UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

Poster Session 1

Commons West, Easel 13

11:00 AM to 12:30 PM

Exploring the Genetic Basis of Thermostability in the RNA Virus phi-6

Kimber Clementine (Clementine) Dunnell, Senior, Biology (Ecology, Evolution & Conservation)
Emily Hsieh, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)
Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Benjamin Kerr, Biology
Mentor: Sonia Singhal, Biology

Thermostability is the capacity of an organism to survive and thrive at high temperatures and is a characteristic with many biotechnological applications, such as food processing. We are seeking to understand the mechanisms of thermostability in viruses by using an RNA virus, phi-6 Cystovirus. We are evolving the phi-6 virus to become thermostable by exposing it to a target temperature of 61C over many generations (wild type viruses grow at 25C). In order to see if exposure to different intermediate temperatures affects the genetic pathway to thermostability, we will expose the phi-6 virus to different temperature regimes prior to reaching the target temperature. The temperature treatments are sudden, where the virus is exposed to the target temperature throughout the entire course of the experiment, moderate, where the virus is exposed to the target temperature halfway through the experiment, and gradual, where the virus is exposed to the target temperature on the final day of the experiment. The genome of phi-6 has already been characterized, which enables us to compare the genome of the evolved, thermostable phi-6 viruses to the ancestor and allows us to study the genetic basis of thermostability. In future studies, we hope to use these thermostable viruses to learn about costs and benefits of maintaining thermostability.

Session 1R

Synthetic Biology and Molecular Biotechnology

Session Moderator: Daniel Ratner, Bioengineering

022 JHN

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Social Dynamics of a Synthetic Cooperative *E. coli* System

Melissa Delaine (Melissa) Arnold, Senior, Biology (General)
Mentor: Benjamin Kerr, Biology
Mentor: Sonia Singhal, Biology

In this project, we explore conditions that are critical for *de novo* evolution of cooperation and altruism in a bacterial system. Previous theoretical work has shown that there is a competitive advantage to defection and selfishness, but the ubiquity of cooperation in nature suggests that cooperation may be adaptive. Here, we aim to uncover environments and genetic conditions that actually promote greater cooperation. We use an engineered cooperative strain of *Escherichia coli*. Through a bistable genetic switch, single cells have the capability to be either a producer cell that makes the cellulase enzyme to break down cellulose, or a consumer cell that eats the byproducts of cellulose breakdown. Our first aim is to characterize the social dynamics of this synthetic system and determine if a social dilemma is in fact occurring—i.e., is there a cost to producing cellulase, and can cheaters that do not produce cellulase displace the cooperators that do? Our second aim is to evolve the system over many generations and analyze how it changes. Uncovering the conditions favoring higher cooperation (e.g., greater production of cellulase) provides insight into how cooperation can evolve and how populations circumvent social dilemmas. We hope to use evolution as a tool to tune the synthetic genetic circuit for maximal cellulose breakdown. On a broader scale, these conditions that favor cooperation may have practical applications in waste degradation and biofuel production.

Poster Session 2

Commons East, Easel 75

12:45 PM to 2:15 PM

A Novel Approach to Show the Mechanism of Artemisinin Anticancer Effect

Ho Wing (Andy) Chan, Sophomore, Bioengineering
Mentor: Narendra Singh, Bioengineering

Artemisinin (ART) is a sesquiterpene lactone (three rings chemical structure), derived from the plant Artemisia annua. Several studies have shown significantly higher cytotoxicity to cancer cells compared to normal cells. However, the mechanism of this is still in debate. The majority of researchers claim that ART reacts with intracellular iron in cancer cells to generate free radicals (species that contain unpaired valence electrons) which are responsible for the cytotoxicity. A recent finding showing ART inhibits cancer cell growth through inhibiting a protein has raised questions about the free radical mechanism for ART to inhibit growth of cancers. We hypothesize that the endoperoxide bridge (two oxygen atoms joined by single bond in a ring structure) in artemisinin forms free radicals by reacting with intracellular iron in cancer cells. To confirm our hypothesis, Molt-4 human leukemia and MDA-MB-231 human breast cancer cell lines will be treated with Deferoxamine (DX) along with Artemisinin. DX is an iron chelating agent, a chemical that could eliminate the iron in cancer cell. Our research shows that the cytotoxicity of ART is DX concentration dependent, lower concentrations (10 μM to 30 μM) reduce the cytotoxicity while higher concentrations (50 μM to 100 μM) increase it. Thus, our results show that lowering iron (to certain levels) by DX inhibits the cytotoxicity of ART. Furthermore, we have also performed experiments about the effect of phenyl-alpha-tert-butyl nitrone, PBN, an antioxidant, on ART anticancer mechanism (data not published). It is our understanding that antioxidant could inhibit the ART anticancer effects. After comparison, we found that the inhibitory effect of DX on ART cytotoxicity is the same as that of PBN. In conclusion, our preliminary results show that iron is necessary for the cytotoxicity of ART and it is free radical mediated.

POSTER SESSION 4

MGH 241, Easel 149

4:15 PM to 5:45 PM

Characterization of Dihydroartemisinin Resistant MOLT-4 Cells

Ross Daniel Jones, Senior, Bioengineering
Mary Gates Scholar
Mentor: Narendra Singh, Bioengineering

Drug resistance is a growing problem in the fights against cancer, malaria, and various bacterial and viral pathogens. Recently, strands of *Mycobacterium tuberculosis* in India, Iran, and Italy have been found to be resistant to nearly all effective drugs, highlighting the need for drug resistance research. The anti-malarial drug artemisinin and its derivatives have been shown to exhibit cytotoxicity and high selectivity in targeting various types of cancer. We selected a strain of MOLT-4 leukemia cells for resistance to the artemisinin derivative dihydroartemisinin (DHA). The project goals were to characterize these cells by analyzing specifics such as growth rate, genetic expression, DNA repair ability, and cell viability across various conditions. Cancer stem cells are known to be involved in drug resistance, thus we hypothesized that the resistant population would contain a higher percentage of stem cells. Techniques employed include the single cell gel electrophoresis assay (Comet Assay), suspension cell culture, and immunoglobulin assays. When applicable, cell samples were counted using a hemocytometer, and statistical tests were conducted using GraphPad Prizm software. We have currently found the resistant cells to exhibit a five times greater LD50 to DHA than the control MOLT-4 cells, a slower growth rate, and the ability to grow in media containing 12.4 μM DHA – an otherwise cytotoxic concentration. Results suggest the mechanisms of resistance include a thicker cellular membrane and improved DNA repair ability, but we have yet to determine whether cytoplasmic iron requital and DHA effusion play a part as well. We expect to find elevated levels of aldehyde dehydrogenase in resistant cells, strongly indicating the presence of stem cells. The information found will be useful for cancer and malaria drug therapy design, and will further the connection between cancer stem cells and drug resistance.