Session C – Biology
(Alphabetical)

Engineering Immunity – A T-independent Immunogen to Elicit Programmed Broadly Neutralizing Antibody Response against HIV
Kiefer P Aguilar
Mentors: David Baltimore and Kenneth Yu

An ongoing project against HIV in the Baltimore Lab is a gene therapy approach to combating the virus by programming B cells with a "Synthetic Switch" antibody gene that both secretes antibodies and expresses surface B-cell receptors (BCRs). The secreted antibody is an engineered variant of the broadly neutralizing anti-GP120 B12 antibody, which targets a highly conserved region of the GP120 HIV surface protein. The corresponding anti-GP120 BCRs are chimeric with both IgM (transmembrane) and IgG (extracellular) isotype fragments, functioning overall as an IgM receptor and in the secreted form as an anti-HIV IgG. The current project aims to develop a method of activating these B-cells through a T-cell independent (TI) pathway for two reasons: (1) patients in a late HIV infection are T-cell depleted, and hence cannot depend on T-cell dependent (TD) pathways of activation of these B-cells; (2) the animal model (mice) in which these B-cells will be tested could have dysfunctional human T-cells because human T-cells are trained on mismatched mouse MHCs, causing them to be unlikely to recognize antigens. As HIV is a TI-poor immunogen, the construction of an artificial TI immunogen for the specific activation of recombinant B12 B-cells is required. Methods for creating polyvalent GP120 (HxBc2 core) immunogens that may activate B-cells through B-cell receptor crosslinking in a TI pathway are described.

Light-Mediated Modulation of a Phosphorelay to Study the Initiation of Sporulation
ZeNan Chang
Mentor: Michael Elowitz, Joe Levine, and Avigdor Eldar

Bacterial sporulation, the process wherein stressed bacterial cells transform into resistant endospores, provides an excellent model system to study how cells make differentiation decisions. In the model bacterium *Bacillus subtilis*, various histidine kinases activate sporulation by phosphorylating the sporulation master regulator Spo0A. These kinases potentially provide a convenient point at which to perturb the sporulation network, but the small molecules which regulate their activity are still unknown. We have pursued an alternative synthetic approach, where the kinases were engineered so that their activities can be controlled optically through a light sensitive LOV domain. We successfully developed and demonstrated protocols to control the activity of these proteins in a time-lapse microscopy setup, allowing us to simultaneously monitor and perturb dynamic gene expression at a single cell resolution. These perturbation protocols open up the possibility of probing various aspects of the natural sporulation network, and also of understanding synthetic versions that may have novel properties.

Testing Whether the Innate Immune System Selects Against Influenza Viruses with CpG Motifs
Veena Chavakula
Mentors: David Baltimore and Jesse Bloom

Influenza is an infectious disease, caused by an RNA virus, contagious among birds and mammals. The influenza virus genome is composed of eight single-stranded and negative-sense viral RNA segments that encode eleven proteins. Membrane-bound or cytoplasmic pattern-recognition receptors (PRRs) trigger the immune system's interferon response by recognizing viral components. It has been noted that the influenza virus suppresses CpG dinucleotides. This project hypothesizes that the virus avoids CpG motifs because they elicit stronger interferon responses when recognized by PRRs. To validate this idea, we test the effects of CpG motifs on the interferon induction of PB1-deficient viruses. Green fluorescent proteins (GFPs) with various numbers of CpG motifs (low, regular, high) are inserted flanked by sequences from the termini of the PB1 gene segment. Cells expressing a full PB1-protein are then infected with the GFP viruses. RNA is collected from the infected cells, converted to cDNA by reverse transcriptase PCR. We then used qPCR to analyze the expression of IFNb (a type I interferon protein) and Mx1-1(an interferon-induced gene) whose upregulation would indicate induction of the interferon response. Based on these findings, it can be determined whether the reason the trend of decreasing CpG motifs occurs is due to the immune system's interferon response.

Molecular interactions between IKappaB kinase and REST
Allen Chen
Mentors: Paul Patterson and Ali Khoshnan

Repressor element 1-silencing transcription factor (REST) is a transcriptional repressor that plays a pivotal role in neuronal stem cell differentiation. REST levels decline during early neural differentiation. Recently, REST has been shown to be a substrate of E3 ubiquitin ligase, βTRCP. The presence of a phosphodegron motif within REST suggests that upstream kinases may direct REST degradation. The goal is to identify

the kinases that may be responsible for the phosphorylation of REST. Here we co-express REST with IkappaB kinase (IKK) and examine if it can promote degradation of REST. Preliminary results in HEK293 transfected cells indicate that REST co-immunoprecipitates with IKKα and IKKβ. Efforts are currently underway to confirm the preliminary results. Understanding the signaling pathways that activate the degradation of REST could provide insight into neurogenesis.

Novel Methods to Study miR-34a Loss-of-Function in Murine Hematopoiesis
Theresa L. Geiger
Mentors: David Baltimore and Dinesh Rao

Recently, a new facet of regulatory function in vertebrates—microRNA-mediated regulation of gene expression—has been uncovered. One way to explore this regulatory function of microRNAs is to perform loss-of-function analyses by achieving microRNA knockdown. Traditional approaches to microRNA knockdown have involved gene targeting and the generation of knockout mice, but these approaches require large investments of time and effort. In this project, we seek to develop an alternative approach to miR-34a knockdown, which has been shown to play a part in the p53 tumor suppressor network (He L, et al., Nature Reviews, 2007). To understand miR-34a function in hematopoiesis and cancer, this project explores novel methods of miR-34a knockdown based on consideration of microRNA biogenesis. Briefly, decoy target sequences for miR34a were introduced into 70Z/3 cells (mouse B-cells) and mouse bone marrow cells. The infected bone marrow was then used to reconstitute lethally irradiated mice. The effects of the decoy sequences were then studied in vitro by qPCR of the 70Z/3 cellular RNA and in vivo by FACS analysis of the peripheral blood of the reconstituted mice.

VENs and Differential Expression of Genes Between Autistic and Control Samples
Pengsu Jiang
Mentors: John Allman and Nicole Tetreault

Autism is a behavioral disorder that involves repetitive behavior, the impairment of social interaction and communication, and other atypical behavioral patterns. There is a strong genetic component connected to autism, although the details of differential gene expression are yet to be discovered. The recently discovered Von Economo Neurons (VENs) have also been shown to be highly involved in autism and are distributed differently in autistic patients versus non-autistic individuals. Through Immunohistochemistry (IHC), antibodies are used to label specific proteins or receptor molecules in attempts to identify which molecules specifically label the VENs in autistic and control individuals. By labeling specific proteins, it is then possible to identify the genes that code for the specific proteins and analyze whether or not the gene is significant in its involvement with autism.

Purification of MECP2 and Characterization of the IKKα-MECP2 Interaction
James Li
Mentors: Paul Patterson and Ali Khoshnan

First described by the Australian pediatrician Andreas Rett, Rett Syndrome (RTT) is a neurodegenerative disease characterized by deceleration of head growth, speech disability, stereotypical hand washing movements and general autistic features. The cause of RTT is a mutation in the methyl-CpG-binding protein (MECP2), which binds to DNA and can act as a transcriptional regulator by recruiting chromatin remodeling complexes. Previous studies have shown that IκB kinase alpha (IKKα), a crucial player in NF-κB activation, is able to regulate chromatin structure. *S*tudies have shown that IKKα is able to elevate the levels of MECP2 and can phosphorylate MECP2 *in vitro*. MECP2 has multiple phosphorylation sites and it is not known where and how IKKα can interact with MECP2. The goal of this project is to study the interactions between IKKα and MECP2. Human MECP2 was expressed in *E. coli* and purified using affinity chromatography. We are currently conducting kinase assays using MECP2 and active IKKα. Results will be analyzed by mass spectrophotometer and other techniques.

Single Molecule Fluorescence in situ Hybridization in Studying Pluripotency in Mouse Embryonic Stem Cells
Kasra Rahbar
Mentors: Michael Elowitz, Fred Tan, and John Yong

Several trancription factors control and maintain pluripotency in mouse embryonic stem (ES) cells. How these factors regulate each other and their target genes is of importance in understanding the intricacies of pluripotency in ES cells. Using single molecule fluorescence in situ hybridization (FISH), the mRNA expression of any gene of interest involved in pluripotency can be tracked at a single-cell level. The fluorescence is observed as dots in the cytoplasm, each corresponding to a single mRNA molecule. In order to precisely count all the mRNA in every cell, widefield microscopy and deconvolution were used to produce three-dimensional z-stacks to image the entire volume of each cell. Other computational tools such as cell segmentation and dot counting software was developed to automatically analyze these

images. Initial FISH studies targeted Nanog mRNA, a transcription factor involved in pluripotency, and Citrine mRNA, which is a fluorescent protein. These studies were performed on ES cells containing a Nanog reporter gene which expresses an H2B-Citrine fusion protein. Preliminary results indicate that the level of Nanog and Citrine mRNA expression as determined from FISH is somewhat correlated to the level of expression of H2B-Citrine (i.e. the level of Nanog promoter activity). Conclusive results will soon be attained as methods are refined. Future FISH studies will be expanded to perform multi-color studies correlating the mRNA expression of multiple genes.

Improving Segmentation of Time-Lapse Microscopy Image Analysis and Cell Morphology Dependent Simulations of Lateral Inhibition Patterns
Arjun Ravikumar
Mentors: Michael Elowitz and David Sprinzak

Despite the widespread use of time-lapse microscopy for the quantitative assessment of the dynamics of genetic circuits, image analysis of the movies produced suffers in the process of differentiating, or segmenting, individual cells. To reduce the error of the current segmentation algorithm, a new technique was explored, which identifies the local maxima of fluorescence intensity, and then uses them to view the image as a surface. Therefore, it readily recognizes ridge lines as the desired edges of cells. When the algorithm is completed, the expectation is for at least a 50% reduction of segmentation errors. The second portion of the research involves the lateral inhibition patterns in cells, produced by the Notch-Delta signaling pathway. These patterns have been mathematically modeled with the simplification of cells as regular hexagons. To determine the effect of the morphologies of cells on these patterns, an algorithm is being developed to create an irregular array of cells. Repeated point mutations and subsequent energy minimizations applied to an initial lattice of regular hexagons will produce a lattice we expect will match the edge length statistics of natural tissues. The existing model can then be adapted for this array to study the pathway’s dependence on cell morphology.

Characterization of Dendritic Cell Behavior in Response to Toll-Like Receptor Stimulation
Qing Yu Weng
Mentors: David Baltimore and Lili Yang

Dendritic cells (DC) are probably the most important antigen-presenting cells to stimulate the adaptive immune system in mammals. DCs are derived from hematopoietic stem and progenitor cells in the bone marrow that differentiate into distinct subsets characterized by markers, function, and distribution in the body. Upon maturation, DCs are able to migrate to draining lymph nodes, where they induce the adaptive immune response via lymphocyte recruitment and stimulation. This experiment investigates the impact of maturation signal on DC behavior through characterization of DC migration pattern and evaluation of cell surface marker levels. DCs harvested from B6 mice are stimulated through eight subtypes of toll-like receptors (TLR1-TLR7 and TLR9). Using flow cytometry, the levels of cytokines and other markers associated with each TLR stimulation pathway can be characterized Through *in vivo* monitoring of GFP and firefly luciferase (Fluc)-labeled BMDCs, it is possible to study the effect of each TLR activation signal on DC trafficking and dynamics within the body. The impact of *in vivo* versus *in vitro* administration of the TLR agonist was also compared. Different maturation signals were found to lead to different marker levels, and different TLRs may stimulate distinct *in vivo* behavior of DCs.