters, please stop and get name tag.***
9:00-9:06	Introduction, Colleen Kearns, Associate Director of Undergraduate Research, Office of Undergraduate Biology
9:06-9:18	**The Effect of Nuclear Envelope Rupture on DNA Damage in Emery-Dreifuss Muscular Dystrophy** **Sushruta Iruvanti**[1,3], Ashley Earle[2,3], Gregory Fedorchak[2,3], Tyler Kirby[2,3], Jan Lammerding[2,3] *Lammerding Lab, [1]Department of Biological and Environmental Engineering, [2]Department of Biomedical Engineering, [3]Weill Institute for Cell and Molecular Biology*
9:18-9:30	**Toward Modeling and Analysis of Coagulation and Fibrinolysis Dynamics using Reduced Order Effective Kinetic Models** **Roanne Yehia**, Nicholas Horvath, Jeffrey D. Varner *Varner Lab, Robert Frederick Smith School of Chemical & Biomolecular Engineering*
9:30-9:42	**Developing Novel Methods and Software Tools for Visualization of Nuclear Architecture** **Zining Chen**, Abdullah Ozer, Judhajeet Ray, Astra E. Hwang, and John T. Lis *Lis Lab, Department of Molecular Biology and Genetics*
9:42-9:54	**Investigating the in vitro synergistic cytotoxic effects of rituximab in combination with chemotherapy on human non-Hodgkin B-cell lymphoma** **Brian Lee**, Tim Pierpont, Kristy Richards *Richards Lab , Department of Biomedical Sciences*
9:54-10:06	**Investigating the production and maturation of Interleukin-18 in astrocytes in Alzheimer's Disease** **Stephanie Becker**, Jenny Tzeng, Douglas Golenbock *Golenbock Lab, Department of Infectious Disease and Immunology*
10:06-10:18	**Using Immunohistochemistry to Explore Semilunar Valve Development in Mice for the Primary Antibodies: SNAIL, VE Cadherin, and Clathrin** **Brian Kauffman**, Duc Pham *Johnathan T. Butcher Lab, Nancy E. and Peter C. Meinig School of Biomedical Engineering*
10:18-10:30	**A Novel In vivo Approach to Examine DNA Damage Response Pathways in Mouse Spermatocytes** **Jenny Yang**, Jordana Bloom, John C. Schimenti *Schimenti Lab, Department of Biomedical Sciences*
10:30-10:42	***Break – coffee, juice, and bagels in lounge outside Room 173***
10:42-10:54	**Investigating the interaction between adipose stromal cells and mammary epithelial cells in a 3D spheroid system** **Yunxin Ouyang**, Lu Ling, Claudia Fischbach *Fischbach Lab, Nancy E. and Peter C. Meinig School of Biomedical Engineering*

10:54-11:06	**Impact of stromal cells and 5-azacytidine on B cell differentiation for treatment of equine common variable immunodeficiency (CVID)** **Luria Greene** , Dr. Julia Felippe *Equine Immunology Laboratory, Department of Clinical Sciences*
11:06-11:18	**Investigating Breast Cancer Stem Cells with starPEG-Heparin hydrogels** **Jason Freedman**[1], Passant Atallah[2], Siyoung Choi[1], Jana Sievers[2], Lucas Schirmer[2], Uwe Freudenberg[2], Carsten Werner[2], Claudia Fischbach[1] *Fischbach Lab, Meinig School of Biomedical Engineering*
11:18-11:30	**Defining the mechanism of melanoma initiation from melanocyte stem cells** **Jerry Zhu**, Hyeongsun Moon, Leanne R. Donahue, and Andrew White *White Lab, Department of Biomedical Sciences*
11:30-11:54	**Applications and Development of Homing Gene Drive** **Joan Chung***, **Chen Liu***, Jackson Champer, Andrew G. Clark, Philipp Messer *Equal contribution *Messer and Clark Labs, Department of Molecular Biology and Genetics, Department of Biological Statistics and Computational Biology*
11:54-12:06	**Neural circuits underlying performance evaluation in mice** **Archana Podury**, Vikram Gadagkar and Jesse Goldberg *Goldberg Lab, Department of Neurobiology and Behavior*
12:06-12:18	**Investigating the identity of an ipsilaterally-projecting excitatory interneuron connection in the neonate mouse spinal cord** **Derek Nie** *Harris-Warrick Lab, Department of Neurobiology and Behavior*

Afternoon session registration from 12:30-1:00 PM (self-serve after 1PM).
Refreshments in lounge outside Room 173.

Afternoon Session (PM)	
12:55-1:00	**Introduction, Colleen Kearns, Office of Undergraduate Biology**
1:00-1:12	**Contamination of Expressed Milk in Real Life Conditions** **Dainelle Allen**[1], Sarah Reyes[2], Anthony Hay[3], Kathleen Rasmussen[2] *[1]Department of Science and Technology Studies, [2]Division of Nutritional Sciences, [3]Hay Lab - Department of Microbiology*
1:12-1:24	**Use of plant small interfering RNAs for virus discovery in cultivated and natural plant populations** **Annika Gomez**, Juliana González Tobón, José Vargas Asencio, Keith Perry *Perry Lab, School of Integrative Plant Science, Plant Pathology and Plant-Microbe Bio.*
1:24-1:36	**OxyPonics** **Rahul Rambhatla, Ashwin Viswanathan, Yannie Mei, Raymond Zhang** *Cornell iGEM, Biomedical Engineering*
1:36-1:48	**Structural and functional characterization of bacterial methyltransferases** **Yiming Niu** *Chappie Lab, Department of Molecular Medicine*
1:48-2:00	**Cobalt(III) Schiff Base Complexes as Potential Anticancer Prodrugs** A. Paden King, **Hendryck Gellineau**, Sam MacMillan, Justin J. Wilson *Wilson Group, Department of Chemistry and Chemical Biology*
2:00-2:06	***Break – refreshments in lounge outside of Room 173***

2:06-2:18	**Genetic analysis of new mutations affecting the bone morphogenetic protein (BMP) signaling pathway in C. elegans** **Gabrielle Villafana** *Liu Lab, Department of Molecular Biology and Genetics*
2:18-2:30	**An investigation on the role of SMOC-1 in the bone morphogenetic protein pathway** **Alice Eastman**, Melisa DeGroot, Jun Liu *Molecular Biology and Genetics REU* *Liu Lab, Department of Molecular Biology and Genetics*
2:30-2:42	**The Effect of Insulin-like Pathway on Caenorhabditis Models of Huntington Disease** **Oanh Tran[1,2]**, Cheng-lin Li[1], Sylvia Lee[2] *Molecular Biology & Genetics REU* *Lee Lab, [1]Department of Molecular Biology and Genetics*
2:42-2:54	**Effect of Rim, CG32834, and btsz on Sperm Competition and CG32277 on Egg Laying in Drosophila Melanogaster** **Hoang V. Bui**, Sofie Y.N. Delbare, and Mariana F. Wolfner *Molecular Biology and Genetics REU* *Wolfner Lab, Department of Molecular Biology & Genetics*
2:54-3:06	**SEMINAL: Evolutionary, Biochemical and Genetic Approaches to Expanding the Sex Peptide Network** **Chris Wilson[1.2]**, Dr. Akanksha Singh[2], and Dr. Mariana Wolfner[2] *Molecular Biology and Genetics REU* *Wolfner Lab, Department of Molecular Biology & Genetics*
3:06-3:18	**The Effect of Chromatin Structure on Recombination Between Divergent DNA Sequences** **Marissa Baccas[1,2]**, Ujani Chakraborty[1], Eric Alani[1] *Molecular Biology and Genetics REU* *Alani Lab, Department of Molecular Biology and Genetics*
3:18-3:30	**Role of phospholipases in the localization of lysosomes** **Gabriela Casanova**, Jennifer Roscoe, William J. Brown *Molecular Biology and Genetics REU* *Brown Lab, Department of Molecular Biology and Genetics*
3:30-3:42	**Functional studies of a family of novel late-Golgi proteins** **Saeed Roschdi**, Laura Thomas, Chris Fromme *Molecular Biology and Genetics REU* *Fromme Lab, Department of Molecular Biology and Genetics*
3:42-3:54	**Arsenic Induces Degradation of Dihydrofolate Reductase** **Alyssa M. Laffitte[1]**, Denise M. Stover[2], Martha S. Field[2], Patrick J. Stover[2, 3] *Molecular Biology and Genetics REU* *Stover Lab, Division of Nutritional Sciences*
3:54-4:06	**Empirical Determination of microRNA-target interactions in the 3' Untranslated Region** **Alexander Girgis**[1,2], Ravi Patel[1], Caterina Schweidenback[1], Andrew Grimson[1] *Molecular Biology and Genetics REU* *[1]Department of Molecular Biology and Genetics*

4:06-4:18	**Investigating the Interaction Between Progranulin and Clusterin, Two Proteins Involved in Neurodegeneration** **Alexander Lacrampe**, Tuancheng Feng, Fenghua Hu *Molecular Biology and Genetics REU* *Hu Lab, Weill Institute for Cell & Molecular Biology, Department of Molecular Biology & Genetics*
4:18-4:30	**Understanding the function and regulation of the nuclear receptor RXRG in neural crest cells** **Rachel Yerden**, Marcos Simoes-Costa *Molecular Biology and Genetics REU* *Simoes-Costa Lab, Department of Molecular Biology and Genetics*

SUMMER INSTITUTE FOR LIFE SCIENCES
SEVENTH ANNUAL UNDERGRADUATE SYMPOSIUM
ABSTRACTS
August 9, 2017

Presentation Time Noted At End of Abstract
All Talks Will Be In Warren 175
Underlined Author is Presenter

Contamination of Expressed Milk in Real Life Conditions
Dainelle Allen[1], Sarah Reyes[2], Anthony Hay[3], Kathleen Rasmussen[2]

[1]*Department of Science and Technology Studies*
[2]*Division of Nutritional Sciences*
[3]*Hay Lab - Department of Microbiology*

Recommendations for human milk (HM) storage are based on studies conducted under sterile conditions, which may not be appropriate for "real-life" conditions. We conducted a randomized control trial to compare bacterial growth in HM pumped with women's own with sterile pumps. We found that HM samples pumped with women's own pumps were more likely to be considered unsafe (>104 cfu/ml; 52% own vs. 11% sterile, p=0.005) and were more likely to contain enteric bacteria (65.7% own vs. 5.7% sterile, p=0.008) than HM pumped with a sterile pump. Recommendations should consider that, even before storage, there are more bacteria in women's pump than sterile pumps. **1:00-1:12 PM**

The Effect of Chromatin Structure on Recombination Between Divergent DNA Sequences
Marissa Baccas[1,2], Ujani Chakraborty[1], Eric Alani[1]

Molecular Biology and Genetics REU
Alani Lab
[1]*Cornell University, Department of Molecular Biology and Genetics, Ithaca, New York*
[2]*Department of Chemistry and Physics, Fayetteville State University, Fayetteville, North Carolina*

Mismatch repair (MMR) proteins detect and correct DNA misincorporation errors that occur during DNA replication. A subset of these proteins is needed for heteroduplex rejection, a process which prevents genetic recombination between divergent DNA sequences. Histone deacetylases cause histones that wrap around DNA to be in a "closed" conformation which I hypothesize suppresses heteroduplex rejection. Work in the Alani lab suggests that the inhibition of Sir2 histone deacetylase increases heteroduplex rejection and that the drug nicotinamide (NAM) has a similar effect, perhaps by making recombination substrates more accessible to the rejection machinery. I hypothesize that the deacetylase inhibitor NAM inhibits the activity of Sir2 or another protein involved in the biochemical modification of histones and as a result improves rejection. To address this, I treated one set of Saccharomyces cerevisiae cells with NAM, another set lacked Sir2, a third was treated with NAM and also lacked Sir2 (Dsir2), and a fourth was a wild-type control. NAM concentrations of 2 mM, 10 mM, and 25 mM were tested. The rates of heteroduplex rejection in strain backgrounds that have either identical

or divergent DNA substrates for recombination were compared. I found the following: 1. In wild type cells, NAM increased the rate of homologous and divergent recombination, but not the overall efficiency of rejection. 2. Compared to wild type, Dsir2 strains showed elevated homologous recombination levels and increased rejection. 3. Curiously, I saw a synergistic increase in rejection in Dsir2 strains treated with 2 mM NAM. However, this synergistic increase was not seen at 10 mM NAM, and at 25 mM NAM rejection was lower than that seen in Dsir2 strains not treated with NAM. I found these results to be interesting, and possibly suggest that NAM acts on different cellular targets at different concentration levels. Future studies will involve repeating these experiments to confirm preliminary results, testing different concentrations of NAM, and examining how and why the rate of rejection decreases in Sir2 null strains as the NAM concentration increases. Understanding heteroduplex rejection mechanisms will allow us to better understand how disease causing chromosomal rearrangements occur. **3:06-3:18 PM**

Investigating the production and maturation of Interleukin-18 in astrocytes in Alzheimer's Disease
Stephanie Becker, Jenny Tzeng, Douglas Golenbock

Golenbock Lab
Department of Infectious Disease and Immunology
University of Massachusetts Medical School, Worcester, Massachusetts

Alzheimer's Disease (AD) is a neurodegenerative disease that features chronic inflammation in the brain. Interleukin-18 (IL-18) and interleukin-1 beta (IL-1β), key cytokines that are produced and secreted typically by monocytes and macrophages to induce inflammation, have been implicated in AD and unpublished data shows that at an early stage of AD, IL-18 plays a protective role. In summary, IL-18 is an important cytokine in AD progression and severity. The current study sought to determine a source of IL-18 in the brain by investigating IL-18 production in astrocytes. Previously published data suggests astrocytes are a potential source of IL-18. Therefore, astrocyte IL-18 production and maturation, as well as the inflammatory cascade upstream IL-18 maturation was observed by western blot and confocal microscopy. Data revealed that astrocytes produce pro IL-18 but not mature IL-18. However, if astrocytes are capable of producing mature IL-18 remains unknown. Preliminary data shows that they do not contain necessary inflammatory cascade components, such as active caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC), and nod-like receptor protein 3 (NLRP3). Further study is warranted to determine if and how astrocytes can produce mature IL-18 in Alzheimer's Disease. **9:54-10:06 AM**

Effect of Rim, CG32834, and btsz on Sperm Competition and CG32277 on Egg Laying in Drosophila Melanogaster
Hoang V. Bui, Sofie Y.N. Delbare, and Mariana F. Wolfner

Molecular Biology and Genetics REU
Wolfner Lab
Department of Molecular Biology & Genetics

In Drosophila melanogaster, reproduction is affected by not just male seminal fluid proteins, but also by the female genotype, which is less well known. Here we investigate the effect of female genotype on sperm competition and egg laying. Ten genes have been identified to influence sperm competition, the number of offspring sired by the first vs. second male to mate, when knocked down in female flies. Interestingly, the majority of these ten genes are heavily expressed in the nervous system. It is possible that these genes help females to actively select for the genotype of the first or second male.

Alternatively, knockdown might affect the success based on the order of mating, independently of male genotype. Here, we further investigate three genes out of the ten (rim, CG32834, and btsz). To distinguish the possibilities, the order of mating is be reversed (brown eyed male first, white eyed male second). If the proportion of offspring of the white vs. brown eyed male remains the same as observed in previous experiments, it is likely that knocking down the genes of interest affects female preference for a male genotype, instead of female preference for the first or second male to mate. Functions of CG32277, which codes for a peptidase that is highly expressed in the sperm-storage organ spermatheca, on egg laying are also investigated. This is done by knocking down gene expression and comparing the number of eggs laid to the control after mating. **2:42-2:54 PM**

Role of phospholipases in the localization of lysosomes
Gabriela Casanova, Jennifer Roscoe, William J. Brown

Molecular Biology and Genetics REU
Brown Lab
Department of Molecular Biology and Genetics

The cell biological mechanisms underlying membrane and organelle trafficking are incompletely understood. The Brown lab focuses on cellular and molecular mechanisms of intracellular membrane trafficking in the secretory and endocytic pathways. One of the main objectives is to understand the role that phospholipid-modifying enzymes (phospholipases and lysophospholipid acyltransferases) play in these processes, as they play an important role in the formation of membrane tubules from the Golgi and endosomes. Phospholipases are enzymes that hydrolyze one of the bonds in phospholipids. For example, phospholipases A2 (PLA2) cleave off the SN2 fatty acyl chain, which is often arachidonic acid, producing a lysophospholipid and free fatty acid. Recent studies suggest that the PLA2 enzyme complex platelet - activating factor acetylhydrolase (PAFAH) Ib, consisting of α1, α2, and LIS1 subunits, has a role in the distribution and function of the Golgi complex and endosomes. Moreover, earlier lab experiments have shown a relationship between overexpression of α1 and α2 and LIS1 binding and regulation of dynein in endosome and lysosome transport towards the centrosome. Also, previous experiments, with knocked out PAFAH1b cells via CRISPR, showed a similar phenotype. Although these results seem consistent, a variable that could have affected these results might have been the length of time (days) for the experiments to be completed. My project focuses on the treatment of control and PAFAH1b KO cells with rapidly acting inhibitors that have an effect in minutes. The changes in lysosome morphology and organization will be observed via antibody staining and immunofluorescence microscopy. In summary, lysosomal morphology change and organization will be observed in PAFAH1b KO cells as the result of the addition of an antagonist that will act within a short time frame. **3:18-3:30 PM**

Developing Novel Methods and Software Tools for Visualization of Nuclear Architecture
Zining Chen, Abdullah Ozer, Judhajeet Ray, Astra E. Hwang, and John T. Lis

Lis Lab
Department of Molecular Biology and Genetics

Nuclear architecture and genome organization serve a critical role in gene expression during development, normal physiology and disease states. In recent years, various Hi-C methods have been developed to understand genome-wide chromatin organization. In addition, softwares such as HiCUP, Juicer, and Juicebox have been developed to analyze and visualize the data generated by these methods. Although HiC methods provide insights about the overall organization of the genome including

Topologically Associated Domains (TADs), long-range loops, and proximity of DNA fragments, they rely on a single crosslinker; formaldehyde (FA). This results in a lack of distance information and sensitivity to detect enhancer-promoter interactions. To overcome these limitations and to better understand the Hi-C protocol, the Lis Lab is developing alternative Hi-C methods with a set of new DNA-DNA crosslinkers with predefined linker lengths, as well as modifications to FA-based HiC methods. Using the heat shock response as a model system of an altered cell condition with FA-crosslinking, we found no significant changes in the chromatin organization compared to non-heat shock conditions in Drosophila Melanogaster despite an earlier publication with contradicting conclusion. We've also analyzed the nature of crosslinked chromatin material used for various HiC methods, and specifically looked at how time and amount of sonication affected size of nuclear chunks and the size of DNA fragments using Chromatin Immunoprecipitation (ChIP) technique. We found that the width of the peaks bound by the target protein (i.e., HSF) was inversely proportional to sonication time; narrower peaks with more sonicated material. Moreover, since the available software tools were developed specifically for existing HiC methods and many are incompatible with one another, adapting these to new methods require development of new tools or repurposing existing ones. For example, I wrote a code that converts a HiCUP generated .bam file to a .hic file so that Juicebox program can be used for visualization of HiCUP analyzed HiC data. Once the aforementioned methods and software tools are in place, we will then be able to study the effects of various changes to cellular condition on the nuclear structure with better sensitivity and higher resolution. **9:30-9:42 AM**

Applications and Development of Homing Gene Drive

Joan Chung*, **Chen Liu***, Jackson Champer, Andrew G. Clark, Philipp Messer
*Equal contribution

Messer and Clark Labs
Department of Molecular Biology and Genetics
Department of Biological Statistics and Computational Biology

During normal sexual reproduction, a parent has a 50% chance of passing on a certain allele to its offspring. However, gene drive systems allow us to overcome this traditional rule, increasing the odds of a specific gene drive allele being passed on to the offspring. Successfully engineered gene drives can either cause a population crash in the target species or modify it to give it a more desirable trait, such as the ability to fight malaria. Because of this, gene drive systems have the potential to revolutionize our strategies for issues such as the prevention of vector-borne diseases and the control of crop pests. Significant strides have been made towards this goal with the discovery of CRISPR/Cas9. We designed different CRISPR/Cas9 homing gene drive constructs and studied their performance in the model organism Drosophila melanogaster. From the experiment, we determined the mechanism of our gene drives and how they form resistance alleles, which prevents the gene drive from functioning. These resistance alleles are the main obstacle that must be overcome to be effective. Additionally, we learned that a gene drive with two gRNAs performs more efficiently and reduces the formation of resistance alleles. We phenotyped several lines of gene drive flies with diverse backgrounds, and found significant differences in their efficiency, which is an important consideration when determining how a gene drive will perform in a natural population. **11:30-11:54 AM**

An investigation on the role of SMOC-1 in the bone morphogenetic protein pathway

Alice Eastman, Melisa DeGroot, Jun Liu

Molecular Biology and Genetics REU
Liu Lab
Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

The bone morphogenetic protein (BMP) pathway is a highly conserved signaling pathway with many important functions. Mutations in the genes functioning in this pathway can give rise to heart and skeletal problems as well as certain cancers in humans. In C. elegans, the BMP pathway regulates several systems including body size. which is the metric I am using to determine how a newly identified secreted protein, SMOC-1, functions in the BMP pathway. smoc-1 null mutants (smoc-1(0)) are smaller, while worms over-expressing smoc-1 (smoc-1(OE)) are longer, than wild-type worms. I have generated double mutants between smoc-1(OE) and null mutations in genes dbl-1, sma-3, lon-2 and lon-1, which encode the ligand, a cytoplasmic transducer R-Smad protein, a negative regulator glypican and a target of the pathway, respectively. I have measured the body lengths of the double mutants and compared their lengths with the corresponding single mutants and with wildtype animals. My results suggest that SMOC-1 functions upstream of the ligand DBL-1 and the negative modulator LON-2 in the BMP pathway. To determine if SMOC-1 is specifically involved in modulating the BMP pathway, I am also testing whether SMOC-1 is involved in a pathway closely related to the BMP pathway, the dauer TGF-β pathway. This is achieved by generating double mutants between smoc-1(0) mutant and null mutants in daf-7 and daf-1, which encode the ligand and type I receptor of the dauer pathway, respectively, and assessing the dauer phenotype of the resulting double mutants. Results from my experiments will help contribute to our understanding of the functions of SMOC-1 in vivo. **2:18-2:30 PM**

Investigating Breast Cancer Stem Cells with starPEG-Heparin hydrogels

Jason Freedman[1], Passant Atallah[2], Siyoung Choi[1], Jana Sievers[2], Lucas Schirmer[2], Uwe Freudenberg[2], Carsten Werner[2], Claudia Fischbach[1]

[1]*Fischbach Lab, Meinig School of Biomedical Engineering, Cornell University, Ithaca, New York*
[2]*Max Bergmann Center for Biomaterials, Leibniz-Institut für Polymerforschung, Dresden, Germany*

In 2017, an estimated 250,000 women will be diagnosed with breast cancer, and an estimated 40,000 will die from the disease, in the US alone. While 89% of women survive the disease 5 years after diagnosis, and the odds of remission are high, survival rates drop dramatically if they experience a relapse. Relapses, often more resilient and aggressive than the initial cancer, are thought to be driven by a subset of cancerous cells called Cancer Stem Cells (CSCs). CSC hallmarks include not only regeneration of heterogenic, treatment-resistant tumors, but also proliferation, progression and metastasis of breast cancer overall.
Two micro-environmental factors that have been implicated in an upregulation of CSC properties have been raised levels of the inflammatory cytokine IL-8, and the stiffness of the surrounding extra-cellular matrix (ECM). These in turn upregulate expression of the transcriptional factor NANOG, which is heavily involved in the renewal of stemness properties. NANOG is thus considered a CSC marker.
In order to study stemness levels in response to these micro-environmental factors, starPEG-Heparin hydrogels that allow for the independent tuning of local IL-8 concentration and stiffness are utilized. Local IL-8 concentration is adjusted by varying the sulfation degree of Heparin, while stiffness is modulated by varying the molar amounts of PEG in each gel. The binding abilities of gels will be assessed

by measuring IL-8 levels in tumor conditioned media (TCM) before and after gel incubation, and storage moduli of the gels are determined via rheology.
Cell stemness will be measured by culturing NANOG-GFP MDA-MB-231 cells on gels and measuring fluorescence from NANOG expression. It is our current goal to characterize and optimize the biochemical and biochemical properties of these gels for the future study of CSCs. **11:06-11:18 AM**

Cobalt(III) Schiff Base Complexes as Potential Anticancer Prodrugs
A. Paden King, **Hendryck Gellineau**, Sam MacMillan, Justin J. Wilson

Wilson Group
Department of Chemistry and Chemical Biology

Current cancer treatments lack biological specificity and lead to adverse side effects. Targeting specific environments or systems, such as tumor hypoxia, yields more selective treatments and minimizes side effects. Tumor hypoxia is a lack of oxygen in the interior of solid tumors, which creates an acidic, reductive environment. We have synthesized (bis)ethylenediamine trifluoroacetylacetone (tfac(en)) complexes of cobalt III for their potential as hypoxia active redox prodrugs. The structures of each complex have been analyzed by NMR and their reduction potentials analyzed by Cyclic Voltammetry. Their stability in solution and their biological activity will soon be evaluated. Selectivity for cancerous and hypoxic cells will be determined. In tandem, cellular uptake and membrane permeability will also be studied. **1:48-2:00 PM**

Empirical Determination of microRNA-target interactions in the 3' Untranslated Region
Alexander Girgis[1,2], Ravi Patel[1], Caterina Schweidenback[1], Andrew Grimson[1]

Molecular Biology and Genetics REU
[1] *Department of Molecular Biology and Genetics, Cornell University, Ithaca NY*
[2] *Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI*

microRNAs (miRNAs) are ~22 nucleotide sequences that influence gene expression by recruiting suppressive complexes to sites within the target mRNA transcript. Most often these regulatory sites are found downstream of the coding sequence within the 3' untranslated region (3' UTR). Computational models such as Target Scan use determinants of miRNA-target interaction such as sequence complementarity and site context to predict miRNA target sites. Through a comparative analysis of the transcriptome in cells with and without miR-1 induction, the Grimson lab has experimentally identified miR-1 regulated, post-transcriptionally repressed gene transcripts. This novel method combined cell transcriptional activity with transcriptome profiling to differentiate directly targeted miR-1 transcripts from indirect target genes. Surprisingly, several identified target transcripts do not correlate with predictive models. False positive targets, for which miR-1 interactions were predicted but not demonstrated, and unpredicted targets repressed by miR-1 have each been identified. I aim to validate targets of each type using a luciferase reporter assay. Luciferase was encoded under the regulation of each target 3' UTR of interest in a reporter construct, and subsequent luciferase expression measured in the presence or absence of miR-1 to empirically identify miR-1 interactions. To control for unanticipated miR-1 binding elsewhere within each 3'UTR, target sites of interest were mutated using site-directed mutagenesis and the assay repeated. Should expression data be consistent with previous results, the modeling algorithms which have incorrectly classified multiple miR-1 targets will require restructuring. **3:54-4:06 PM**

Use of plant small interfering RNAs for virus discovery in cultivated and natural plant populations
Annika Gomez, Juliana González Tobón, José Vargas Asencio, Keith Perry

Perry Lab
School of Integrative Plant Science, Plant Pathology and Plant Microbe Biology Section

Grapevine (*Vitis vinifera*) hosts a multitude of viruses due to the way it is cultivated; it has been vegetatively propagated for millennia. There are 64 viruses known to infect grapevine, more than any other cultivated plant. Although related grapes (Vitis sp.) grow wild across North America, little is known about viruses in these natural plant populations. In order to detect emerging grapevine viruses and to track the spread of common viruses, a virus non-specific diagnostic technology is required. Virus-specific small interfering RNAs (siRNAs) ranging from 20-25 bp in length are produced by the host in response to virus infection. In plants, they are part of the RNA interference (RNAi) pathway that defends the plant against viral infection by inhibiting expression of viral genes. Previous studies have demonstrated that sequencing of siRNAs is a powerful tool for the detection and discovery of viruses in plants, including grapevines. Virus-derived siRNAs from members of the genera *Foveavirus, Maculavirus, Marafivirus, Closterovirus, Ampelovirus and Nepovirus* have been shown to be derived from either the genomic or antigenomic strands of viral RNA. Additionally, the de novo reconstruction of complete viral genomes from siRNAs has been described for members of the families *Caulimoviridae* and *Geminiviridae*. siRNA sequencing generates several million reads, only a fraction of which are virus-derived. The pipeline VirusDetect can be used to identify sequences derived from known and novel viruses. BLASTN and BLASTX local database searches are used to identify known virus sequences, and size profile analysis of unmapped reads is used to identify putative novel virus sequences. Following analysis using VirusDetect, we have performed conserved encoded protein domain searches in an attempt to identify more distantly related viruses. In addition, we are able to map reads to more distantly related viral genomes, which will aid in the characterization of novel viruses. We have successfully applied these techniques to datasets of both cultivated and wild vines and have identified known and putative novel viruses from these datasets. Future work will aim to confirm the presence and identity of novel viruses and to analyze additional datasets to be generated from library preparations that are currently being prepared. These datasets will allow for a comparison of viruses present in wild versus cultivated grapevines.
1:12-1:24 PM

Impact of stromal cells and 5-azacytidine on B cell differentiation for treatment of equine common variable immunodeficiency (CVID)
Luria Greene , Dr. Julia Felippe

Equine Immunology Laboratory
Department of Clinical Sciences

Common variable immunodeficiency (CVID) is a heterogeneous, late onset disorder characterized by spontaneous faulty B cell development in the bone marrow and hypogammaglobulinemia. Human and horse patients have recurrent bacterial infections such as pneumonia, sinusitis, hepatitis, and meningitis. There is no cure for CVID, and the cause of CVID is unknown in most cases. Horses are the only model organism for this disease. The objectives of this research are to determine the impact of stromal cells on B cell development and to determine the impact of using 5-azacytidine ex vivo as a DNA demethylating agent to modulate B lymphocyte differentiation of hematopoietic precursor cells from horses with CVID. In the initial phase of the research project, to quantify the impact of stromal cells on B cell development, hematopoietic stem cells (HSC, CD34+) from archive equine bone marrow aspirates

were sorted and cultured with and without equine stromal cells in α minimum essential medium with cytokines. In the second phase of the research, HSCs co-cultured with stromal cells and treated with 5-azacytidine were compared to control samples without 5-azacytidine. Flow cytometry was used for cell phenotyping and to count the number of CD19+ B cells produced. The results indicated that with stromal cells, HSC organize into niches based on cell lineage. Without stromal cells, HSC do not organize and B cell differentiation does not occur. Additional trials are needed to determine if using 5-azacytidine results in a statistically significant difference in B cell production. **10:54-11:06 AM**

The Effect of Nuclear Envelope Rupture on DNA Damage in Emery-Dreifuss Muscular Dystrophy

Sushruta Iruvanti[1,3], Ashley Earle[2,3], Gregory Fedorchak[2,3], Tyler Kirby[2,3], Jan Lammerding[2,3]

Lammerding Lab
[1]*Department of Biological and Environmental Engineering*
[2]*Department of Biomedical Engineering*
[3]*Weill Institute for Cell and Molecular Biology*

The nuclear lamina is a fibrillar network of intermediate filaments, located primarily on the inner face of nuclear envelope (NE). There are two types of lamin proteins, A-type and B-type, however mutations in A-type lamins are the primary cause of diseases in humans. A-type lamins have structural and functional roles such as providing mechanical support to the nuclear envelope, participating in chromatin organization, and assisting in DNA replication and transcription. Mutations in LMNA have been shown to cause several different diseases (laminopathies) such as Hutchinson-Gilford progeria syndrome, dilated cardiomyopathy, and Emery-Dreifuss Muscular Dystrophy (EDMD) that preferentially effect mechanically active tissues.

On the macroscopic level, EDMD is characterized by muscular atrophy and loss of function. EDMD was modeled with an A-type Lamin knockout Lmna –/–, and also using point mutations LmnaH222P/H222P and LmnaN195K/N195K. Primary myoblasts were harvested from mice and grown and differentiated to maturity at 10 days. We observed weakened and ruptured nuclear lamina with evidence of protrusion of chromatin across the NE and into the cytoplasm. We hypothesize that weakened lamins break down and rupture under mechanical stress causing the release of DNA, subsequent DNA damage and ultimately apoptosis leading to muscle loss. γH2AX is one of the earliest cellular responses to double-stranded DNA damage, and acts as a coordinator for the damage response signaling pathway. To test our hypothesis that DNA damage is caused by rupture, I evaluated the presence and intensity of damage in differentiated muscle fibers in vitro at mid- and late-stages of differentiation in all three EDMD models. To confirm these findings in vivo, I isolated single fibers from hindlimb muscles and investigated the incidence of rupture and DNA damage. In vitro data shows increased levels of DNA damage and protrusion at later differentiation time points in all three genotypes. In vivo data shows γH2AX concentrated around rupture sites, providing a linkage between rupture and DNA damage. Additional studies to look at other downstream apoptotic signing, such as 53BP1 staining and activation are needed to confirm that damage is leading to apoptosis, however initial results are promising that rupture may be a contributor to the disease mechanism. **9:06-9:18 AM**

Using Immunohistochemistry to Explore Semilunar Valve Development in Mice for the Primary Antibodies: SNAIL, VE Cadherin, and Clathrin

Brian Kauffman, Duc Pham

Jonathan T. Butcher Lab
Nancy E. and Peter C. Meinig School of Biomedical Engineering

Using immunohistochemistry, we examined semilunar valve development within mice heart embryos. The significance of studying aortic and pulmonary valve formation is to help understand and treat congenital heart disease, one of the most common types of birth defects in people. It was recently thought that apoptosis or programmed cell death played a significant role in initiating these pre-valvular structures to mold from bulbous tissue into semilunar shapes via specific chemical expression along the arterial side. However, our data suggests not only that EMT or epithelial to mesenchymal transition is more so a factor in the formation of the aortic and pulmonary valve, it also similarly correlates the older findings in that the arterial side of the endocardial cushions expresses more chemical transition, rather than the ventricular side. In addition, EMT is also widely shown throughout excavation whereas before it was thought to stop prior. Our methods involved binding the antibodies: Snail, VE Cadherin, and Clathrin at the peak of excavation, using a fluorescent staining tag. The results showed that Snail was successfully expressed on the arterial side of the endocardial cushion and Clathrin was expectedly present but not oversaturated. However, VE Cadherin, a cell bridge protein, was expressed inside the cushion as opposed to the endocardial barrier only. The current mechanism for epithelial to mesenchymal transition is not fully understood in semilunar valve development. Therefore, ongoing research must be maintained. Upon application in clinical research, understanding the chemical processes of semilunar valve development can help prevent heart valve disease. **10:06-10:18 AM**

Investigating the Interaction Between Progranulin and Clusterin, Two Proteins Involved in Neurodegeneration

Alexander Lacrampe, Tuancheng Feng, Fenghua Hu

Hu Lab
Weill Institute for Cell & Molecular Biology, Department of Molecular Biology & Genetics, Cornell University, Ithaca, New York

Mutations in the progranulin (PGRN) gene, which encodes a secreted growth factor and regulator of lysosome function, are a leading cause of frontotemporal lobar degeneration (FTLD). Mutations in the clusterin (CLU) gene, which encodes a secreted chaperone protein, are associated with Alzheimer's disease. Although a large amount of research has investigated the roles of PGRN and CLU individually in these diseases, little research has investigated possible interactions between these two proteins.

Recent data collected in the Hu lab, however, suggests that CLU can bind to PGRN. In order to understand the effect CLU has on PGRN protein levels and function, we used CRISPR-Cas9 gene editing to produce a Hela CLU knockout cell line. Guide RNAs targeted near the start codon in the CLU genes were cloned into a lentiviral vector expressing Cas9, and lentiviruses were produced to infect Hela cells. Stable cell lines were selected using puromycin.

Western blotting confirmed the success of CLU ablation with one of the guide RNAs. In ongoing and future experiments, western blots and immunostaining will be used to determine if the CLU ablation has an effect on PGRN levels and trafficking, as well as on lysosome morphology. This research will provide insight into functional relationship between PGRN and CLU and the molecular mechanisms of FTLD with PGRN mutations. **4:06-4:18 PM**

Arsenic Induces Degradation of Dihydrofolate Reductase

Alyssa M. Laffitte[1], Denise M. Stover[2], Martha S. Field[2], Patrick J. Stover[2, 3]

Molecular Biology and Genetics REU
Stover Lab
[1]*Department of Biology, University of Miami, Miami, FL 33124*
[2]*Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853*
[3]*Graduate Field of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY*

Folic acid supplementation prevents neural tube defects (NTDs), a specific type of birth defect that results from failure of neural tube closure during development. Environmental arsenic exposure causes NTDs, but recent studies in mice indicate that arsenic-induced NTDs are not folic acid-responsive. Folic acid is a synthetic, oxidized form of folate that must be converted to a biologically active form of folate within the cell. This conversion requires the enzyme dihydrofolate reductase (DHFR), which preliminary data suggests may also be sensitive to arsenic-induced degradation.

This project focuses on the effect of arsenic exposure in mouse embryonic fibroblast (MEF) cell lines cultured in medium containing either folic acid or reduced folate. We hypothesize that if arsenic degrades DHFR, the cells cultured in folic acid-containing medium will be sensitized to arsenic exposure, whereas MEFs cultured in reduced folate will be protected. To test this hypothesis, MEFs cultured in each condition will be treated with arsenic-containing medium. Then, DHFR levels will be quantified using Western Blot. Furthermore, we will determine whether there is a dose-response relationship between arsenic (0, 1 uM, 5 uM, and 10 uM) exposure and cell viability, and whether viability is affected by the type of folate (folic acid versus reduced folate) in which MEF cells are cultured using an MTT assay to measure cell viability. **3:42-3:54 PM**

Investigating the in vitro synergistic cytotoxic effects of rituximab in combination with chemotherapy on human non-Hodgkin B-cell lymphoma

Brian Lee, Tim Pierpont, Kristy Richards

Richards Lab
Department of Biomedical Sciences

Non-Hodgkin lymphoma (NHL) is a heterogenous group of disorders that ranks seventh in cancer incidence and mortality. Rituximab, a CD20 monoclonal antibody, binds to B-cells and has improved survival in many NHL subtypes. It has been shown to potentiate the apoptotic response for many chemotherapeutics. The addition of rituximab to the CHOP chemotherapy regimen (cyclophosphamide, doxorubicin, vincristine, prednisolone) results in a higher complete response rate in patients compared to that from CHOP alone. Despite this marked improvement in treatment outcomes, little is known about the synergy between Rituximab and CHOP. Furthermore, the mechanism of rituximab-mediated cell death in vivo has not been fully elucidated. Therefore, identifying cooperation of any of these four drugs with rituximab would shed light on the mechanism of action of this combination

immunochemotherapy. Rituximab can induce cell death through three main mechanisms: (1) direct apoptosis, (2) antibody dependent cellular cytotoxicity (ADCC), and (3) cell dependent cytotoxicity (CDC). In this project, dose-response curves were established with the individual constituents of CHOP with or without rituximab-induced apoptosis to investigate the cooperation between rituximab and CHOP. Our findings indicate that rituximab-induced apoptosis does not potentiate CHOP cytotoxicity (cyclophosphamide has not been tested yet). Therefore, rituximab may synergize with CHOP through different concentration ratios, through cyclophosphamide only, through the ADCC or CDC pathway, or through a combination of the agents in CHOP. **9:42-9:54 AM**

Investigating the identity of an ipsilaterally-projecting excitatory interneuron connection in the neonate mouse spinal cord

Derek Nie

Harris-Warrick Lab
Department of Neurobiology and Behavior

Central pattern generators (CPGs) are relatively simple neural networks that coordinate the muscle contractions behind repetitive behaviors, such as walking. Adding neurotransmitters, such as NMDA, serotonin and dopamine, to an isolated neonate mouse spinal cord can induce "fictive locomotion," the rhythmic bursting of CPGs, in vitro. In the intact spinal cord, this is characterized by alternating bursting of flexor and extensor ventral motor roots on one side of the cord. By performing extracellular recordings of action potentials of these motor roots, it is possible to identify and record fictive locomotion.

Previous research has shown that the addition of strychnine and picrotoxin, antagonists of the inhibitory neurotransmitters GABA and glycine, induces synchronous instead of alternating bursting patterns in ipsilateral flexor and extensor ventral motor roots. By testing fictive locomotion using longitudinally-bisected spinal cords, or hemicords, the effect of ipsilaterally-projecting interneurons can be isolated because input from contralateral commissural inhibitory interneurons, which project from the other side of the spinal cord, are disrupted. I have found that strychnine and picrotoxin, applied to a hemicord locomoting preparation, can evoke synchronous rhythmic bursting between the flexor and extensor ventral roots. I have also noticed changing phase shifts between flexor and extensor root bursts, which drift modestly from pure synchrony. This proves that contralateral commissural input is not necessary to generate this synchronous activity, and implicates the existence of a currently unknown excitatory connection between flexors and extensors.

I now seek to identify the ipsilaterally-projecting interneurons that generate this synchronous activity. The V2a neurons are the major class of ipsilaterally projecting excitatory interneurons, and are identified by embryonic expression of the transcription factor, Chx10. For future experiments, I will use Chx10::DTA mice, in which the V2a interneurons are killed during the embryonic stage. Previous studies have shown that these mice are still viable for such an approach. For these experiments, I will repeat the hemicord preparation with strychnine and picrotoxin; if the flexor and extensor roots are not synchronously active, or if their bursting patterns drift markedly, this result would support my hypothesis that V2a interneurons mediate the excitatory connection between ipsilateral flexor and extensor networks. **12:06-12:18 PM**

Structural and functional characterization of bacterial methyltransferases
Yiming Niu

Chappie Lab
Department of Molecular Medicine

DNA methyltransferases (MTases) catalyze the addition of methyl groups to DNA bases. DNA methylation is critical for modulating gene expression and mediating the epigenetic landscape of prokaryotes and eukaryotes. In prokaryotes, DNA methylation is also important in distinguishing host DNA from foreign DNA, which ensures proper and efficient activity of restriction endonucleases and other bacterial defense systems. Although MTases share a conserved structural core, different enzymes have evolved distinct specificities that target either adenines at the N6 position or cytosines at the N4 or C5 position. Understanding how a MTase recognizes its substrate and exerts is catalytic function is critical for understanding how it functions in a given biological context. Several poorly characterized bacterial MTases are associated with the atypical restriction systems LlaJI and LlaI. LlaJI contains two cytosine MTases (LlaJI.M1 and LlaJI.M2) that regulate the expression of the cleavage machinery (LlaJI.R1 and LlaJI.R2). The R1 and R2 proteins are distant homologs of McrB and McrC respectively, which together form a prototypical modification-dependent restriction system. The LlaI operon, in contrast, consists of a control protein (LlaI.C), a MTase (LlaI.M), and three proteins (LlaI.1, LlaI.2, and LlaI.3) presumed to form a restriction complex. To gain insight into the molecular mechanisms of these systems, I have endeavored to express in E. coli and purify the LlaJI.R1, LlaJI.R2, and LlaI.M proteins from Lactococcus lactis for structural and biochemical studies. Preliminary efforts show low expression yields for each MTase and unfavorable interactions with numerous purification tags. LlaI.M, however, demonstrated promising purification results: the protein could be isolated and remained soluble to a final concentration was 2.0 mg/mL. Future efforts will be aimed at scaling up this procedure to obtain enough material for crystallization trials as well as optimizing expression of the other proteins. Once purified, I will also develop an assay to test the activity and specificity of each enzyme in vitro.
1:36-1:48 PM

Investigating the interaction between adipose stromal cells and mammary epithelial cells in a 3D spheroid system
Yunxin Ouyang, Lu Ling, Claudia Fischbach

Fischbach Lab
Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY

The microenvironment of breast cancer, a heterogeneous group of malignancies derived from the ductal epithelium, comprises of extracellular matrix and numerous stromal cell types, including fibroblasts, adipocytes, immune cells, etc. and is now considered as an important prognostic factor in tumor progression. Previous studies in our lab showed that obesity can promote adipose stromal cells (ASCs) to deposit stiffer extracellular matrix and contribute to increased levels of fibrotic remodeling, thereby mimic tumor associated stroma and promoting carcinogenesis (Seo et al., 2015). However, how the different types of ASCs interact with breast epithelial cells with various degrees of malignancy remains unclear and has yet to be elucidated. Therefore, the goal of this study is to characterize the growths and interactions between mammary epithelial cells and ASC cells, and to investigate how ASC cells may promote tumor migration.

We utilized a 3D in vitro platform in which cells form a multicellular spheroid to mimic tumor microenvironment. ASCs from lean (wild type, WT) and obese (ob/ob) mice were cultured individually or co-cultured with MCF10A (normal epithelial cells) and MCF10AT1 cells (pre-malignant epithelial cells) to form spheroids. The proliferation and growth rate of spheroids from each condition were tracked by DNA quantification and bright-field imaging analysis. In addition, spheroids were fixed, cryosectioned and immunofluorescence imaged on day 1, 3, and 5. MCF10A and MCF10AT1 mono-cultures become more densely packed over time, while interestingly, both their co-cultures with ASC cells develop irregular shapes with protruded structures. In addition, during their natural integration, the epithelial cells tend to form a layer and envelop the ASC cells in the core region of the spheroids. To evaluate their migratory behaviors, spheroids from each condition were imbedded into collagen gel and their spreading were tacked by bright field images as well as immunofluorescence imaging. Results indicated that spheroids containing only epithelial cells were not able to migrate at all, but they require ASC cells to exhibit sprouting behaviors. Furthermore, the imbedded co-culture spheroids form hollow-cage structures with a thick ring of sprouting cells and a small, dense core.

In conclusion, ASC cells might promote the proliferative, and invasive capacities of mammary epithelial cells and provide guidance cues for their migration. Ongoing studies evaluate the functional connections and cellular regulations of ASCs and mammary epithelial cells in their roles during tumor development and progression. **10:42-10:54 AM**

Neural circuits underlying performance evaluation in mice
Archana Podury, Vikram Gadagkar and Jesse Goldberg

Goldberg Lab
Department of Neurobiology and Behavior

Virtually all human behaviors are learned through a trial-and-error process of exploration followed by pruning of variability. For instance, with enough repetition, a tennis player learns to direct an inconsistent forehand into a reliable region of the court. While we know that practice leads to stereotyped movement patterns, the neural circuitry that drives this learning remains unclear. Behavioral models show that external rewards, such as food and juice, can drive animals to learn complex motor sequences. In nature, however, most motor learning occurs in the absence of any external reward; a reward-independent internal evaluation circuit must therefore drive motor learning. We recently showed that songbirds internally evaluate their song performance during practice, and their ongoing evaluation drives them towards a successful, stereotyped sequence of notes. This mechanism of performance evaluation is analogous to the presence or absence of an external reward: hitting the correct note evokes the same dopaminergic (DA) response as a monkey that receives juice, while missing the note drives the same DA pause that is observed when the juice is withheld from the monkey. Thus, the internal evaluator circuit modulates learning just as an external reward would. To better characterize the role of performance evaluation in motor learning, we aim to uncover the mammalian analog to this evaluation circuit.

First, we are developing a behavioral paradigm to study performance evaluation independent of external reward. We will allow a mouse to spontaneously run on a motorized running wheel while occasionally inducing cued changes in the resistance of the wheel. The change in resistance requires the mouse to utilize a different motor template to avoid tripping. On a fraction of cued trials, resistance changes will not occur, causing the mouse to trip and engage performance evaluation circuits. During

the task, we will record from DA neurons in the ventral tegmental area (VTA), a midbrain nucleus implicated in reward, using a calcium-dependent imaging technique known as fiber photometry. Cre-dependent expression in transgenic mice allows for cell-type specific access to DA neurons; viral tracing with calcium-dependent fluorophores and implanted optical fibers allow for in-vivo visualization of neuronal activity. We predict that properly cued trials will elicit increased activity of DA neurons in VTA, while improperly cued trials will lead to a DA pause, analogous to the evaluation mechanism in songbirds. Elucidating performance evaluation circuits in mammals would provide insight into how humans learn complex motor sequences. **11:54-12:06 PM**

OxyPonics
Rahul Rambhatla, Ashwin Viswanathan, Yannie Mei, Raymond Zhang

Cornell iGEM
Biomedical Engineering

Hydroponics is one of the fastest growing areas of agriculture, expected to be a $400 million market by 2020. However, hydroponic farmers still face low crop yields due to disease and nutrient imbalances. One promising solution for deep-water hydroponic systems lies in regulating reactive oxygen species (ROS), which play significant roles in processes ranging from tumor angiogenesis to plant growth to signaling. Oxidative stress in controlled amounts is well-documented to aid crop growth, boost plant immunity, and improve nutrient uptake. Currently, due to difficulty in sensing and controlling stress levels, few systems exist that optimize and provide real-time monitoring of oxidative stress. Cornell iGEM hopes to develop a novel self-regulating biopathway in Escherichia coli to offer greater versatility and sensitivity for monitoring oxidative stress in hydroponic systems. The pathway contains two parts: a ratiometric biosensor made by fusing a redox-sensitive fluorescent protein (rxRFP) and yeast peroxidase (Tsa2), and a light-sensitive downstream pathway known as the pDawn/pDusk system that controls the expression of products to adjust environmental ROS levels surrounding the hydroponic plants. Testing will include accuracy of oxidative stress control and plant biomass evaluations in presence of the genetically modified E. coli. To create a comprehensive system and deliver the bacteria to the plants, an optogenetic hardware system was designed in collaboration with Rev, a hardware startup incubator, using input from hydroponic farmers. The system has a camera which detect fluorescence intensity from the bacteria in the presence of ROS. Past a specified ROS threshold, the camera triggers LEDs to activate pDawn/pDusk light sensitive production of antioxidant molecules. This feedback system optimizes oxidative stress levels in each individual plant's local environment. End users can optimize their hydroponic systems by manipulating the threshold level of fluorescence which triggers to biochemical pathway. Respective crop yield can hence be greatly improved. In another context, the ratiometric oxidative stress pathway, and pDawn/pDusk response peptide, can be used in other applications where self-contained oxidative stress monitoring system may be needed. **1:24-1:36 PM**

Functional studies of a family of novel late-Golgi proteins
Saeed Roschdi, Laura Thomas, Chris Fromme

Molecular Biology and Genetics REU
Fromme Lab
Department of Molecular Biology and Genetics

The understanding of trafficking within the Golgi is essential to understanding how eukaryotic cells function. Through the use of model systems such as Saccharomyces cerevisiae (budding yeast) we can

gain insight into the trafficking mechanisms of eukaryotic cells which is needed for the cells to properly function. The TVP family of proteins, made up of TVP-15, 18, 23, and 38, was identified in a screen of proteins associated with late Golgi/endosomal compartments in yeast. The goal for this summer was to determine the function(s) of these proteins by creating a quadruple knockout of all TVP proteins in yeast. The reason for creating the quadruple knockout is to see if the nonessential TVP proteins share any redundant functions. The first step of this process was to create the quadruple knockout by replacing the TVP's coding sequences with selectable markers. The quadruple knockout was viable, so we tested whether loss of TVP proteins caused a growth defect and saw a lessened growth speed at 37 degrees Celsius and above. I also used fluorescence microscopy with established Golgi markers to test whether Golgi morphology is altered in the TVP mutant. Through these experiments I saw colocalization between Sec-7 and Tvp-23 and an increased concentration of Snc1 in the cells. These results give us important insight into possible functions of the Tvp proteins and verifies their functioning at the late-Golgi. **3:30-3:42 PM**

The Effect of Insulin-like Pathway on *Caenorhabditis* Models of Huntington Disease

Oanh Tran[1,2], Cheng-lin Li[1], Sylvia Lee[2]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca NY; [2]Deptartment of Undergraduate Natural Science, University of Texas at Austin, Austin TX

Huntington disease (HD) is a neurodegenerative disease caused by glutamine expansion (polyQ) in the *htt* gene that results in protein aggregations in the nerve cells. To understand the toxic effects of polyQ aggregation, *Caenorhabditis elegans* transgenic models expressing polyQ (Q0::yfp, Q40::yfp, and Q67::yfp) and exhibiting progressive motility defects have been established. Since HD is an age-dependent disease, manipulations that influence aging may affect the motility decline associated with polyQ aggregation. In *C. elegans*, the insulin-like pathway is well known to modulate longevity, metabolism, and development. Thus, analysis of the thrashing rate (BBPS) would determine whether downregulating the insulin-like pathway, which is known to prolong lifespan, can delay the motility defects of polyQ worms. Using RNAi to knockdown major components of the insulin-like pathway (*daf-2, age-1,*and *daf-16*), polyQ worms are tracked to monitor the bending rate of the treated worms. Because of the overlap in motility rate in WT, Q0, Q40, and Q67 worms at different time points, the result dictates that the insulin-like pathway has no significant effect on polyQ HD worms. Daf-2 deficient Q67 worms show relatively improved motility rate on day 1-2 in comparison to other Q67 RNAi deficiency worms. However, the data displays no significant difference which concludes that longevity does not significantly affect polyQ HD worms. Thus, this project helps to better understand how longevity modulating impact polyQ-mediated neurodegeneration. **2:30-2:42 PM**

Genetic analysis of new mutations affecting the bone morphogenetic protein (BMP) signaling pathway in C. elegans

Gabrielle Villafana

Liu Lab

Department of Molecular Biology and Genetics

The bone morphogenetic protein (BMP) signalling pathway is responsible for regulating many different developmental processes. Defects in this pathway can cause various human diseases. Thus, there are

many levels of modulation to ensure that this pathway is activated in the right way at the right level spatiotemporally. In C. elegans there is a BMP-like pathway that regulates body size, male tail patterning and mesoderm development. The Liu Lab conducted a large scale genetic screen in order to identify new genes functioning in the BMP pathway. Whole genome sequencing of 42 mutations isolated from the screen showed that there are 8 mutant strains that did not have mutations in the coding regions of known BMP pathway genes on chromosome III. I have been conducting pairwise bi-directional complementation tests for these 8 mutations in order to determine the number of complementation groups that they belong to and whether each group affects one of the five known BMP genes on chromosome III. From this we hope to identify new genes functioning in the BMP pathway.
2:06-2:18 PM

SEMINAL: Evolutionary, Biochemical and Genetic Approaches to Expanding the Sex Peptide Network
Chris Wilson[1,2], Dr. Akanksha Singh[2], and Dr. Mariana Wolfner[2]

Molecular Biology and Genetics REU
Wolfner Lab
Molecular Biology & Genetics Research Experience for Undergraduates
[1]*Reed College*
[2] *Department of Molecular Biology, Cornell University*

The seminal fluid proteins (Sfp) made by male flies are responsible for inducing several post mating responses in females. One of these Sfps, sex peptide (SP), has been shown to play a major role in several post mating responses, namely – stimulating egg laying and decreasing receptivity (meaning making the female less likely to mate again). However, it has been shown that the long-term functionality of sex peptide is dependent on the protein binding to sperm and that this event requires a complex network of Sfps, female reproductive tract proteins and sperm proteins. My goal for this summer has been to expand the currently defined SP network.
Previous members of the Wolfner lab have performed evolutionary rare covariation (ERC) and mass spectrometry studies, and have found 21 proteins to be possible involved in the SP network. By knocking-down the expression of each protein of interest using RNAi driven by GAL4/UAS, and mating males carrying this KD will wild-type females, I have been able to subsequently monitor whether the long-term post-mating responses correlated with SP-sperm binding (receptivity and egg laying) are conserved, check for the long-term retention of SP in the female reproductive tract using western blots, and identify the effects on production, transfer and processing these KD have been on previously identified members of the SP network (also using western blots). Both the receptivity and egg laying assay have been done over a period of 4-5 days in able to ensure the long-term effects of SP are being targeted. I have found that 4 of the 15 proteins I screened through this summer showed statistically significant increases in receptivity in comparison to controls (determined via fisher's exact test), indicating that these proteins may be involved in the SP network. The SP retention studies are still underway and will hopefully serve to reinforce these results. Additionally, I have performed western blots on female reproductive tracts mated with KD males 1 hour after the start of mating and probed these blots for members of the SP network that have been previously identified by members of the Wolfner lab. So far, this work has shown that one of the ERC candidates, Spn43Ab, is likely responsible for the transfer or stability of several members of the SP network in the females. **2:54-3:06 PM**

A Novel In vivo Approach to Examine DNA Damage Response Pathways in Mouse Spermatocytes
Jenny Yang, Jordana Bloom, John C. Schimenti

Schimenti Lab
Department of Biomedical Sciences
Undergraduate Researcher

DNA double-stranded breaks (DSBs) are a crucial component of meiosis and their correct repair ensures proper synapsis, crossover, and chromosome segregation events. Repair of DSBs is facilitated by p53-binding protein 1 (53BP1), which localizes to DNA DSBs generated either endogenously or exogenously. Typically, the DNA damage response (DDR) in meiosis is studied using antibody-based immunofluorescence staining. This antibody-based approach is limited to fixed samples and may not always be able to capture cellular mechanisms in vivo. To better capture DNA damage repair dynamics during male meiosis our lab has generated a transgenic mouse model that expresses the chromatin-binding domain of 53BP1 fused to a mCherry fluorophore. I am currently in the process of selecting a transgenic reporter line that best expresses the reporter construct. Preliminary results from meiotic spreads of different mouse lines reveal that some reporters express more strongly than others and that under normal conditions the reporter localizes to the sex body of pachytene-stage spermatocytes. However, if subjected to exogenous DNA damage induced with irradiation, within one hour after the DNA damage, distinguishable 53BP1 foci are observed throughout the nuclei of the spermatocytes. I have confirmed that these observed 53BP1 foci from our reporter do indeed localize to sites of DNA DSBs lesions by co-staining these cells with markers for DNA damage, including γH2AX and RAD51. In the future, we plan to use this transgenic reporter to examine DNA damage in numerous meiotic mutant animals. Through these studies, we hope to gain a better understanding of how DNA damage repair pathways operate during male meiosis in vivo. **10:18-10:30 AM**

Toward Modeling and Analysis of Coagulation and Fibrinolysis Dynamics using Reduced Order Effective Kinetic Models
Roanne Yehia, Nicholas Horvath, Jeffrey D. Varner

Varner Lab
Robert Frederick Smith School of Chemical & Biomolecular Engineering

Trauma accounts for one of the leading causes of death in the United States and is especially fatal for people 36 and younger. It accounts for 30% of the years of life lost in the US and causes an economic burden of $671 billion per year. Counteracting trauma presents a challenge when it comes to controlling a hemorrhage, especially when there are coagulation disorders in the patient. In response to this, the Varner Lab has developed pharmacokinetic models which can simulate the body based on physical and biochemical principles that describe the injury. Since this can be very complex, they have also developed reduced-order kinetic modeling tools which are both effective and simple. Prior to modeling the human body, it is essential to begin with the very essence of all beings—the cell. Understanding the biochemistry at this level will enable successful modeling of large scale systems, such as the human body. In this study, we modeled a simple batch culture experiment to predict a synthetic dataset for substrate uptake and biomass production. We wrote mole balances around the substrate and biomass, modeled as a system of ordinary differential equations. Using the programming language Julia, and Euler's method for solving differential equations, we implemented an algorithm to minimize the error between our simulations and the synthetic dataset and solve for the optimal parameter values. We then performed local sensitivity analysis on the five model parameters to determine which parameter

induced the largest effect on the biomass production and substrate intake. While there was not one parameter which had the highest impact during every time interval tested, the maximum growth coefficient had the largest sensitivity impact overall. The maintenance constant had by far the smallest effect on sensitivity. The system that we chose to study is known as the "black box" model of the cell. The only processes being considered are the ones related to uptake and growth; it does not model the internal cellular stoichiometry. To more thoroughly model intracellular processes, we will use flux balance analysis, a method that uses linear programming to solve for the fluxes of reactions occurring in the cell. This will make it possible for us to start applying pharmacokinetic models to whole body systems to better understand hemorrhage. **9:18-9:30 AM**

Understanding the function and regulation of the nuclear receptor RXRG in neural crest cells
Rachel Yerden, Marcos Simoes-Costa

Molecular Biology and Genetics REU
Simoes-Costa Lab
Department of Molecular Biology and Genetics

The neural crest is a transient structure located between the epidermis and the neural plate of a vertebrate embryo, and the neural crest contains populations of multipotent cells. These progenitors can give rise to over thirty different types of cells that form facial structures, pigmentation, parts of the gut, parts of the heart, and cartilage. Because these cells are multipotent, they have been considered for possible uses in stem cell therapies. In addition, if the neural crest forms incorrectly it can lead to various cancers and congenital birth defects. Therefore, neural crest cells are crucial to human health. Many genes and transcription factors contribute to the formation of the neural crest. RXRG is a nuclear receptor expressed specifically in the neural crest cells. It has been shown that RXRG is an essential part in the retinoic acid signaling pathway, which has a vital function in craniofacial development. However, the function of RXRG in neural crest development is not known. I will employ a variety of techniques to better understand the function of the RXRG gene in neural crest development. I will use in-situ hybridization to identify neural crest cells in which RXRG is present and to understand the timing of RXRG expression. To understand the regulation of RXRG, I will analyze the chicken genome to identify enhancers that may regulate expression of this gene by looking at open regions of chromatin that are present prior to the RXRG sequence. Moreover, by inhibiting the function of the RXRG gene with morpholinos, which will block translation of proteins, we will see the resulting effects of RXRG knockdown on neural crest development. Results from these experiments show that RXRG is crucial in neural crest development, is regulated by the chromosome regions 8.957 and 8.958, and begins to be expressed around 30 hours post-fertilization. This data is important in better understand the neural crest and its development. **4:18-4:30 PM**

Defining the mechanism of melanoma initiation from melanocyte stem cells
Jerry Zhu, Hyeongsun Moon, Leanne R. Donahue, and Andrew White

White Lab
Department of Biomedical Sciences

Melanoma is considered the deadliest of skin cancers and is responsible for the majority of skin cancer related deaths despite representing fewer than five percent of skin cancer cases. In the past 30 years, the annual mortality rate of melanoma has increased and melanoma is responsible for claiming approximately 9,000 lives in the US annually. Although recent research has focused on developing

molecular targets against established tumors, the aggressive nature and low survival rate of late stage melanoma make preventing tumorigenesis a compelling goal. Therefore, to develop effective preventative strategies in melanomas, identifying the cancer cell of origin and elucidating tumor initiation from its cellular origins are critical.

In our recent study, we found melanocyte stem cells as cancer cells of origin and delineate the environmental causes and cellular conditions responsible in early steps of melanoma initiation. To achieve our aims, we used genetically engineered mouse models of melanomas in combination with a lineage tracing method to identify the cellular dynamics and molecular mechanisms inherit to melanoma initiation. Of note, melanomas arise from tumor-competent melanocyte stem cells upon stimulation by UVB irradiation inducing melanocyte stem cell activation and translocation via an inflammation dependent process. Conversely, we further found that melanocyte stem cell originating melanoma initiation can be reduced by suppression of acute cutaneous inflammation. These findings provide a new framework for melanoma initiation and suggest potential molecular targets for preventative therapeutic applications for melanoma. **11:18-11:30 AM**