Online Proceedings

SESSION 2B

GENETIC EVOLUTION ACROSS THE LIFESPAN

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems
228 MGH
3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

The Effects of Human Paralogs and Allelic Variants of Yeast Bim1 on Signaling in the *Saccharomyces cerevisiae* Pheromone Response Pathway

Derek Michael (Derek) Britain, Senior, Bioengineering, Biochemistry
Levinson Emerging Scholar, Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Roger Brent, Division of Basic Sciences, Fred Hutchinson Cancer Research Center

The three mammalian MAPRE proteins play important roles in the microtubule dynamics of the cell cytoskeleton, and facilitate the binding of multiple +TIP proteins to microtubule plus ends. Work conducted by the 1000 Genomes Project, the Exome Sequencing Project, and many other groups has led to the identification of non-conservative coding sequence allelic variants of the MAPRE proteins in the human population that result in the production of mutant MAPRE proteins. Disruption of MAPRE protein function could decrease cell signaling fidelity (how well a cell receives and transmits information) and microtubule function. The MAPRE proteins are related by descent from a common ancestor to the Bim1 protein in budding yeast. Here I designed and constructed mutant versions of Bim1 that contained mutations corresponding to known MAPRE variants and expressed them in yeast. The yeast pheromone response pathway was utilized as a model signaling system to test the effects of the MAPRE variants on cell signaling. The output of the pheromone response pathway, and therefore amount of signal received and transmitted by the cell, was quantified using fluorescent reporters under the control of a pheromone responsive promoter in yeast strains expressing the mutant versions of Bim1. By comparing the pheromone pathway output via fluorescence intensity of the mutant MAPRE strains to the output of the wildtype MAPRE strains, I was able to discern effects of the Bim1 mutants on cell signaling. Deletion of Bim1 resulted in increased variation in the pheromone pathway output. Also, a mutant MAPRE allele consisting of a C-terminal domain deletion resulted in diminished pathway output and increased output variation. Adverse effects on cell signaling could result in poor decision making and environmental response, potentially leading to the generation of disease. By better understanding the effects of allelic variants in the human population, we will be able to develop individualized health plans and therapies.

SESSION 2B

GENETIC EVOLUTION ACROSS THE LIFESPAN

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems
228 MGH
3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

Do Centromere-Like Regions (CLRs) Regulate DNA Replication Timing?

Seungbeen (Steven) Lee, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Bonita Brewer, Genome Sciences

Chromosomal DNA synthesis begins at origins of replication, with some origins activating earlier than others during S-phase. Neither the mechanism by which origin activation time is determined nor the biological significance of this temporal program is fully understood. Interestingly centromeres, sites where kinetochore complexes form to correctly separate chromosomes during mitosis, replicate early. In the budding yeast *Saccharomyces cerevisiae*, centromeres actively promote their own early replication by advancing the activation time of their neighboring origins. We previously showed, in cells lacking the mitotic checkpoint, an artificially delayed, late replicating centromere causes a dramatic increase in chromosome instability. In this study, I am investigating the function of centromere-like regions (CLRs) in *S. cerevisiae*. These sequences can bind the centromeric histone (Cse4), especially when *CSE4* is overexpressed. Under this condition, additional kinetochore proteins can build up and CLRs take on some of the properties of functional centromeres. Because

the kinetochore protein Ctf19 recruits a replication initiation factor, I hypothesize that the centromere-mimicking DNA sequences are also capable of recruiting the kinetochore protein associated with early replication to advance the initiation time of nearby origins. To test this possibility, I will examine the effects of the CLR on replication origin activation using a plasmid that contains two identical origins. I will clone a CLR next to one of the origins and determine whether this origin is now the earlier activated origin on the plasmid when *CSE4* is overexpressed. The CLR I will use resides in an early replicating part of the yeast genome. In a complementary experiment I will delete the genomic copy of this CLR and measure its effect on firing time of the adjacent origins. The results of this study will provide us deeper insights on how cells orchestrate when and where in their genome to start duplication.

SESSION 2B

GENETIC EVOLUTION ACROSS THE LIFESPAN

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems

228 MGH

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

Identifying the Genetic Basis of Uniquely Human Phenotypes Through Comparative Genomics Analyses of Neanderthals and Modern Humans

Ann Bucher, Senior, Biology (General)
Mentor: Joshua Akey, Genome Sciences
Mentor: Benjamin Vernot

Anatomically modern humans and Neanderthals overlapped in time and space, and recent genomic studies show that limited amounts of mating between these two groups occurred. The goal of my project is to analyze genomic patterns of Neanderthal sequences that survive in present day individuals. Of particular interest are regions of the human genome where there are no surviving Neanderthal sequences, which suggest genetic incompatibilities or selective pressure between human and Neanderthal DNA. Specifically, I am focusing on a 16 Mb region on chromosome 7 in which no Neanderthal sequences persist in modern humans. Strikingly, this region includes the gene *FOXP2*, which is involved in speech and language. I am performing a number of computational analyses to catalog all sequence differences between modern humans and Neanderthals in this region, and prioritizing variants that are most likely to be functionally important using comparative and functional genomics data. Ultimately, the goal of my project is to narrow down the set of variants in this region that may contribute to uniquely human phenotypes.

SESSION 2B

GENETIC EVOLUTION ACROSS THE LIFESPAN

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems

228 MGH

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

A Screen for Genetic Regulators of rDNA Copy Number

Xiaobin (Summer) Wang, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Bonita Brewer, Genome Sciences
Mentor: Elizabeth Kwan, Genome Sciences

Eukaryotic genomes contain many copies of ribosomal DNA (rDNA) encoding the RNA components of ribosomes, ranging from 150 tandem repeated copies in the budding yeast *Saccharomyces cerevisiae* to approximately 700 copies in diploid human cells. Recent work in the Brewer/Raghuraman and Bedalov labs used *S. cerevisiae* to identify connections between rDNA copy number and cellular processes such as genome replication and replicative aging. Based on their findings, I hypothesize that a cell with too many rDNA copies may have difficulty with genome-wide DNA replication, while a cell with too few copies will not be able to satisfy ribosome demand from protein synthesis. What dictates the size of rDNA region remains unknown, but we hope to learn about the relationship between the size of rDNA region and DNA replication by identifying its genetic regulators. A preliminary survey found single gene deletion strains with altered numbers of rDNA repeats, supporting the idea that rDNA copy number is under genetic control. Encouraged by this result, I screened 400 mutants from *S. cerevisiae* deletion collection for rDNA copy number. I also examined the putative link between rDNA size and longevity: half of the mutants chosen in this screen were reported to have longer replicative lifespan and the other half were randomly selected. I found that the average rDNA copy number is not significantly different between the two groups, suggesting that the relationship between the size of rDNA region and replicative lifespan is not direct. I did observe a notable enrichment for mutants with altered rDNA size in genes that have mitochondrial function, defects in cell cycle progression, and abnormal cytoskeleton phenotypes. By continuous investigation and exploring the molecular mechanisms that govern rDNA copy number, I hope to gain a deeper understanding of how the rDNA region interacts with genome-wide DNA replication.

Session 2B

Genetic Evolution across the Lifespan

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems

228 MGH

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

Stability of a Large Amplification in *Saccharomyces cerevisiae*

Jamie Lynn (Jamie) Pogachar, Senior, Anthropology: Medical Anth & Global Hlth, Biochemistry
Mary Gates Scholar
Mentor: Celia Payen, Genome Sciences
Mentor: Maitreya Dunham, Genome Sciences

Large chromosomal amplicons and deletions or copy number variation (CNV) are found in many different cancers. However, the inheritance, stability, and fixation of these amplicons and deletions within a population are widely debated and poorly understood. In an effort to better understand how the stability of this large chromosomal rearrangement, I analyzed the stability of a large segmental amplification in the yeast *S. cerevisiae*. This amplification is commonly observed in clones evolved under sulfate-limited conditions and contains the sulfate transporter *SUL1*. This amplification is an important adaptive strategy used by the cells to improve their ability to extract the limited supply of sulfate available in the media. High copy number of this gene confers a competitive fitness advantage over other clones that have only one copy of the gene. We used a GFP marker integrated next to *SUL1* to differentiate green clones, which contain a single copy of the *SUL1* gene, from “super green” clones that have multiple copies of the gene. A super green evolved clone was grown in steady state growth vessels for ~30 generations in media that is non-selective for adaptation and our selective media as a control. I monitored the population using flow cytometry and quantitative PCR to find clones that have lost the amplification. These green clones were isolated using flow sorting and then had their genome sequenced to look at the scar that was left behind. This tells us how efficiently the cells can remove amplifications without causing detrimental effects to their fitness.

Session 2B

Genetic Evolution across the Lifespan

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems

228 MGH

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

An Evolutionary Molecular Arms Race between Mitochondrial and Nuclear Genomes and its Effect on Male Fitness

Ganeshkumar (Ganesh) Miriyala, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Harmit Malik, Basic Sciences (UW Genome Sciences), Fred Hutchinson Cancer Research Center
Mentor: Maulik Patel, Basic Sciences Division, Fred Hutchinson Cancer Research Center

Genetic conflict ensues between two parties that have an antagonistic relationship, where fitness gains in one party necessitate fitness losses in the other. As a result, there is constant selective pressure on both parties to maintain evolutionary dominance. One such conflict may exist internally between the mitochondrial and nuclear genome. This arises from the difference in DNA inheritance patterns between the two parties. Nuclear DNA is transmitted maternally and paternally in Mendelian fashion whereas mitochondrial DNA is only transmitted maternally, leaving males as an evolutionary dead end for mitochondrial DNA. This suggests that male-harming mutations could arise and fixate in mitochondrial DNA as long as those mutations are beneficial or neutral to female fitness. One way for males to counteract such mutations is for the nuclear DNA to evolve suppressors of these mutations. This conflict may not commonly exist in nature because males with mutant mitochondria would appear wild-type if their nuclear genomes contain suppressors of these mutations. To reveal if a conflict exists, we took an experimental evolution approach, using *Drosophila melanogaster*, to break the coevolution of mitochondrial DNA and Nuclear DNA and allow mitochondrial DNA to evolve on its own. After 34 generations, we performed a variety of assays and sequence analysis to identify any mitochondrial male harming mutations. We isolated a single mutation and an associated fertility decrease in males. We are currently trying to identify if any suppressors of this mutation exist in nuclear DNA of other strains of melanogaster by introducing the nuclear genome of these strains into a male with the mutation. We predict that if such nuclear genomes exist, then the fertility of mutant males should be restored when this nuclear genome is introduced. This study will provide an evolutionary insight into disease and defects of males associated with mitochon-

drial mutations.

SESSION 2E

NEW TOOLS FOR EXPLORING PROTEIN FUNCTION, STRUCTURE AND PATHOLOGY

Session Moderator: Douglas Fowler, Genome Sciences
238 MGH
3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

Solving Protein Structure with Deep Mutational Scanning
Margaret Griset, Fifth Year, Biology (Molecular, Cellular & Developmental)
Mentor: Douglas Fowler, Genome Sciences

Because a protein's three-dimensional structure defines its function, improved methods for resolving structure are an important objective in molecular biology. For example, the structures of many pharmaceutically relevant proteins are difficult to characterize with current experimental approaches. Computational techniques that predict structures from amino acid sequences obviate problematic physical manipulation of proteins, but are unreliable. Computational prediction improves, however, when supplemented with limited structural data. We propose generating data that describes spatial constraints with deep mutational scanning, a method we developed to measure the functional consequences of hundreds of thousands of variants simultaneously. I will use large-scale mutagenesis to create single- and double-mutant variants of two essential yeast proteins, cdc42 and guk1. These constructs will be transformed into Tet-Off yeast, in which the endogenous cdc42 or guk1 promoter is replaced with a repressible tet promoter. I will then employ high-throughput DNA sequencing to track variant frequencies before and after competitive growth in doxycycline. Stable, functioning variants should rescue growth and increase in frequency while deleterious variants will decrease in frequency. From these frequencies, I will derive functional scores. We hypothesize that functional scores given by two single mutations will predict the functional score of those mutations combined in a double mutant; double mutants with unexpectedly high or low scores would suggest interaction between the mutated positions. Interacting pairs associate amino acids in space, revealing spatial constraints that may enhance computational approaches for determining otherwise intractable three-dimensional protein structures.

POSTER SESSION 3

Balcony, Easel 118
2:30 PM to 4:00 PM

Genome-Wide Kinase-Chromatin Interactions in Human Embryonic Stem Cells
Katherine (Kate) Gerull, Senior, Business Administration, Pre-Health Sciences
Mary Gates Scholar, UW Honors Program
Mentor: David Hawkins, Medicine & Genome Sciences, University of Washington School of Medicine

Currently, kinases are thought to function primarily in signal transduction cascades by phosphorylating target proteins. Based on emerging evidence, a novel role for kinases is promising: direct kinase binding of DNA and chromatin. To analyze the role of kinases in the nucleus and investigate whether direct kinase binding of DNA or chromatin takes place, I will generate genome-wide histone phosphorylation and kinase localization maps for multiple kinases in human embryonic stem cells. We first verified the presence of specific kinases in the nucleus of H1 human embryonic stem cells using mass spectroscopy. To visualize protein presence in the nucleus, I stained whole cells and whole nuclei using immunofluorescence. I then studied specific kinase-chromatin interactions and histone phosphorylation sites using ChIP-seq (chromatin immunoprecipitation coupled with high-throughput sequencing). Sequencing data are expected to show genomic locations where direct kinase binding of DNA and chromatin occurs. We predict we will see kinases present at promoter and enhancer elements, as phosphorylation of proteins in the complexes that bind these elements or the surrounding nucleosomes may be important for gene regulation.

POSTER SESSION 3

Commons East, Easel 76
2:30 PM to 4:00 PM

A Tale of Two Transporters: Amplification of Sulfate Ion Transporters in *S. cerevisiae* during Evolution under Constant Sulfate-Limitation
Erica Alcantara, Sophomore, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Kolena Dang, Freshman, Pre-Sciences
Anne Elisabeth (Annie) Young, Senior, Neurobiology
Mary Gates Scholar
Mentor: Aaron Miller, Genome Sciences
Mentor: Maitreya Dunham, Genome Sciences

We are interested in identifying the spectrum of mutations that arise when performing the same evolution experiment many times. We have previously grown 32 replicates of hap-

loid yeast cells in continuous cultures under constant sulfur limiting environments for 300 generations. Mutants with increased fitness will increase in prevalence and can be characterized using an array of comparative genomic hybridization (aCGH) or next generation sequencing (NGS). To date we have used aCGH on a subset of our evolutions, and in 16/16 cultures, found amplification of *SUL1*, a high affinity sulfate ion transporter gene used by the cells to aid in increasing import of sulfate. We are also interested in identifying alternative means for adaptation in this environment and have evolved a a strain in which we'd knocked out *SUL1* (*sul1*Δ). With this background, we observed *SUL2* amplification in 4/4 cases in the absence of *SUL1*. A second focus of our work is to track changes in fitness as specific mutations arise. To assay fitness, we periodically compete an evolving culture against a Green Fluorescent Protein (GFP) expressing ancestral strain of yeast. This work has shown that the dynamics of adaptation of *wt* and *sul1*Δ yeast differ, which suggests very different fitness landscapes during the evolution for these two genotypes. Collectively these data show that *SUL1* amplification is a first target of evolutionary selection and that in its absence, amplification of *SUL2* becomes the primary means for adaptation in constant sulfate limitation.

POSTER SESSION 4

Commons East, Easel 45

4:00 PM to 6:00 PM

Epigenetic Analysis of Naïve Human Embryonic Stem Cells

Leone (Sophie) Hopkins, Senior, Extended Pre-Major
Mentor: David Hawkins, Medicine & Genome Sciences, University of Washington School of Medicine
Mentor: Stephanie Battle, Genome Sciences

Embryonic stem cells can be used to study development and diseases. Mouse embryonic stem cells (mESC) are used as a model in developmental research and display several pluripotent properties not seen in the current human embryonic stem cell (hESC) lines. A newly derived line of naïve hESCs is thought to recapitulate an earlier stage of development, and resemble mESCs. To understand how the naïve hESCs differ from typical hESCs we are investigating DNA methylation, a form of epigenetic regulation, on a global scale at nucleotide resolution. The underlying genomes of cells are highly similar, yet their epigenomes change with cellular differentiation and development. Epigenetic modifications are important factors controlling gene regulatory elements. To analyze whole genome DNA methylation of the naïve hESCs, I am using MethPipe, a computational tool, to determine how the naïve hESC line differs from a later stage hESC line, and if the naïve line is more similar to mESCs at the epigenetic level. I will compare global methylation levels, hypo/hyper-methylated regions, and partially methylated domains. I will determine which regulatory elements, such as promoters, enhancers and CpG islands, show similarities and differences. We also expect to determine differential non-CG methylation (a characteristic of ESC). This characterization of naïve hESCs will provide a foundation for studying the earliest stages of human development.