

Session B – Biology (Alphabetical)

Using Meiotic Drive in *Drosophila melanogaster* for Population Control

Shamili Allam

Mentors: Bruce Hay and Geoff Pittman

In a genetic drive system, a selfish genetic element is included in an organism such that it spreads itself through a population at higher than Mendelian ratios. For this specific project, we use a meiotic drive system in *Drosophila melanogaster*, which biases the progeny of a male fly to have a certain gene. This is done by ensuring through a toxin-antidote system that the sperm without this gene also lack a necessary mRNA for fertility. In this case, this mRNA is Mst77F, a gene essential in histone-like compaction of DNA during spermatogenesis. The toxin is a set of miRNAs which silence the endogenous Mst77F mRNAs while the antidote is the gene itself attached to a post-meiotic promoter. In this project, we created this meiotic drive construct and inserted into *D. melanogaster* to show that it serves as an effective drive system.

2-Hybrid Assay for Mitochondrial Stress in Parkinson's Disease

Gal Barak

Mentors: Bruce Hay and Kelly Dusinger

Parkinson's Disease is a chronic, neurological disorder that is characterized by the loss of dopaminergic cells in a region of the midbrain. Studies have shown that mitochondrial dysfunction plays a role in the development of Parkinson's disease, with dopaminergic neurons undergoing cell death in response to oxidative stress on the mitochondria. To monitor cell stress, we designed a 2-hybrid assay for mitochondrial rupture in *Drosophila melanogaster*. First, a DNA binding domain was cloned downstream of a mitochondrial targeting sequence to direct one part of a transcription factor to the mitochondria. In addition, the activation domain of a transcription factor was generated to remain free in the cytoplasm; therefore, when stressed mitochondria rupture, both domains will be available to go the nucleus. Since both constructs were given leucine zipper domains that are known to have tight interactions, the heterodimerization of these domains will generate a chimeric transcription factor for a promoter region upstream of a Green Fluorescent Protein sequence. Transcription of this gene will serve as a reporter for oxidative stress. By transfecting cells with plasmids containing these constructs, we expect to answer empirical questions about the accuracy of a screen for cells that are preparing for death.

Analysis of Receptor Specificity of Insulin Analogues

Evan Biggs

Mentors: Charles Roberts and Ellen Rothenberg

Long-acting insulin analogues are widely used in the management of diabetes mellitus. There are two distinct issues with the use of these long-acting analogues: firstly, the possible tumorigenicity of insulin glargine (Lantus); and secondly, the biological basis for the lack of weight gain in patients treated with insulin detemir (Levemir). It has been hypothesized that these possibilities may both be due to differential activation of the two insulin receptor isoforms or (in the case of glargine) crosstalk with the insulin-like growth factor-I receptor. Here we show that the nature of glargine signaling does support some concern for its potential tumorigenicity, given its increased activation through the insulin-like growth factor-I receptor, and that the lack of weight gain associated with detemir is not due to differential activation of the A isoform of insulin receptor as previously conjectured. Furthermore, we confirm that the hyperoxia associated with standard cell culture does not effect the signaling of these analogues.

Building a Lethal Engineered Underdominance Construct in *Drosophila*

Margaret Chiu

Mentors: Bruce Hay and Kelly Dusinger

A new approach for eliminating mosquito-borne disease is creating transgenic insects that are unable to carry disease. Transgenic mosquitoes can be released, fixing a refractoriness gene in the wild population by genetically linking a gene drive system with the gene. We are developing the gene drive system required, and are focused on a strategy called Engineered Underdominance (EUD). EUD is a desirable gene drive system because it only fixes in a population if a higher number of engineered organisms are released than exist in the wild habitat, allowing for the removal of the engineered gene from a population if necessary. Our EUD system is a two-chromosome system. Each chromosome consists of a lethal toxin and an antidote for the toxin on the opposite chromosome. The chromosomes must be built individually, so, for the animal to survive, it must carry a conditional rescue. We have two versions of the EUD system with two different promoters, which express at different times in development and in different tissues. Four transgenic fly lines have been created: two chromosomes for each promoter. The lines with matching promoters will be crossed and the conditional rescue removed to test for a gene drive phenotype.

Antisense: The Critical Features Identified for Constructing a Stable and Specific Alternative to Micro- and siRNA

Daniel Leighton

Mentor: Bruce Hay

Antisense RNA was a popular tool for the translational inhibition of specific genes in the 1990s. Due to the extreme inconsistency in its effectiveness from one target to another, this field died out at the invention of siRNA in the early 2000s. In this paper, we describe the influence of a variety of design features on the effectiveness of an antisense construct. These features include the presence, position, and length of open reading frames; the presence of introns; and the 3'UTR chosen for the construct. These design features may influence the steady state levels of the construct, as well as its ability to inhibit its target.

Repression and Derepression of mRNA Constructs using Targeted microRNA

Ang (Alan) Li

Mentor: Bruce Hay

In nature, microRNAs (miRNA) have the ability to degrade mRNA at specific binding sites. By doing so, translation of mRNA is inhibited. However, by synthesizing mRNA with special folding properties, we reversed the function of the miRNA, and now use it to enable translation of reporter proteins. The native states of our mRNA constructs are folded transcripts with very low levels of translation. Upon the addition of miRNA, the folded regions are excised and the translation is recovered. In this way, translation of any protein in an organism can be regulated by miRNAs. The constructs are tested in *Drosophila* S2 cells, derived from embryos. S2 cells were co-transfected with both reporter and modifier plasmids. Green fluorescent protein and firefly luciferase serve as reporter proteins for measuring translational levels. The modifier constructs carries a miRNA gene. From all of the constructs we have tested, we have found that the kissing stem-loop and antisense-homology constructs have the most promising repression and derepression in S2 cells. In the future, optimized stem loop or antisense constructs may initiate cell death in the presence of cancer-originated miRNA to prevent metastasis.

Temporal-Spatial Expression of rbms3 in Developing Zebrafish

Jennifer Li

Mentor: Marianne Bronner-Fraser

RNA-binding proteins (RBPs) play important roles in RNA metabolism. Genetic screening in fish has identified an RBP called rbms3 which has been found to possibly upregulate homeobox transcription factor Prx1, which is involved in cranial facial development. It may also play a role in regulating the transcription of human $\alpha 2(I)$ collagen COL1A2, which is involved in cartilage development. Because of this, it is possible that rbms3 is important in regulating RNA stability of transcription factors involved in cranial-facial development. To effectively study rbms3, a zebrafish model has been developed in which a citrine signal is attached to the rbms3 gene. We investigated 1) when and where rbms3 is expressed in earlier stages of zebrafish development and 2) how closely the citrine model resembles wildtype rbms3 expression. Whole mount *in situ* hybridization and immunohistochemistry are used to determine mRNA expression and protein expression of citrine-tagged rbms3 and wildtype rbms3. Results determine when and where rbms3 and citrine-tagged rbms3 expression begins in early stages of zebrafish development. If citrine expression closely resembles wildtype rbms3, then it validates the use of the citrine-tagged model as a tool to study the function of rbms3 *in vivo*.

Imaging the Heart Contraction in Developing Quail Embryos During Cardiac Looping

Jason Lunn

Mentors: Scott Fraser and Jennifer Yang

The embryonic vertebrate heart begins as a straight tube. Through a process of cardiac looping, the chambers and valves of the heart are created. By measuring heart contractions in developing quail embryos during cardiac looping, we aim to learn more about the cellular aspects related to heart morphogenesis. This was principally accomplished by using laser scanning confocal microscopy (LSCM) in conjunction with a Tie1 transgenic line of quail that expresses H2B::eYFP in endothelial cells. Quail embryos from different stages of development (Hamburger-Hamilton 9:14) were imaged under a LSCM microscope using line scans to measure heart contractions across different regions of the developing heart tube. Preliminary results show that the heart contracts most strongly in the upper ventricle region of the heart. It also appears that the thickness of the cardiac jelly is not consistent throughout the entire heart tube and may play a vital role in proper heart development.

What Does It Mean to Be Social? Fundamental Differences in Motor Response to Human and Electronic Motion in Competitive Reaction-Based Game

Luke Moryl

Mentor: Shinsuke Shimojo

In the past few years, the fledgling area of social interaction has attracted a substantial amount of thought within the neuroscience community. Although a great body of work has grown around the perceptual and preferential changes evoked by visual stimuli such as faces or simulated human motion, little attention has been given to the behavioral changes that occur at an implicit level during visuomotor interaction with another person. This project uses a modified version of the traditional Japanese game, *acchi muite hoi*, to investigate behavior during play against a human and play against a computer opponent. Our findings show significant differences between reaction times, success rates, and other measures for these two conditions, and these disparities provide preliminary insights into the fundamental visuomotor changes that appear in human interaction.

Control of Endoreduplication in *Arabidopsis* by CYCLIN D3 and LGO

Will Suh

Mentor: Elliot Meyerowitz

In many plant species including *Arabidopsis*, a specialized form of the cell cycle known as endoreduplication occurs where cells undergo expansion in volume and replicate their genome, but forgo mitosis. Endocycles are regulated by incredibly complex machinery that is not fully understood as of yet. However, it has been shown that three genes in the *CYCLIN D3* family are negative regulators of endocycling, and their loss of function results in increased endoreduplication, even in organs such as petals where endoreduplicated cells are normally not observed. Conversely, *Arabidopsis* plants with a mutation in *Igo* strikingly showed no highly endoreduplicated cells in its sepal. Here we successfully identified *Arabidopsis* that are triple loss-of-function mutant in *cycd3; 1-3*, and quadruple loss-of-function mutant in *cycd3; 1-3* and *Igo*. Both triple and quadruple mutants have narrow sepals, which often do not cover the entire flower. In addition, both *cycd3; 1-3* triple and *cycd3; 1-3 Igo* quadruple mutants have larger petal cells than wild type, which hints that these cells have undergone endoreduplication. Confocal microscopy of the quadruple mutant surprisingly showed lack of giant cells, which is akin to the *Igo* mutant's phenotype. Therefore, CYCD3 does not act downstream of LGO in regulating the endoreduplication in the sepal.

The Differentiation of MGE from Mouse Embryonic Stem Cells

Leslie M. Tong

Mentors: David Hansen, Arturo Alvarez-Buylla, and Marianne Bronner-Fraser

The aim of this project is to derive and characterize medial ganglionic eminence (MGE) from mouse embryonic stem cells (mES cells). The MGE is an embryonic population of neuronal precursor cells that migrate extensively into the striatum and neocortex and ultimately differentiate into GABAergic interneurons. Disorders of these inhibitory interneurons can lead to conditions such as epilepsy or Parkinson's Disease, and cell-based therapies to restore this GABA-mediated inhibition have shown promise in mouse studies. To develop a differentiation protocol, four transcription factors known to be involved in normal MGE development—*Dlx2*, *Gsh2*, *Lhx6*, and *Nkx2.1*—are used. They are delivered by lentivirus, retrovirus, and if time allows, direct protein incubation to pluripotent mES cells and mES cells differentiated into neural stem cells by previously established protocols. The resulting cells are assayed by immunocytochemistry for the transcription factors of interest, GABA, GAD enzymes, and neuronal markers, as well as electrophysiology. The outcome of these experiments will contribute to our understanding of MGE differentiation and if successful, can be expanded to human ES lines and ultimately to clinical applications.