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The Role of Polycomb Group Proteins in the Maintenance of Embryonic Stem Cell Pluripotency

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Embryonic stem (ES) cells have the remarkable characteristic of being able to self-renew indefinitely in culture and yet differentiate into any cell type. Elucidating the mechanisms behind pluripotency is crucial to understand early development and the potential clinical application of embryonic stem cells. Polycomb group (PcG) proteins are evolutionarily conserved transcriptional repressors that have essential roles in embryonic development and in the maintenance of stem cells. In this study, we used genome-scale location analysis (chromatin immunoprecipitation coupled to a microarray platform) to identify binding sites for PcG proteins in mouse ES cells to explore whether the silencing of certain genes plays a role in cell differentiation and developmental progression. We found that PcG proteins bind and repress a remarkable number of transcription factors with important roles in development and that these genes are preferentially upregulated upon ES cell differentiation. The methylation mark characteristic of silent chromatin (H3K27me3) also correlated with the PcG-bound regions. PcG-target genes were derepressed in ES cells deficient for the PRC2 component Eed. Our results indicate that repression of developmental pathways by Polycomb complexes is critical for maintaining ES cell identity and for developmental plasticity during early development.

Faculty Supervisor: Rudolf Jaenisch
Postdoc Mentor: Laurie Boyer

mMps1 and the Mammalian Mitotic Spindle Checkpoint

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Chromosome instability has been associated with many human cancers, and inactivation of the mitotic spindle checkpoint is thought to contribute to errors in segregation. *Mps1* is one of the core components of the mitotic checkpoint, and it appears that mutating this gene in conditional mouse embryonic fibroblasts (MEFs) causes disruption of the checkpoint and increased cell death. When *p53* was also mutated, *Mps1* conditional MEFs expressing Cre Recombinase became immortal in culture, showed increased aneuploidy, and demonstrated numerous anaphase defects. Based on nocodazole assay this *Mps1* mutation did not completely cripple the mitotic checkpoint; nevertheless, it is evident that *Mps1* is essential for maintaining controlled cell division. These results also support *in vivo* data, suggesting that mutating *Mps1* in cells lacking the *p53*-mediated apoptotic pathway leads to increased rates of cell proliferation, which is correlated with increased incidence of random mutation and accelerated tumorgenesis.

Faculty Supervisor: Peter Sorger
Postdoc Mentor: Stephanie Xie

Potential New Method in Receptor Engineering: Insertion of Anti-fluorescein scFv into *Escherichia coli* FecA Receptor Protein

Annie Phuong Vo
Main Faculty Advisor: Drew Endy
Other faculty advisors: Tom Knight, Natalie Kuldell,
Kate Schneider, and Gerry Sussman.

The engineering of receptor-ligand or enzyme-substrate interactions has become an increasingly common technique to create new tools for manipulation and study of biological systems. There are two current methods: the mutation and the polar interaction change. The first generates cavities in the binding pockets so that only ligands with introduced bumps will bind. The second switches polar interactions of charged residues as another way of generating new binding. The inherent problems with these methods lay in the non-specific binding and the significant decrease of the receptors' activities. Inserting a sequence with high affinity and specificity for a different ligand into a receptor may be a better method. In this study, I design a testing system to investigate the possibility and the potential of this insertion method. The anti-fluorescein scFv, which has high affinity and specificity for fluorescein molecules, is inserted into the receptor FecA, an outer membrane protein in ferric citrate transport pathway in *Escherichia coli*. Comparisons of dissociate constant and specificity assay results will confirm that the insertion is indeed a better method.

This project was part of a competition called iGEM, with multiple faculty supervisors. The primary supervisor is Drew Endy. The others are: Tom Knight, Natalie Kuldell, Kate Schneider, and Gerry Sussman.

The Bacterial Two-Hybrid Approach to UmuDC in *E. coli*

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Most bacteria, including *Escherichia coli*, have a coordinated inducible response to DNA damage known as the SOS response to combat damage to their genomes. The SOS response is triggered by the blockage of normal replication, possibly caused by a lesion on the DNA. This event leads to the expression of at least 40 SOS regulated genes, among which are the proteins of interest: a specialized DNA polymerase called DNA Pol V or UmuDC. The *umu*DC gene products have at least three roles in the cell, which are temporally regulated. $UmuD_2C$ appears to have a role in delaying the cell cycle and DNA replication, allowing time for accurate DNA repair processes to occur. After approximately 20 minutes of $UmuD_2$ existing as a full-length protein, it self-cleaves to form $UmuD'_2$. The new $UmuD'_2C$ complex, together with several other proteins, is capable of low fidelity translesion replication. Additionally, $UmuD_2$ and $UmuD'_2$ interact physically with several of the replicative DNA polymerase subunits and $UmuD'_2C$ is capable of inhibiting homologous recombination while $UmuD_2C$ is not. To gain a better understanding of how UmuDC works, a screen has been performed for UmuDC protein interactions. The Stratagene BacterioMatch® II Two-Hybrid System has been used to detect the protein interactions between the gene products of *umu*DC and a library of *E. coli* proteins.

So far 67 different proteins have been identified as having an intereaction with either UmuD, UmuD', UmuC, UmuC N-terminus, or UmuC C-terminus. Characterization of positive proteins will include cloning the genes into appropriate vectors, making rational site-directed mutations to test specific interactions between target and bait, purifying the proteins, quantifying their interactions with the bait proteins, and testing their interactions with UmuDC mutants that have interesting phenotypes.

Faculty Supervisor: Graham Walker
Postdoc Mentor: Penny Buening

Why Are You Such a Nervous Wreck? The cellular basis for temperature sensitivity in *nwk* mutants

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Neurons elaborate a complex arbor of axons and dendrites that allow the formation of synaptic connections that mediate information transfer in the brain. These neuronal connections are not structurally static, and can be dynamically altered by changes in neuronal activity. Alterations in cell morphology are controlled through the actin cytoskeleton, but how neurons modulate actin is unknown. We are trying to elucidate these pathways by identifying neuron-specific actin regulators. Nervous Wreck (Nwk) is a member of a conserved family of actin regulatory proteins and necessary for *Drosophila* synapse formation and function. *nwk* mutants have two predominant phenotypes: reduced synaptic transmission coupled to overgrowth at the neuromuscular junction (NMJ) and temperature sensitive seizing (TS). The cellular basis of TS is not understood. I isolated a suppressor of the *nwk* TS mutant through a mutagenesis screen. Surprisingly, the suppressor eliminates TS by restoring Nwk expression in some cells without reducing NMJ overgrowth, suggesting that the NMJ overgrowth does not cause TS. To identify neurons that cause the temperature-induced seizure phenotype, I began a screen using the UAS-GAL4 expression system. I have collected neuron-specific GAL4 transcriptional drivers to express a *nwk* transgene in distinct brain regions in animals otherwise lacking Nwk. Some drivers rescue the *nwk* TS phenotype. Adult brains from these lines have been stained with anti-Nwk antibodies to identify a sufficient common subset of cells that rescue TS. In addition, to determine Nwk temporal requirements, I am utilizing the drug-induced GeneSwitch construct to initiate Nwk expression at specific points in development. This will indicate whether Nwk plays a role in neuronal development or has a more acute function in synaptic transmission. Through these approaches, I hope to pinpoint when and where Nwk is required for function, and how its natural activities regulate the actin pathway inherent to synaptic organization and transmission.

Faculty Supervisor: J. Troy Littleton
Postdoc Mentor: Avital Rodal

The Phosphatase Gene cdc-14 Exerts Cell-specific Control Over the Cell Division Cycle

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The postdeirid is a small sensory structure in the nematode worm C. elegans which consists of four cells: two neurons (the PVD and the PDE) and two glial-like cells (the PDEsh and the PDEso). Because postdeirid development can be directly observed in the living animal, it provides a useful system in which to investigate the development and differentiation of specialized tissues. We isolated the mutation *n3444* in a screen designed to recover animals with an extra dopaminergic neuron in the postdeirid. We then mapped *n3444* using single nucleotide polymorphisms to a 70 kb region on chromosome II and rescued the mutant phenotype by transgenesis with either of two overlapping cosmids. Complementation and sequencing showed that *n3444* is a likely null allele of *cdc-14* (cell division cycle–related), which encodes a putative phosphatase whose homolog is responsible for mitotic exit in the budding yeast S. cerevisiae. We directly observed the development of the postdeirid of cdc-14(_) animals *in vivo* and determined that the dopaminergic PDE neuron undergoes an extra cell division in approximately 40% of mutant animals (n = 24). No other cell fate or cell division defects were observed during postdeirid development. Loss of *cdc-14* has been previously reported to cause ectopic divisions in other tissues in *C. elegans*‡. We are pursuing our finding that, in animals completely lacking cdc-14 function, specific cells are subject to ectopic divisions, while others appear completely unaffected. To investigate this question, we plan to observe other developing lineages and to use transgenic cell-fate markers to examine the extent and specificity of cell cycle defects normally prevented by *cdc-14* function.

‡Saito, RM et al. (2004) Nature Cell Biology. 6, 777-783

Faculty Supervisor: Bob Horvitz
Postdoc Mentor: Hillel Schwartz

SEC31 Acts in the Internal Amino Acid Sensing Pathway in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae regulates the trafficking of the high capacity, low specificity general amino acid permease, Gap1p, in response to internal amino acid levels. When internal amino acid levels are low, Gap1p is sorted to the plasma membrane where it imports amino acids into the cell. However, when internal amino acid levels are high, Gap1p is poly-ubiquitinated, sorted to the vacuole, and degraded. To determine how the cell senses and responds to amino acids, a screen was done to identify mutants that sort Gap1p to the plasma membrane in the presence of high internal amino acid levels. Eleven mutants were isolated, two of which contain an S367T mutation in the Sec13p-binding region of SEC31. Sec31p is a member of the COPII vesicle coat, which coats vesicles that transport proteins from the endoplasmic reticulum to the Golgi. In the COPII vesicle coat, Sec31p is in a complex with Sec13p, which was previously identified as having an effect on Gap1p trafficking. Because of this, it was hypothesized that Sec13p-Sec31p binding may be affected by amino acids. The finding that the sec31-1 temperature sensitive, COPII defect is more severe on amino acid containing medium supports this hypothesis. Further tests have shown that Sec31p is involved either in trafficking from the pre-vacuolar compartment to the Golgi or from the pre-vacuolar compartment to the vacuole. These findings support a role of Sec13p and Sec31p in the amino acid dependent sorting of Gap1p.

Faculty Supervisor: Chris Kaiser
Postdoc Mentor: April Risinger

Gene Expression Profiling of Early Lesions in a Genetically Defined Mouse Model of Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the fifth leading cause of cancer death in the United States. The non-invasive neoplastic precursor to this cancer is pancreatic intraepithelial neoplasia (PanIN). Alternative schemes for the development of PanINs have been proposed; yet the identity of the cell of origin has remained elusive. Candidate cells represent the exocrine unit that includes the duct and the acinar cells, and the endocrine unit that consists of the islets cells. Identification of the cell-of-origin of PDAC is essential to determine the pathways that are affected by oncogenic mutations early in tumour development, at a point when chemopreventative measures might still be effective. Towards this end we are performing a thorough molecular characterization of normal ductal and acinar cell populations versus PanINs generated in a genetically defined mouse model using gene expression profiling. Over 90% of the invasive pancreatic adenocarcinomas harbour activating mutations in the K-ras proto-oncogene that are thought to be an initiating event. A mouse model of PDAC was initially developed in our laboratory, which harbours a Cre-activatable allele of oncogenic Kras ($Kras^{LSL\text{-}G12D}$) in the endogenous K-ras locus. Targeting the expression of $Kras^{G12D}$ specifically to the pancreas by crossing the $Kras^{LSL\text{-}G12D}$ mouse with the Pdx-1-Cre strain generates progressive PanIN lesions.

We are using laser capture microdissection (LCM) to harvest the different cell types from frozen pancreatic tissue sections of normal mice and from *Pdx1-Cre: LSL-*$Kras^{G12D}$ mice. We then process the RNA for analysis using an Affymetrix mouse array. The Microarray analysis will explore whether PanIN samples have gene expression profiles that are distinct from normal acinar and ductal epithelium. The identification and understanding of the biochemical and biological changes that lead to the transformation of these cells may provide new tools for better early PDAC detection and possible approaches for therapy of this devastating disease.

Faculty Supervisor: Tyler Jacks
Postdoc Mentor: Sharon Gidekel

Characterization of Oceanic Cyanobacterial Viral Isolate Infection Using One-step Growth Curve Analysis

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The marine cyanobacteria *Prochlorococcus* and *Synechococcus*, are the numerically dominant photosynthetic species on Earth, and thus play a significant role not only in marine ecosystems, but also in the planet's atmospheric composition. The viruses (cyanophages) that infect them play a fundamental role in cyanobacterial population dynamics. Curiously, cyanobacterial photosynthesis genes appear to be a regular feature of cyanophage genomes and are even expressed during infection. These genes are thought to be selected for because of long periods of infection during which host cell carbon and energy stores likely become limiting for the production of viral progeny. To begin to test this hypothesis, and to better understand the interactions between marine cyanophages and cyanobacteria in the environment, we characterized basic physiological interactions between cyanophage-host pairs from laboratory isolates. In contrast to other well-characterized model virus-host systems (e.g., coliphages) with established protocols for examining viral production, the sensitivity of open-ocean host isolates prohibits standard experimental perturbations including large dilutions and viral titering by plaque assay. We have overcome these problems by optimizing multiple methods of obtaining an accurate viral titer. Here we report the results of one-step growth curve experiments (commonly used to characterize virus-host physiology) between two virus-host pairs–Syn1 infecting *Synechococcus* WH8101 and P-SSM4 infecting *Prochlorococcus* MED4. These marine cyanophages require over 12 hours to proceed through a single lytic cycle, a remarkably long time as compared to the 20-minute lytic cycles exhibited by coliphages. It is likely that such long periods of cyanophage infection are made possible by the expression of 'host' proteins encoded in these cyanophage genomes that maintain photosynthesis as previously hypothesized. Furthermore, these data offer the first quantification of marine phage lytic cycles–critical parameters for modeling efforts aimed at further understanding of virus-host interactions in the world's oceans.

Faculty supervisor: S.W. Chisholm
Postdoc Mentors: Matt Sullivan

Elucidating the Neurotoxic Mechanism of Alzheimer's Disease Peptides

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The beta-amyloid peptide ($A_{}_{1-42}$) is the prominent peptide species that makes up the fibrillar aggregates found in the extracellular brain tissue of Alzheimer's Disease (AD) patients. Here, we use the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay for cell viability to determine if oligomeric $A_{}_{1-42}$ neurotoxicity occurs through interaction with cell surface ionotropic glutamate receptors. Preliminary results show that undifferentiated IMR-32 cells lacking functional glutamate receptors are sensitive to toxicity by non-fibrillar (i.e. non-aggregated) $A_{}_{1-42}$, thereby showing that $A_{}_{1-42}$ can induce cell death by a mechanism not involving the receptors. In addition, even though glutamate itself can induce cell death in some experiments, suggesting possible sporadic expression of glutamate receptors, the presence of glutamate receptor antagonists does not reduce the toxicity of $A_{}_{1-42}$. In fact, the antagonists alone produce some toxicity that seems to be additive to the $A_{}_{1-42}$ effect. Thus, our current results strongly suggest that oligomeric $A_{}_{1-42}$ does not induce cell death by interacting with glutamate receptors.

Faculty Supervisor: Vernon Ingram

Deamidation of Human γS Crystallin Influencing Folding and Stability

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Human γS crystallin is a two domain, primarily b-sheet protein whose stability and native conformation is critical for the maintenance of lens transparency. Loss of stability and solubility is associated with the eye disease cataract. Proteins found in the insoluble aggregates of cataractous lenses have high levels of covalent damage, including glutamine deamidation, the products of which are L, D-glutamate and L, D-isoglutamate. Deamidation of residues Gln16, Gln63, Gln120, and Gln148 in HγS-crys has been found in the insoluble protein aggregates. This suggests that deamidation may induce cataractogenesis by destabilizing the protein and causing population of an aggregation prone intermediate. In this study, we analyzed the effect of deamidation at these residues by using site-specific mutagenesis to mimic the deamidation event and studied the thermodynamic and kinetic properties of the mutants at physiological pH. Our studies show that deamidation at surface exposed residues do not perceptibly alter the concentration midpoint of unfolding, but does decrease the native stability of the protein. Deamidation at an interface residue was found to significantly decrease the stability of the protein and the concentration midpoint of unfolding. Kinetic refolding properties for all mutants were affected by the mutagenesis event.

Faculty Supervisor: Jonathan King
Graduate Student Mentors: Shannon Flaugh, Ishara Mills