The Role of CTCF in Regulating Escape of X-linked Gene Shroom4 from X-Chromosome Inactivation
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In mammals, males have sex chromosomes XY while females have XX. To balance out the extra X sex chromosome, females undergo X-chromosome inactivation (XCI) which silences most genes on one of the two X chromosomes. However, some genes escape XCI and continue to be expressed on the inactive X chromosome causing a high expression level of these genes in females compared to XY males, leading to potential sex differences in health and disease. Shroom4, an X-linked gene that encodes an essential protein for cytoskeletal architecture, is an example of a gene that escapes XCI in mice. How Shroom4 escapes XCI is unclear. It has been proposed that CTCF, a master chromatin regulator that controls gene transcription through histone or chromatin modifications, could play a role in insulating escape genes from the silencing environment on the inactive X chromosome. Indeed, we found there is a strong CTCF peak between Shroom4 and the neighbor silenced gene Bmp15. To functionally test this insulation model, I am using CRISPR/Cas9 to edit the CTCF binding site and examining the effects of deletion and inversion of the site on Shroom4 allelic expression levels. This analysis will show whether the CTCF binding site and its correct orientation are necessary for Shroom4 escape from X inactivation. Through this project we are able to improve our understanding of the complex nature of XCI.

Estimating the Rate of Plasmid Transfer with an Adapted Luria-Delbrück Fluctuation Analysis
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Bacteria have the ability to transfer certain small, mobilizable pieces of DNA from one cell to another, nearly regardless of species, through a process called conjugation. During conjugation, a donor cell containing a plasmid donates a copy to a recipient cell lacking a plasmid to form a transconjugant (a recipient cell now containing a plasmid). This has strong implications for the spread of antibiotic resistance among bacterial communities, as plasmids often harbor genes which confer resistance to certain antibiotics. Sharing of these plasmids between bacteria can increase the amount of resistant individuals in a population, which can produce infections that can be difficult to treat clinically. Therefore, having an accurate method to predict the rate at which these transconjugant cells form within a bacterial population can provide key insights to the spread of antibiotic resistance through plasmid-mediated gene transfer. Current models rely heavily on the presumed deterministic nature of transconjugant formation; however, we revealed that experiment estimates with these currently available methods can lead to biased estimates. In need of a more accurate and robust method to estimate the conjugation rate, we developed a novel stochastic model and accompanying lab protocol that effectively provides an accurate estimate of the conjugation rate. Our approach was inspired by the classic experiments of Luria and Delbrück, which revealed that mutation (changing a normal cell into a mutant cell type) was a stochastic process (i.e., random). Similarly, we hypothesized that this stochastic framework could be useful for creating a method for estimating conjugation (changing a recipient cell into a transconjugant cell type). We found using experiments and simulations that our method is accurate and robust under a variety of conditions. In conclusion, we developed a new method for the accurate estimation of plasmid conjugation rate.

Microbial Expression of Potentially Therapeutic Paraoxonase-2
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Paraoxonases (PONs) are a family of three closely related enzymes that hydrolyze organophosphorus and organochlorine insecticides and pesticides, and are responsible for the degradation of these hazardous compounds in vivo. However, the current structures and substrates of PONs such as PON1 and PON2 are not known, and this research project explores microbial expression as a way to elucidate the PON2 enzyme. This research is based on work done by Dr. Furlong in investigating whether PONs are expressed in bacteria, which serves as a starting point for the development of new therapeutic targets for enzyme inhibition. Using the TetR-based lambda system to inductively express PON2 in E. coli, we aim to determine its structure and function, and demonstrate the potential of using these enzymes for therapeutic medicine.
genes found on the long arm of chromosome 7. The genes encode PON1, PON2, and PON3, which are primarily involved in metabolizing oxidized lipids and modulating oxidative stress. However, each of the PON enzymes are involved in important secondary reactions. PON2 is an intracellular enzyme localized in the mitochondria that plays a vital role in modulating oxidative stress and inactivating microbial quorum sensing factors. Individuals with PON2 deficiencies are sensitive to oxidative stress. It may be possible to restore PON2 function by creating an injectable protein for individuals with a PON2 deficiency. Our goal is to actively express recombinant PON2 in an *E. coli* expression system and inject PON2 into *PON2* knockout mice to determine if PON2 function can be restored. To express PON2 in *E. coli* we designed a synthetic DNA sequence by removing the transmembrane sequence of PON2 and replacing it with the signaling sequence from PON1, which facilitates the purification of the chimeric protein. We transformed the synthetic PON1/PON2 plasmid in *E. coli* and are currently performing gel electrophoresis and activity assays to analyze the expression of PON2. If we see protein expression as expected, then we will purify the recombinant PON2 for injection using a histidine tag that we added to the end of the protein coding sequence in the construct. The histidine tag allows for single-step purification via affinity chromatography, which we will then inject into PON2 knockout mice to observe their response to oxidative stress. Findings from this experiment will allow for further understanding of PON2 function and its restoration via an injectable protein, as well establishing that *E. coli* expression systems can be used as a more cost-effective method for pursuing further PON2 related research.

**Humanized Histones in the Yeast Epigenome Cause Mutations That Affect the Monomer-Dimer Equilibrium in the Dam1 Complex.**

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*Mentor: Trisha Davis, Liberal Arts*

*Mentor: Alex Zelter, Biochemistry*

During mitosis, the kinetochore plays a central role in ensuring the proper segregation of chromosomes in the parent cell. It does so by forming attachments to spindle microtubules, which facilitate the equal distribution of chromosomes to daughter cells. In budding yeast, the Dam1 and Ndc80 complexes are essential protein complexes that bind the kinetochore to spindle microtubules. The Ndc80 complex functions as the direct contact between the kinetochore and the dynamic microtubule tip and it is required for the Dam1 complex to associate with kinetochores. The Dam1 complex strengthens the kinetochore-microtubule attachment. In the presence of microtubules, Dam1 complex oligomerizes into a sliding ring. This self assembly has been observed to occur with nanomolar concentrations of the complex in the presence of microtubules but in the absence of microtubules, appreciable oligomerization occurs at concentrations of the complex in the micromolar range. Dimers of the complex appear to predominate in high salt concentrations (500 mM NaCl) in comparison to monomers. This is thought to be due to electrostatic interactions between the monomers. When yeast histones were swapped for human histones, several mutations occurred in the Dam1 complex, and one mutation in the Ndc80 complex, that rescued the yeast cells from defects in mitosis. Preliminary characterization of the mutant Dam1 complexes lead to the hypothesis that the mutations that allow the yeast cells to adapt to the humanized histones changed the monomer-dimer equilibrium for the Dam1 complex. To measure the affinity of Dam1 complex monomers for each other, I will purify the protein complex and use size-exclusion chromatography and western blotting to quantify the relative abundance of the monomer and dimer at different concentrations of the complex. This will contribute to a greater understanding of mitosis and in turn cancer because it focuses on the dynamics that control proper chromosome segregation in cells.

**Rapid Isolation of Large Protein Complexes Stabilized With Formaldehyde Cross-Linkage**

*Daphnee Michelle Marciniak, Senior, Biochemistry*

*Mentor: Shao-En Ong, Pharmacology*

Proteins often interact with other proteins to relay signals or to cause physiological effects. Knowing the components of cell signaling pathways is important for understanding diseases and developing treatments. However, it can be difficult to isolate and detect protein complexes because some experimental conditions are too harsh on the weak interprotein interactions. We therefore questioned whether we could use cross-linking to maintain native protein complexes for rapid isolation and detection. I used formaldehyde (FA), a protein cross-linker, in conjunction with a 100k molecular weight cut-off (MWCO) spin filter to isolate protein complexes above 100k molecular weight (MW). FA treatment for a few minutes should only cross-link proteins that are near each other. Protein complexes formed through cross-linkage should be massive (>100k MW) compared to most unlinked proteins. Therefore, complexes of closely-associated proteins can be rapidly purified from a 100k MWCO filter. I specifically studied the proteins associated with epidermal growth factor receptor (EGFR) in response to extracellular epidermal growth factor (EGF) treatment. EGFR itself is above 100k MW, but the proteins that associate with it when EGF is bound to it, such as MAPK and GRB2, are below 100k MW. The presence of the smaller proteins in filtered cell lysate samples prepared with EGF and FA treatment would show that the described experimental setup can be used to isolate smaller proteins in complexes above 100k MW. This can be further applied in structural and mechanistic studies of protein complexes involved in normal and pathological physiological processes, in determining novel complexes after fur-
ther purification and identification steps, and in time course experiments—where different proteins may arrive in a complex at different times post treatment. The aforementioned applications can help pave the way for novel and more effective disease treatments and provide a better understanding of the inner workings of cells.

Investigation of Kinetochore Force Transmission by Quantification of Subunit Affinities

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The kinetochore is a protein complex responsible for transmitting force between microtubules and the centromeric region of DNA on chromosomes. The OA and Mif2 protein subunits of the kinetochore make direct attachments to the chromosome. They both bind to another subunit called MIND, which forms a bridge to the microtubule-binding elements of the kinetochore. Preliminary research shows that OA and Mif2 exhibit different binding preferences for the MIND complex. OA binds constitutively, regardless of whether MIND is in an open or closed conformation, whereas Mif2 strongly prefers the open conformation. However, the binding affinities for both OA and Mif2 for MIND have not yet been measured. This study developed the experimental methods for performing immunoprecipitation assays with OA, Mif2, and MIND. By performing phosphomimetic and truncation mutations to promote the MIND open conformation, the binding affinities of OA and Mif2 will be quantified. It is important to quantify the subunit binding affinities to gain a deeper understanding of kinetochore assembly and force transmission between microtubules and chromosomes. Ultimately, this research has applications in cellular division and cancer research.