UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

SESSION 1Q

BIOLOGICAL STRUCTURE AND FUNCTION

Session Moderator: Matt Kaeberlein, Pathology
JHN 022
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Rigid Uso1 Protein Construction and Testing for Tethering Mechanism
Weisha Liu, Senior, Bioengineering
Mentor: Alexey Merz, Biochemistry

Membrane trafficking in the eukaryotic cell is a highly controlled and significant process that is related to various inherited disorders and cancers. Among numerous regulatory proteins, Uso1 protein – an essential, long, coiled-coil protein – plays a key role in the tethering process, capturing and pulling the vesicle toward the target membrane. Despite many years of work, the tethering mechanism has not been fully understood. Although several tethering models have been proposed, none of them were tested with Uso1 protein. Hence, to test these models, we engineered a mechanically deficient version of Uso1 protein, which lacks the critical tethering region for functioning. After the protein characterization, in cell survival tests and in test tube tests in chemically defined fusion system have been performed. The failure of the trafficking event, which would support our hypothesis, along with other functional test data, would provide a promising demonstration for the tethering mechanism of the long coiled-coil tether and the role of Uso1 protein.

POSTER SESSION 2

Balcony, Easel 89
1:00 PM to 2:30 PM

Genetic Analysis of the AP-3 Protein Complex
Malia Clark, Junior, Biochemistry
Mentor: Alexey Merz, Biochemistry
Mentor: Rachael Plemel, Biochemistry

My research project involves the AP-3 (adaptor protein) complex, which plays a key role in membrane trafficking cells. Within all eukaryotic cells, there are membrane-bound sections of the cell that interact with each other in various ways to drive the cell's function. Vesicles mediate the transport of proteins and lipids among cellular organelles. These vesicles are created in various ways by proteins throughout the cell that form a "coat" around the vesicle as it travels to its destination. AP-3 is a protein complex that mediates vesicular transport from the Golgi apparatus to the lysosome. The current aim of my project is to analyze evolutionarily conserved features of the AP-3 complex by mutating subunits of the complex and observing resultant phenotypes in our model organism, *Saccharomyces cerevisiae* (baker's yeast). In doing so, we utilize a gene reporter system called GNSI to analyze AP-3 function in different genetic strains. This reporter system is mainly analyzed through a colorimetric assay (chemical test to determine components), using qualitative observations of the intensity of colored halos around yeast colonies on a gel plate. We also use fluorescence microscopy by using signals from the Green Fluorescent Protein (GFP) to determine whether AP-3 was successful in trafficking to the lysosomal vacuole. So far, our results have shown that targeted truncations of proteins within the AP-3 subunit Apl6 yielded loss of function in trafficking. We are continuing our analyses by focusing on other proteins within AP-3. These basic studies will further our understanding of membrane trafficking and may provide insight into diseases linked to AP-3 function, including HIV-1 particle assembly and human genetic disorders.