Online Proceedings

Poster Session 2

MGH 206, Easel 174

1:00 PM to 2:30 PM

Determining the Genes of an Essential Replication Complex that Are Involved in Evolutionary Divergence

Navinder (Navin) Gurmit Singh, Junior, Biology (Molecular, Cellular & Developmental)
Mentor: Maitreya Dunham, Genome Sciences
Mentor: Bryce Taylor

DNA stores genetic instructions that are required to program cell activities. Therefore, it is important for DNA to replicate in order to provide a copy of genetic material to daughter cells during mitosis or meiosis. Origin recognition complex (ORC) is a six-subunit assemblage that promotes the initiation of DNA replication. ORC proteins bind in all eukaryotes to a specific sequence found within an origin of replication to assemble and bring together other key initiation factors. Although the ORC proteins themselves are highly conserved throughout evolution, dramatic changes in the origin DNA sequence motif have been observed across species. These changes are most likely accompanied by changes in one or more subunits within the ORC complex. The aim of this project is to understand how changes in the ORC's binding preferences across species are determined by changes in ORC protein sequences, specifically within the yeast phylogeny. Using genetic engineering approaches, I will introduce foreign ORC alleles into knockout strains of *Sacchoromyces cerevisiae* which we have generated. Previous data generated by members of our lab and others show how ARS (autonomous replication sequence) Consensus Sequences (ACS, the binding site for ORC) differ across the yeast species. The data indicate that *Lachancea kluyveri* and *Lachancea waltii* have intermediate levels of ACS differentiation from *Sacchoromyces cerevisiae*, so I am introducing alleles from these species as a starting point. Differences in the cell growth rate, size, and fitness will be observed within haploid strains of *Sacchoromyces cerevisiae* containing the foreign ORC protein in order to determine which ORC subunit(s) is/are responsible for binding preferences for different ACS sequences within the yeast phylogeny. This research can provide a fundamental understanding of evolution of the genome sequence and the proteins that bind it.

Session 2S

Modulation of Cell Behavior and its Components

Session Moderator: Valerie Daggett, Bioengineering

JHN 175

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Determining the Contributions of Genetic Modifiers to Complex Cell Aggregation Traits in *Saccharomyces cerevisiae*

Kolena Dang, Senior, Biochemistry
Mary Gates Scholar
Mentor: Maitreya Dunham, Genome Sciences
Mentor: Elyse Hope, Genome Sciences

Model yeast *Saccharomyces cerevisiae* normally grows in the lab as isolated cells but can evolve the ability to clump, protecting the cells from stress factors including antimicrobial treatments. Clumping can occur either due to a failure of cell separation during division or through physical cell adhesion ("flocculation"). Understanding the genetics behind these traits could give us insight into how microbes develop antimicrobial resistance. Previously, we evolved *S. cerevisiae* from a non-clumping ancestor strain for 300 generations in miniature chemostats, multiplexed culture devices that maintain a constant environment. Using a combination of whole genome sequencing and bulk segregant analysis, a pooling and sequencing approach, we identified causal mutations in 23 independent, clumping clones from the evolution experiments. We identified two strains with cell separation defects which had mutations in both budding gene *BEM2* and cell separation gene *ACE2*. In the remaining clones, the majority of the causal mutations affected the regulation of known flocculation gene *FLO1*. Despite this shared mutation, a number of these clones exhibited varying flocculation phenotypes. We hypothesized that other "modifier" mutations might also be contributing to the complexity of the phenotype. For one strain with unusual cell morphology, targeted sequencing of candidate modifier genes revealed that the modifier mutation is either in the *IRA1* or *HSL7* gene, which are genetically linked. The goal of our current experiments is to determine the contributions of *BEM2* and *ACE2* to the separation defect phenotype and of *HSL7* and *IRA1* to the flocculation pheno-

type. We are using a method called complementation testing, in which the wild-type gene is added to the mutated strain using molecular cloning techniques to see if the mutant phenotype is repaired. This will enable us to identify which genes are contributing to the complexity of these traits and facilitate a better understanding of their underlying mechanisms.