Undergraduate Research Symposium May 20, 2016 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Balcony, Easel 87

11:00 AM to 1:00 PM

Creating a Diverse Dental Pulp Stem Cells Bank

Yan Ting (Blair) Zhao, Senior, Biochemistry
McNair Scholar
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Damien Detraux, Biochemistry
Mentor: Jason Miklas, Bioengineering

Most of the tissues in our body contain adult stem cells that in theory could combat many aging phenotypes observed in human. However, these stem cells do not regenerate the tissue during aging. To understand, and in the future govern adult stem cell regenerative capacity we need to study the regulators of human adult stem cells. DPSCs are adult mesenchymal stem cells with powerful regenerative capacity that can easily be isolated from waste product, extracted wisdom teeth. Due to low cost and easy accessibility, dental pulp is a more feasible source for mesenchymal stem cells than bone marrow. DPSCs are thought to have the ability to differentiate into multiple cell lineages, and thus hold great promises in tissue regeneration. However, little is known about the molecular markers, cellular regulators, and differentiation capacity of these cells. The goal of my research project is to generate a large diversity set of DPSC to carry on a careful analysis of true common markers and differentiation capacity of these cells. At the moment I have optimized the procedures and isolated DPSCs from over 50 patients in partnership with UW dental clinic. We have now identified the molecular markers of DPSCs, and evaluated their differentiability to better understand the key criteria in the self-renewal and differentiation capacity of these cells. Western blot and flow cytometry analysis shows that most of our DPSCs have a robust expression of CD29 and CD146, both molecular markers of mesenchymal stem cells, but displaying an unexpected diversity on the levels of these markers among individuals. In differentiation tests, we also observe diversity in cartilage and adipocyte differentiation efficiency. With this unprecedented diversity set of easily accessible adult stem cells we will now identify the key components and metabolites that enhance the stemness and regenerative capacity of these adult stem cells.

POSTER SESSION 1

Balcony, Easel 88

11:00 AM to 1:00 PM

Mechanisms and Factors of Loss of Heterozygosity in Cancer

Kevin John Khoo, Senior, Biochemistry
Mentor: Nancy Maizels, Immunology and Biochemistry
Mentor: Luther Davis, Immunology
Mentor: Henry Olson, Biology, South Seattle College
Mentor: Kostantin Kiianitsa

Loss of heterozygosity (LOH) frequently occurs in tumor cells and is associated with the expression of deleterious recessive phenotypes. However, relatively little is known of its mechanisms or the various functional/regulatory factors involved. The goal of our research is to characterize the mechanisms of LOH and identify the factors involved. One cause of LOH is homology-directed repair (HDR), which uses the homologous chromosome as a donor to repair DNA damage like DNA nicks or double stranded breaks (DSBs). In HDR, the homologous chromosome functions as a template for repairing the damaged DNA strand. In order to study the mechanisms and factors involved in LOH, we developed a novel flow-based reporter model that utilizes an endogenous gene. This model was developed by making genetic modifications in a human cancer cell line (HT1080) in such a way that would allow the detection of a LOH event. Using our flow-based reporter, we will target DNA nicks and DSBs using CRISPR/Cas9 and quantify the resulting LOH events using our flow-based reporter. We will also use siRNA knockdowns to assay other candidate factors potentially involved in the mechanisms and regulation of LOH. To simulate mutations seen in tumors, we will downregulate the expression of common tumor suppressors (BRCA1, BRCA2 and PTEN) with siRNA and determine how these factors affect LOH. When common tumor suppressors are downregulated in our model, mutations that take place are expected to follow a similar mechanism to mutations seen in tumors. Using our model in this way, we will verify which common tumor suppressors are involved in the mechanisms of LOH often seen in tumors. With a better understanding of the factors involved in LOH, prophylactic therapies can be developed to minimize occurrence of LOH and tumorigenesis.

POSTER SESSION 1

Balcony, Easel 86

11:00 AM to 1:00 PM

***In-vivo* Screen for Drugs that Can Target Cancer Stem Cells Utilizing *Drosophila* Germline Stem Cell Model**

Debra Del Castillo, Fifth Year, Biochemistry
Zachary Carl (Zach) Gottschalk, Senior, Biochemistry, Physics: Comprehensive Physics
Jennifer Emily (Jenny) Merrill, Senior, Biochemistry
Deepkiran Singh, Senior, Biochemistry
Lavinia Ema Turian, Senior, Biochemistry
Randy Lu, Senior, Biochemistry
Samuel Joachim (Sam) Marbaix, Senior, Biochemistry
Dana M Rothwein, Junior, Biochemistry
Ashley Ou, Senior, Biochemistry
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Yalan Xing, biochemistry

Agressive cancers are resistant to apoptosis and stem-like cancer cells may be responsible for re-growth of cancerous tumors. This inspired this search for compounds that could aid in the destruction of cancer cells with stem-like properties. *Drosophila* germ-line stem cells under radiative or chemotoxic stress are protected by a molecular signal from apoptotic daughter cells via the TIE receptor. Thwarting this protective signal by the introduction of a small molecule may lead to the discovery of compounds that can be effective against stem-like cancer cells. The NCI Diversity Set of 1,596 small molecules was screened by undergraduate research students, in-vivo, to find compounds that can induce apoptosis in germline stem cells of *Drosophila melanogaster*. Four compounds have been found that induce apoptosis in both the male and female germ-line stem cells at a low 5 uM concentration. Future investigations will uncover the mechanism of action and efficacy against stem-like cancer cells.

POSTER SESSION 2

MGH 241, Easel 142

1:00 PM to 2:30 PM

Characterizing PICK1 in *Caenorhabditis elegans*

Samuel Keylon (Sam) Skavaril, Senior, Biochemistry
Madhuri Kasa, Senior, Biology (Physiology)
Mentor: Michael Ailion, Biochemistry
Mentor: Jill Hoyt, Biochemistry

Our project is to characterize the *Caenorhabditis elegans (C. elegans)* ortholog of *PICK1* and we will henceforth refer to this *C. elegans* gene as *pick-1*. In other systems, PICK1 is involved with dense-core vesicle (DCV) biology and has been shown to affect the trafficking of AMPA glutamate receptors to the cell membrane, which are believed to be trafficked in a non-DCV process. DCVs are a specialized organelle in neuron and neuroendocrine cells that are loaded with, and release, several important neuromodulators. These DCV-release neuromodulators regulate a broad spectrum of biological processes such as blood glucose homeostasis, the plasticity of the nervous system, and even appetite. AMPA trafficking is crucial to signaling in the brain. Glutamate is the major transmitter of excitatory signals in the brain. Our project aims to figure out if, and how, *pick-1* is affecting these processes in *C. elegans.* To investigate *pick-1's* involvement in DCV biology we tested its interaction with RIC-19 (ICA69 in mammalian systems), a protein that is known to interact with PICK1 systems. To test *pick-1*'s interaction with AMPA receptors, we tested how PICK1 affects the localization of GLR-1 (an AMPA receptor). If PICK1 does not affect the localization of GLR-1, then we would expect to see GLR-1 not properly trafficked to the synapse. If PICK1 is involved in DCV biology, we would expect a deletion of *pick-1* would create lower levels of RIC-19 in the worms. To this point, our raw data has suggested that PICK1 does not play a role in DCV biology or AMPA receptor trafficking in *C. elegans.*

POSTER SESSION 2

MGH 241, Easel 145

1:00 PM to 2:30 PM

Response of *Caenorhabditis elegans* to Hydrogen Sulfide and Hydrogen Cyanide

Corey Andrew Coombs, Senior, Biology (General), Biochemistry
Mentor: Joe Horsman, Biochemistry
Mentor: Dana Miller, Biochemistry

Hydrogen sulfide (H_2S) is an important signaling molecule in humans, however the mechanisms behind H_2S signaling and its toxicity, which is thought to be similar to hydrogen cyanide (HCN), are poorly understood. *Caenorhabditis elegans (C. elegans)* is a convenient model to study exposure to H_2S and HCN as the proteins that mediate the organismal responses are highly conserved. Our previous work has shown that low levels of H_2S can increase thermotolerance and lifespans in *C. elegans*, but at high levels H_2S has toxic effects. The disparate effects of signaling and toxicity of H_2S on *C. elegans* led us to ask how the response to HCN compares to that of H_2S. To investigate this, we exposed different *C. elegans* strains to gaseous H_2S and HCN in sealed chambers and scored the survival rates between the two gases. We see that while both H_2S and HCN are toxic to *C. elegans*, HCN leads to death at lower concentrations, suggesting different modes of toxicity. We confirmed that HIF-1, a key transcription factor in the response to both H_2S and HCN, is necessary for survival in both H_2S and HCN. To determine if there are mechanistic similarities between the responses to these gases we exposed mutants that suppress hif-1 lethality

in H_2S to both H_2S and HCN, and found that mutants that increased survival in H_2S did not necessarily increase resistance to HCN. This allows us to identify proteins that play a role in the response to H_2S. Understanding the proteins which differentially mediate toxicity in H_2S versus HCN will help us better understand the signaling roles of H_2S.

POSTER SESSION 2

MGH 241, Easel 147

1:00 PM to 2:30 PM

Regulation of the Golgi SM Protein Sly1

Tom Duan, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program,
Undergraduate Research Conference Travel Awardee
Mentor: Alexey Merz, Biochemistry
Mentor: Rachael Plemel, Biochemistry

Sec1/Munc18 related proteins (SM) are essential cofactors of SNARE–mediated membrane fusion. They function in conjunction with SNARE proteins, a family of zipper-like proteins that drive the two opposing membranes toward fusion, and other SNARE cofactors. Membrane fusion is one of the most fundamental cellular processes. However, universal mechanisms of SM function and regulation have been elusive to membrane biologists for a long time. Sly1 is an SM protein that functions at the Golgi. Here, we present genetic and biochemical results, which show how a SNARE–mediated tethering mechanism of Sly1 is regulated, and which suggest that key aspects of SM function are evolutionarily conserved across the SM family. Unlike other SMs, Sly1p contains a loop that covers a portion of domain 3a on the protein. Previous studies show that mutations at the tip of the loop can suppress requirements for the Rab GTPase Ypt1 and tethering protein Uso1. It was hypothesized that the loop serves an auto–inhibitory function. Moreover, the recent crystal structure of another SM, Vps33, shows that domain 3a serves as a binding site for the cognate R–SNARE. We performed a screen for Sly1 mutants that suppress the loss of the Golgi tether, Uso1. The new mutants, as well as limited proteolysis experiments, suggest that the entire loop swings away to reveal a SNARE binding site on domain 3a, thereby activating the tethering and SNARE assembly functions of Sly1. These experiments reveal new features of Sly1 regulation, and in addition to the results from Vps33, suggest a universal mechanism of SM–SNARE interaction during vesicle tethering.

POSTER SESSION 2

MGH 241, Easel 148

1:00 PM to 2:30 PM

Computational Design of *de novo* Cyclic Protein Homooligomers

Vanessa Thuy Anh Nguyen, Junior, Bioengineering
Mentor: George Ueda, Biochemistry
Mentor: Jorge Fallas

Protein homooligomers, multibodied assemblies built from identical polypeptide chains, comprise a large fraction of known cellular proteins. Homooligomers prove to be particularly amenable for many biological applications; they hold the potential as oligomerization domains, often have enzymatic functionality as a byproduct of their oligomeric configuration, and can serve as structural scaffolds for bionanomaterials. While there exists a multitude of protein homooligomers in the Protein Data Bank, the finite number of existing homooligomers limits the potential for custom applications. Our current work involves designing novel cyclic protein homooligomers from a set of *de novo* designed repeat proteins that share no sequence or structural homology to any known protein structures. We chose these proteins because of their modular properties, large variety of topologies, and unprecedented thermostability. Using the Rosetta software suite, we generated a set of *de novo* homooligomer models by designing the oligomeric interface to direct self-assembly into a target configuration with two to six identical chains. After a round of refinement, we will express the designs in *Escherichia coli* and purify them by immobilized metal affinity chromatography. Their oligomerization state will be validated by measuring the molecular weight in solution by size exclusion chromatography paired with multi-angled light scattering and comparing it to the predicted molecular weight of the design. Designs that exhibit the desired molecular weight will be submitted to collaborators for small angle X-ray scattering data and X-ray crystallography. The exclusive use of *de novo* proteins in homooligomer design grants a greater control over the shape and stability by nature of the repeats, thus making one successful interface design useful for a multitude of shapes and sizes. This variability opens up a wide scope of scaffolds for bionanomaterial engineering.

POSTER SESSION 2

MGH 241, Easel 146

1:00 PM to 2:30 PM

Identifying the Role of *Eve1* and *Vent* in Zebrafish Embryogenesis

Alice Xin (Alice) Dong, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: David Kimelman, Liberal Arts

Gastrulation is a stage in embryogenesis in which most progenitor cells commit to a muscle or neural fate. However, a population of bipotential progentitor cels does not differentiate during gastrulation and allows the embryos to dynami-

cally allocate neural and muscle tissue in the correct proportion as the body axis is formed. The regulation of this process is crucial for proper neuromuscular development. The maintenance of these bipotential progenitor cells requires the repression of *tbx16*, a transcription factor that progenitors express when they commit to a muscle fate. Here, we examine two genes of interest termed *eve1* and *ven*t, and investigate their roles in the regulation of *tbx16*. While previous studies relied on Morpholino anti-sense oloigonucleotides (MOs), the credibility of MOs has recently become seriously questioned because they can cause off-target effects, generating misleading effects that don't reflect genetic mutants. Revolutionary new gene-editing techonology known as CRISPR, is hailed as the new standard for gene analysis because it allows the production of true genetic mutants in specific genes. CRISPR accurately and efficiently creates small deletions in target genes, which can result in complete loss of function. Using the CRISPR method, we will generate F1 lines of zebrafish with *eve1* and *vent* mutations. Through high-resolution melt anaylsis (HRM) and DNA sequencing, we have thus far identified two *eve1* F0 founder fish. We will then test how the absence of *eve1* and *vent* affects the expression of *tbx16* during zebrafish embryogenesis using in-situ hybridizations. By analyzing how embryogenesis in normal zebrafish embryos differs from that of *eve1* or *vent* mutant embryos, we can identify the role that these genes play during development. Ultimately, our goal is to gain a better understanding of how serious birth defects and genetic disorders arise from complications in development.

POSTER SESSION 2

MGH 241, Easel 144

1:00 PM to 2:30 PM

Mechanisms of IpaH4.5, a Bacterial E3 Ubiquitin Ligase

Thomas Paul (Thomas) Schweppe, Senior, Biochemistry, English
Mentor: Peter Brzovic, Biochemistry
Mentor: Matthew Cook

In order to successfully invade and survive within a host cell, pathogens such as the bacteria Shigella flexneri have to suppress the host cell's immune response. To do this, Shigella flexneri inject bacterial effector proteins into the host cell that hamper the host cell's ability to detect and respond to invasion. One such protein is IpaH4.5, a bacterial E3 ubiquitin ligase, which hijacks the host cell's ubiquitylation machinery to transfer ubiquitin, a small signaling protein, to a substrate. Ubiquitin signaling impacts all aspects of eukaryotic cell biology. Often modification of a protein by ubiquitin serves to mark protein for destruction. IpaH4.5 targets proteins involved in the host cell's immune response, dampening the host cell's ability to respond to invasion by Shigella flexneri. I am investigating IpaH4.5 and its interactions with both host substrates and the host ubiquitin machinery. After sub cloning out the various domains of TbK1, a known substrate of IpaH4.5, I will perform ubiquitilation assays in order to determine which domain of TBK1 is polyubiquitilated, indicating that IpaH4.5 interacts with this domain. Then, this domain can be used for structural and mechanical analysis of IpaH4.5, such as determining how mutations in IpaH4.5 affect ubiquitilation through biochemical assays. Understanding how IpaH4.5 binds to and interacts with its substrates will increase our understanding of how Shigella flexneri dampens host immune response as well as help us better understand the mechanisms of bacterial E3 ubiquitin ligases. Since IpaH4.5 functions differently than eukaryotic E3 ubiquitin ligases, increasing our understanding of its mechanisms could allow us to use it as a target for future drug development. Finding new targets for drug development is becoming increasingly important with the spread of antibiotic resistant bacteria.

POSTER SESSION 2

MGH 241, Easel 143

1:00 PM to 2:30 PM

Improving SUMO: Making a Powerful Biotechnical Tool for Protein Expression Even Better

Kara Lau, Junior, Pre-Sciences
Tessa Anne (Tessa) Howard, Senior, Public Health-Global Health
UW Honors Program
Mentor: Christopher Bahl, Biochemistry

Proteins are the executors of life's most essential processes, and the ability to express and purify proteins of interest is critical to studying their structure and function. Genetic fusions to small ubiquitin-like modifier (SUMO) tags generally increase the solubility and stability of target proteins during recombinant expression. Following purification, the fusion protein can be cleaved by the highly specific SUMO protease, leaving the target protein intact and unmodified. This specificity attests to the use of the SUMO protease as a powerful biotechnical tool. The current SUMO system relies on the *Saccharomyces cerevisiae* protease Ulp1. However, this protein has considerable limitations in that it is temperature sensitive and requires detergent to maintain stability, which can interfere with downstream applications. Furthermore, Ulp1 instability often leads to incomplete digestion of SUMOylated constructs and can nucleate aggregation of target proteins. We sought to improve the current system by two orthogonal approaches. First, we mined genomic information from thermophilic organisms for homologous proteins. We have identified and experimentally characterized a novel SUMO-protease system from, *Chaetomium thermophilium*, a eukaryote that can grow in compost up to 60C. Second, we used computational protein design with Rosetta to reengineer *S. cerevisiae* Ulp1 to enhance the stability and solubility of

this protein. Using a novel fluorescence resonance energy transfer (FRET) based reporter assay that we have developed to monitor protein cleavage, we can characterize the SUMO systems and evaluate their efficacy. This assay allows for assessment and optimization of protease digestion in varying temperature, pH, and salinity conditions. These SUMO protease systems that we have uncovered and redesigned allow for the ability to circumvent many of the issues associated with the current Ulp1 system by offering an alternative, enhanced tool for the purification and expression of recombinant protein in laboratory research settings.

POSTER SESSION 2

MGH 241, Easel 141

1:00 PM to 2:30 PM

Characterizing the Genetic Complexity of Hybrid Sterility

Meenakshi (Meena) Palanisamy, Senior, Biology (Physiology)
Mentor: Michael Ailion, Biochemistry
Mentor: Piero Lamelza, Molecular and Cell Biology Program / Biochemistry

When setting up interspecies crosses between female *Caenorhabditis species 29* (*C.sp.29*) and male *Caenorhabditis nouraguensis* (*C.n.*), the resulting progeny are all dead. However, the reciprocal cross (mating male *C.sp.29* with female *C.n.*) still results in mostly dead progeny, but there are rare progeny that survive. These rare, surviving progeny can either be sterile or fertile. My research focuses on studying what causes the sterility of the rare offspring genetically. Thus, I set up crosses between female *C.n* and male *C.sp.29* and collect the resulting rare offspring to genotype them. Then I characterize these offspring based on their morphology and genetic differences such as homozygous or heterozygous type inheritance at each chromosome. The worm genome consists of six chromosomes and I am currently testing chromosome I and IV. So far, fertility is associated with only maternal inheritance at chromosome I, while sterility is associated with offspring being a hybrid (heterozygous) at chromosome I. The fertile progeny are able to survive possibly due to a process called gynogenesis. Gynogenesis is when the egg is activated by the sperm but sperm's DNA is excluded in the offspring. Some offspring exhibit a homozygous genotype at one chromosome while a heterozygous genotype at another. Therefore, if a reoccurring pattern is observed where a sterile worm has a homozygous genotype at chromosome I and a heterozygote genotype at chromosome IV, then I could hypothesize that sterility of the offspring is due to this particular pattern of inheritance. This implies that there could be more than one combination of inheritance contributing to the degree of sterility of each offspring. The main goal of the project is to investigate the complexity and the genetic requirement for sterility that is caused as result of these crosses.

POSTER SESSION 4

Balcony, Easel 102

4:00 PM to 6:00 PM

Designing Protein Oligomers and Fibers Using Parametrically Generated Helical Bundles

Sereno Luis Roberto (Sereno) Darwin Lopez, Sophomore, Pre-Sciences
Mentor: Chunfu Xu

De novo protein design can be used to create many types of biomaterials. In this project we explored if helical bundles (structural motifs consisting of several alpha helices arranged with rotational symmetry) could be used to create two different nanomaterials: fibers and oligomers. Both structures have broad industrial and experimental applications including small-molecule transport, structural support, and catalysis, so developing a strategy for specific and stable design is an important task. The oligomers (structures made from a small number of repeating units) consisted of two cylindrical layers of helices connected by short loops. The fibers were made with tapered helical bundles which docked into one another and bound strongly to form a string. Both the fibers and the oligomers were designed parametrically; we set intervals for several geometric variables and used the macromolecular modeling program Rosetta to determine which combinations of variables created the most energetically favorable shape for protein folding, as well as which amino acid sequences were most likely to fit these shapes. We then used the HBnet protocol to insert hydrogen bond networks (polar amino acid groups which form hydrogen bonds with one another to increase binding specificity). For each round of design, we chose four designs to express in E. coli based on amino acid ratios, solubility, and hydrogen bond network quality. For the fiber expression, no 6-helix bundles formed correctly, but several 5-helix bundles were successful. We have selected and expressed the oligomers and have used mass spectroscopy and SAXS (small angle x-ray scattering) data to confirm structure. In the future, we will use this design strategy to make stable oligomers and fibers with larger internal radii that can accommodate large molecules such as DNA.