SESSION 1C

MOLECULAR CONTROL OF THE CELL

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 171
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

CRISPR–Cas–Mediated Chemical Control of Transcription in Yeast
Brianna Lee Fernandez, Senior, Biochemistry
Mary Gates Scholar
Mentor: Jesse Zalatan, Chemistry

Synthetic CRISPR–Cas transcription factors enable the construction of complex gene expression programs, and chemically–inducible systems allow for precise, rheostatic-like control over the transcriptional dynamics. We have constructed a bio-orthogonal, chemically–inducible synthetic CRISPR regulatory system to activate and repress gene expression in yeast. By fusing chemically inducible transcriptional regulators to specific RNA binding proteins, we have expanded the tunability of this system of constructs. The RNA binding proteins are fused to half of one chemically-inducible system while the other half is fused to an effector. Upon addition of drug, the two halves come together to form the complete chemically-inducible system and either activate or repress the target gene. We use reporter gene assays to probe the dose-dependence, time-dependence, and reversibility of the systems. The use of multiple, orthogonal chemically-inducible systems and unique guide RNAs allows for more sophisticated, multi-gene programs that still maintain precise control of gene expression dynamics independently at different sites.

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Engineering DNA Loops Using a Protein Switch DNA Sensor
Kieran Elliott Lewis, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Jesse Zalatan, Chemistry

New techniques in genome mapping have revealed a high degree of 3D spatial organization in the nucleus. Long-range loops connect enhancers to their gene targets to regulate expression. In order to assess the mechanisms and functions behind 3D spatial organization of the nucleus we need a system that allows us to engineer DNA loops. We use programmable CRISPR-Cas DNA binding domains to target specific sites in the genome. The CRISPR-Cas complex is tethered to a targeting domain (TD) that can dimerize with another TD at a distant DNA locus. To promote interactions between TDs that are bound to DNA, we have designed an allosteric switch that assembles the TD only when the CRISPR-Cas complex has engaged its DNA target. To validate that our switch proteins can act as a DNA sensor we have developed a simple reporter assay; upon successful switch protein activation on DNA a transcription factor is recruited to drive expression of a fluorescent protein. Our results indicate that the protein switches activate when they are recruited to DNA, effectively acting as a sensor for DNA binding. Future steps include optimizing our reporter assay design and modifying the design for DNA looping.

POSTER SESSION 2

MGH 241, Easel 163
1:00 PM to 2:30 PM

Identification of Abiotic Stress Responsive Genes in *Zea mays* (Maize) Dependent on MOP1-mediated Epigenetic Regulation and the Plant Hormone ABA
Rachel Christine Calder, Senior, Biology (Bothell Campus)
Mary Gates Scholar
Mentor: Thelma Madzima, STEM - Biological Sciences
Mentor: Jesse Zaneveld, STEM, Division of Biological Sciences, University of Washington Bothell

Zea mays (maize, corn) is an essential crop plant; important to global agriculture and the U.S. economy. However, maize productivity and yield can be drastically affected by abiotic

environmental stress. Therefore, a priority for many plant breeding programs is to select for crops displaying phenotypic traits of enhanced tolerance to abiotic stress. A subset of abiotic stresses induce the plant hormone, abscisic acid (ABA). The mediator of paramutation1 (mop1) gene encodes an RNA-dependent RNA polymerase that functions in the RNA-directed DNA methylation (RdDM) pathway. The mop1-1 mutation results in the loss of DNA methylation which in turn causes a variety of genes to be expressed abnormally. We determined how a mutation in a mop1-1 affects RNA expression under abiotic stress by conducting a computational analysis of multiple RNA-seq datasets of stress-treated maize seedlings. We compared RNA-seq data from mop1-1 and WT seedlings treated with exogenous ABA control (no ABA treatment) with a publicly available dataset of WT maize plants treated with heat, cold, drought, salinity, and control (no stress treatment). Genes commonly down-regulated in the four stresses and in MOP1 WT ABA, but up-regulated in mop1-1 ABA represent genes potentially silenced under stress that require MOP1 for gene silencing. The presence of these genes in the given stress treatment allows us to identify the abiotic stress responsive genes that require ABA and MOP1 mediated regulation.

POSTER SESSION 4

Balcony, Easel 109

4:00 PM to 6:00 PM

Identifying Sites Under Positive Selection on Influenza Hemagglutinin

Jonathan Charles (Jon) Mah, Senior, Microbiology, Biochemistry, Applied & Computational Mathematical Sciences (Biological & Life Sciences)

Mary Gates Scholar

Mentor: Jesse Bloom, Division of Basic Sciences

Mentor: Sarah Hilton, Genome Sciences

The influenza virus is known for its rapid evolution, or the ability to fix many mutations over a short period of time. Some of these mutations lead to amino acid substitutions in regions of the virus targeted by the immune system. Such changes are often selected for because they confer a fitness advantage by allowing the virus to ”escape” immune response. This pattern of repeated immune escape is a detriment to public health because it necessitates an annual update to the influenza vaccine. Therefore, identifying sites on the influenza virus which are targeted by the immune system could help predict which influenza strain will circulate in the future, inform vaccine design, and help understand basic evolutionary questions. Using molecular phylogenetic techniques, we can identify sites potentially targeted by the immune system by looking for ”positive selection”, a phenomenon which manifests as a higher than expected rate of evolution. To identify sites evolving faster than expected, we defined a null expectation of the evolutionary rate of influenza in the absence of immune pressure. This null model is defined using empirical measurements from a high-throughput functional assay known as deep mutational scanning. This null model differs from traditional phylogenetic models in that it describes the constraints on influenza on a site-specific basis and, as a result, has been shown to be a more accurate and powerful null model. I have implemented an empirical Bayes approach to identify sites which deviate from the null model by an unexpectedly high evolutionary rate, suggesting positive selection. Preliminary results show that my method outperforms other methods for identifying sites under positive selection. Next, I will apply these methods to the influenza virus surface protein, hemagglutinin, which is a major target of the immune system.