Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Commons East, Easel 73

11:00 AM to 12:30 PM

Identification of Functional Interactions of an Ubiquitin Ligase Involved in DNA Damage Repair

Alyssa Kaelyn (Ally) Mueller Ponto, Senior, Biochemistry
Mentor: Rachel Klevit, Biochemistry
Mentor: Katja Dove

Ubiquitin (Ub) is a small post-translational signaling protein with roles in DNA double stranded break (DSB) repair, apoptosis, and protein degradation. The pathway for attaching Ub to a substrate involves sequential interactions of three enzymes: Ub activating enzymes (E1), Ub conjugating enzymes (E2), and Ub ligases (E3). Substrate proteins can be poly-ubiquitinated and the nature of the poly-Ub chain determines the fate of the substrate. One type of poly-Ub chains signal for a DSB repairing complex, as opposed to another type of poly-Ub chains that signal for proteasomal degradation. RNF168 is an E3 involved in the DSB repair pathway. Whereas the E3 ligase binds its substrates, in ubiquitination mechanism it is the E2 that determines the type of Ub moieties formed. There are dozens of E2s found in the human cell; therefore, it is important to study and understand the E2:E3 pairwise interactions. Currently, the published investigations of RNF168 provide varying conclusions about RNF168's relationship to specific E2s and with that its role in DSB repair. In order to remove the ambiguity, I examined *in vitro* protein interactions between RNF168 and a library of human E2s using yeast-two hybrid experiments. The Klevit Lab has investigated the process of poly-ubiquitination with other E3s, BRCA1 and RNF8, and found involvement of different E2s at different stages of ubiquitination. To further clarify the poly-ubiquitination mechanism of the RNF168:E2 pairs, I conducted similar activity assays to those done for BRCA1 and RNF8. These *in vitro* assays consist of autoubiquitination reactions, where RNF168 itself is used as a proxy-substrate to determine different types of Ub product formation utilized with different E2s. By yeast-two hybrid I have found that several E2s bound to RNF168. These interactions will be further confirmed and further tested with activity assays to determine the type of Ub modification by the different RNF168:E2 pairs.

POSTER SESSION 1

Commons East, Easel 56

11:00 AM to 12:30 PM

Identifying the ZVM10 Mutation that Causes Early Complete Blindness in *Danio Rerio*

Gail Ruth (Gail) Stanton, Senior, Biochemistry
Mentor: Susan Brockerhoff, Biochemistry

Blindness is a condition that impacts people across the globe. Some of these people are born blind due to a genetic defect, while others become blind through an accident or as the result of disease. Thus dissecting the underlying molecular causes of blindness is a critical component in improving the lives of many people. To study causes of blindness, we are analyzing disease causing mutations in the model organism *Danio Rerio*, or zebrafish. The recessive ZVM10 mutation causes very early degeneration of both rod and cone retinal photoreceptors, causing complete blindness. To study this mutation we have determined the identity of the mutated gene. I helped in this endeavor by first determining the position of the mutation within the zebrafish genome (i.e. map the mutation). This was done by mating the ZVM10 AB fish with a polymorphic fish strain. The crossovers between the different DNAs were traced, and used to identify the area homozygous for the AB allele. By continually reducing this region we specified a small group of genes that potentially were mutated. I then helped sequence these genes. A single base pair mutation that introduces a premature stop codon was identified in the gene encoding N-ethylmaleimide-sensitive attachment protein, beta (NAPB). I am currently working on a reliable genotyping protocol based on this mutation to sort mutated from non-mutated fish. The mechanism of photoreceptor degeneration in this mutant will further be studied by examining the role of this gene in wild type cells. The identification of this mutation will permit the identification of people who are blind due to this genetic mutation, through genetic screening of the patient. Finally, if this gene is also involved in human blindness, it will become a candidate for rescuing blindness through gene therapy.

POSTER SESSION 1

Commons East, Easel 74

11:00 AM to 12:30 PM

Small Heat Shock Proteins: Investigating Client Binding and Oligomer Diversity
Emily Duncan, Senior, Biochemistry
Ashwin Nitin (Ashwin) Karnik, Senior, Biochemistry, Anthropology: Medical Anth & Global Hlth, Neurobiology
Mentor: Rachel Klevit, Biochemistry
Mentor: Scott Patrick Delbecq

Proteins perform a vast array of functions within all living organisms. These functions are heavily dependent on the protein's 3-D structure. The loss of protein structure can lead to a wide variety of problems, one being the formation of insoluble protein aggregates, which can result from cellular stress. Aggregates can inhibit proper protein function, disrupt cellular homeostasis, and are implicated in many diseases. In order to combat aggregate formation, a family of proteins exists that interact with misfolded and aggregate prone proteins (clients). This family, known as the small heat shock proteins (sHSPs), delays the formation of insoluble aggregates in the cell. Their expression and activity as molecular chaperones have been seen to increase under stress conditions. However, how sHSPs delay aggregation is not well understood. We seek to better understand binding between sHSPs and their clients. Interactions between the sHSP αB crystallin and the model client $\Delta131\Delta$ (a mutant of staphylococcal nuclease) have been characterized by Nuclear Magnetic Resonance (NMR). We hope to test the observations made from this simplified system in more functionally applicable assays. sHSPs not only interact with clients, but also amongst themselves. There are ten known human sHSPs, some of which have been shown to interact with each other. While this interaction is not well understood, we do know that sHSP monomers associate non-covalently to form dimers, which in turn form higher ordered oligomers. To begin understanding these interactions, we seek to investigate whether sHSPs can exchange their monomer subunits to form heterodimers. The ability to heterodimerize would suggest an even greater diversity of oligomer structure and function. We are interested in characterizing the properties of this proposed heterodimerization. Through site directed mutagenesis, experimental based assays, and gel electrophoresis, we seek to gain a detailed description of these proteins that play such a critical role in cellular health.

SESSION 1O

HEALTH IN GLOBAL COMMUNITIES

Session Moderator: Stephen Gloyd, Global Health
288 MGH
1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Farmworker Morbidity and Mortality in Eastern Washington State
Spencer May, Senior, Biochemistry, Biophysics and Molecular Biology, Whitman College
Mentor: Jim Russo, BBMB, Whitman College
Mentor: Jennie McLaurin, Migrant Clinicians Network
Mentor: Adam Hoverman, Director, Clinical Skills I; Director of Global Health and Research, Pacific Northwest University of Health Sciences

Immigrant farmworkers have been an integral part of Eastern Washington communities for decades. Their health and well-being are essential for the health of our communities. But for many farmworkers, health care access is unavailable or limited by a lack of insurance, health providers, discrimination, and social marginalization, making it difficult to assess their health status and burden of disease. A lack of basic information about farmworker health complicates the decision-making of state and local officials, physicians, and other advocates dedicated to improving farmworker health. In partnership with the Migrant Clinicians Network and the Pacific Northwest University of Health Sciences, I used community-based research techniques to design a farmworker public health study which could provide the necessary data to inform policies to improve farmworker health. Our survey research was designed to assess the serious injuries, diseases and causes of death among farmworkers and their families.

SESSION 1T

MOLECULAR AND CELLULAR BIOLOGY

Session Moderator: Hannele Ruohola-Baker, Biochemistry
111 JHN
1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

The Role of Metabolites in Stem Cell Differentiation
Sandra Shannon, Senior, Biology (General)
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Henrik Sperber, Biochemistry

Recent reports have shown that metabolic signatures are highly characteristic for a cell and may act as a leading cause for cell fate changes. In particular, we have shown that during development from naïve to primed embryonic stem cells, the cells undergo a dramatic transition from metabolically bivalent to highly glycolytic. However, this state of inert mitochondria rapidly changes to highly potent mitochondria during further differentiation. It is not yet understood how and why the pluripotent cells enter the highly glycolytic, metabolically cancer-like (Warburg effect) stage and how a differentiating cell leaves this stage. We have now performed

metabolomics profiling of naïve and primed pluripotent cells using mass spectrometry. Interestingly, primed cells accumulate the tryptophan degradation product kynurenine. Kynurenine (KY) can act as a ligand for the transcription factor AHR. In cancer cells AHR activation by KY incudes growth while in surrounding T-cells KY based AHR activation will inhibit T-regulatory cells. My hypothesis is that kynurenine is a key metabolite in determining stem cell stage and in helping protect the primed stage embryo against the mother's immune cells. I will now test this hypothesis by analyzing the levels of the key KY metabolism enzymes by qPCR. My prediction is that the tryptophan degrading enzyme IDO1 will be unregulated in the primed stem cells. To test whether kynurenine can change the state of naïve stem cells, I will add different concentrations of kynurenine to the stem cell media and test potential cell fate changes. Since primed ESC-like metabolism is also observed in cancer cells, it is of great interest to understand how the cellular transitions in and out of this metabolic state are controlled during normal development. These studies will shed light on the function and regulation of metabolism during normal and disease developments.

SESSION 2L

CANCER MECHANISMS

Session Moderator: Michael Lagunoff, Microbiology
271 MGH
3:45 PM to 5:15 PM
* Note: Titles in order of presentation.

Error Limit in Cancer: A Novel Approach to Treating Human Malignancies
Joshua T. (Josh) Genstler, Junior, Biochemistry
Mentor: Edward Fox, Pathology

Cancers contain large numbers of mutations. This reservoir of genetic variation can be used by cancer to produce new phenotypes in response to environmental barriers. In order to accumulate these mutations we have proposed that cancers express a mutator phenotype, i.e. an increased rate of mutation. Previous results in the lab demonstrate that sequential selection of cancer cell lines (colon cancer cells HCT116 and SW620) for novel phenotypes enriches for cell lineages with elevated mutation rates. After three rounds of sequential selection for resistance to non-mutagenic drugs (diphtheria toxin, puromycin, staurosporine), both cancer cell lines exhibit an increase in mutation rate. Specifically a 3-fold for HCT116 and 6-fold for SW620 was measured. I will extend these experiments using additional selection conditions (e.g., elevated temperature, hypoxia and nutrient deprivation) and will determine mutation rate of the resulting cell lines using the PIGA gene mutation assay. The random mutation load will be characterized using Duplex Sequencing. We believe the process of sequential selection favors mutator lineages as has been observed in bacteria. However, because an increased rate of mutation is potentially deleterious to a cell, we propose the existence of a mutation limit beyond which cells are no longer viable, i.e. the cells undergo apoptosis. I will screen mutagenic nucleosides for their potential to increase the mutation rate of cancer cells beyond this threshold. Targeting cancer's error threshold may represent a novel and innovative therapeutic strategy for the treatment of human malignancies.

POSTER SESSION 3

Commons East, Easel 56
2:30 PM to 4:00 PM

Automated Protein Extraction and Analysis
Arrika LaSalle, Junior, Chemical Engineering, Biological Engineering, Montana State University
Howard Hughes Scholar, McNair Scholar
Mentor: Ed Dratz, Montana State University

Proteins usually contain many post-translational modifications in response to biological or environmental stimuli, which affect their activity, cellular localization, or protein partners. Mass spectral analysis is able to identify proteins and characterize post-translational modifications, but target proteins must first be isolated and extracted. Separation by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is a powerful protein and protein isoform separation technique. An average of 40 protein isoforms from each eukaryotic gene may be visualized with fluorescent labeling, some of which are potential disease markers. They are often present in low abundance, making it challenging to recover them for mass spectral analysis. Current methods suffer from sample losses, contamination, and long, manual proccesses. Our protein extraction system will avoid these problems using automated electrokinetic extraction with in-line protein digestion and mass spectral analysis. Nanogram levels of fluorescently-labeled Bio-Rad proteins are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Target proteins are isolated and rapidly eluted directly from 2-D gel into our micro-capillary system using a high-voltage electric field. Proteins are digested in a microfluidic bioreactor, and peptides are deposited onto a trap column of a Chip LC and resolved for mass spectral analysis. The bioreactor and trap column will eventually be coupled with the extraction system using automated switching. Our micro-capillary system is high-throughput (˜90% recovery of proteins), and 1-2 orders of magnitude faster than traditional electroelution processes. The principles of electophoretic migration may be applied not only to separation of proteins onto gradient gels, but also to routine extraction and resolution of low-nanogram protein quantities directly from 2-D gel. Automation will decrease contamination by limiting the manual portion of the

process to loading the gel onto the device, and may greatly improve recovery of low-abundance proteins.