Online Proceedings

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 Δ131Δ (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.

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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.

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.

Leidensdruck: Gender Confirming Surgical Procedures, Sociocultural Factors, Economic Issues and Quality of Life of Transgendered Individuals in Germany and the United States

Baya Walls, Senior, Anthropology, Comparative Religion
Mentor: Rachel Chapman, Anthropology

President Obama's recent inaugural speech included the call for equal rights for members of the GLBT community. Among the issues transgender people especially face are access restrictions to transition related health care. This project examines the implications for transgendered people in terms of access to gender-confirming surgical procedures and how this affects their quality of life. The sites of this study were the United States and Germany, two countries using the same parameters to determine if a person suffers from Gender Identity Dysphoria, the clinical diagnosis required to undergo gender-confirming procedures. In the U.S. these procedures are widely not covered because they are labeled elective or cosmetic. German law on the other hand mandates full health insurance coverage as surgery is considered a necessary and effective treatment of a clinically established condition. This project uses quantitative data collected with questionnaires and qualitative data in the form of life history interviews. It compares the results of the National Transgender Discrimination Study (n=6,540) with responses to the same questionnaire by transgender Germans (n=88). Findings indicate that access to transition related care via health insurance improves the mental and physical well-being of individuals whereas access limited due to lack of coverage can be detrimental. Life histories provided by five individuals, three Americans and two Germans, evaluated in open and closed coding according to the Grounded Theory approach, also indicate lower rates of depression and drug use in people after gender-confirming procedures. Yet due to the small size of the German sample these findings may not be representative. Furthermore, even though gender-confirming procedures are effective, they should not be understood as mandatory for everyone diagnosed with Gender Identity Dysphoria.

POSTER SESSION 2

MGH 241, Easel 150

12:45 PM to 2:15 PM

Utilizing Nitrification Rates to Characterize Microbial Physiology in Hood Canal

Daniel James Noteboom, Junior, Computer Engineering
NASA Space Grant Scholar
Mentor: Rachel Horak, School of Oceanography

Nitrogen (N) is an essential element of life that organisms need to make DNA and proteins. Unlike other essential elements such as phosphorous, nitrogen takes many different forms (NH_4^+, N_2O, NO, NO_2^-, NO_3, N_2) which are constantly cycling in the recently revised oceanic nitrogen cycle. One group in the Archaeal domain of life, ammonium-oxidizing Archaea (AOA), has recently been discovered to be a key player in the oceanic nitrogen cycle. In this cycle, Archaea oxidize ammonium (NH_4^+) into nitrite (NO_2^-) in a process called nitrification. We hypothesized that different abiotic factors such as copper, light, and temperature regulate the physiology and depth distribution of the AOA. To test our hypothesis, we conducted $^{1}5NH_4^+$-oxidation experiments in July 2012 aboard the R/V Clifford A. Barnes in Hood Canal, Washington. Samples of seawater were filled in bottles, spiked with $^{1}5NH_4^+$, and incubated for 10 hours. We measured the oxidation of the $^{1}5N$ isotope using mass spectrometry, and compared the rates for control treatments to rates for treatments amended by copper (Cu), light, and other factors. $^{1}5NH_4^+$-oxidation rates were consistently low at the surface, increased until 30-40 m, and decreased to 115 m depth. Our experiments clearly showed light inhibition of $^{1}5NH_4^+$-oxidation, but the data did not clearly determine whether sunlight-produced peroxides inhibited AOA. Copper additions in Cu-deplete regions increased nitrification, possibly because the key enzyme in ammonium oxidation (amoA) is Cu-dependent. TETA, a chelator that binds Cu, did not show any effect when added during incubations. All together our results suggest that Archaea are Cu-limited, but may store Cu in small amounts. This project is part of a larger interdisciplinary effort to understand the oceanic microbes and the impacts they have on biodiversity and greenhouse gases such as N_2O and CO_2. Future studies in 2013 will extend these experiments to the open ocean.