

Biology **MIT** 2007 Undergraduate Research *Symposium*

All are welcome!

Invited Speakers:

1:00 pm

Opening Remarks

1:15–2:15 pm

Brianne Holmbeck, Chisholm lab

Chin Chin Yao, Orr-Weaver lab

Jerry Trejo, Bartel lab

Laura Jacox, Sherley lab

2:25–3:10 pm

Heidi Holley, Sive lab

Liz Gillenwater, Krieger lab

Ryan Pester, Jacks lab

3:20–4:05 pm

Rishi Puram, Sharp lab

Shuai Chen, Rich lab

Stephanie Wang, Lodish lab

4:15 pm

Presentation of Awards

*Thursday,
February 1st
1:00-4:30 pm
Room 68-181
refreshments served*

Organized by:

The Biology Undergraduate Committee

Biology Undergraduate Student Association (BUSA)

Biology Education Office

Sponsored by the Howard Hughes Medical Institute

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Mutation Frequencies and Mutagenesis of *Prochlorococcus*

Brianne Holmbeck, Marcia Osburne, Penny Chisholm

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The cyanobacterium *Prochlorococcus* is the smallest oxygenic phototroph known and plays an important role in the global carbon cycle. It numerically dominates the phytoplankton community in the tropical and subtropical ocean basins, and is responsible for up to 79% of primary productivity in the North Atlantic. The *Prochlorococcus* community encompasses related strains, roughly designated as high-light and low-light, each with different depth distributions in the ocean. These “ecotypes” define distinct ecological niches with respect to light and nutrients. Obtaining useful mutants of *Prochlorococcus* is important for understanding many aspects of its biology and ecology. Yet this process remains difficult due to the lack of a robust genetic transfer system. We first attempted (unsuccessfully) to select faster-growing mutants of high-light strain MED4 by serially transferring a culture into fresh medium, assuming that fast-growing variants would eventually dominate. We then sought to determine whether MED4’s spontaneous mutation rate was high enough to select for mutants, and then to develop a mutagenesis procedure to increase the mutation rate and introduce multiple mutations.

Because *Prochlorococcus* does not form colonies efficiently, we devised a liquid culture method to estimate the spontaneous mutation rate of resistance to the antibiotics rifampicin, kanamycin or ciprofloxacin, each of which has a different cellular target. Resistance to these antibiotics is caused by a single base change within the genome. The cell density at which resistant mutants appeared enabled us to approximate the mutation rate for MED4 (1 in 10^7 to 10^8 cells). We then assessed several other strains, including a low-light variant, and found their mutation rates to be similar.

We next devised a protocol to mutagenize *Prochlorococcus* with UV light, resulting in a 100-fold increase over the spontaneous mutation rate. We are now analyzing multiply-mutagenized cultures and will use them to select for mutants of interest.

Faculty Supervisor: Penny Chisholm
Mentor: Marcia Osburne

Genetic Screen for Enhancers and Suppressors of *Cortex* in *Drosophila melanogaster*

Chin Chin Yao, Jillian Pesin, Terry L. Orr-Weaver

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Cortex is an important gene controlling completion of female meiosis in *Drosophila melanogaster*. Mutations in this gene cause sterility in females: fertilized eggs abnormally arrest in meiosis II and never progress to embryogenesis. Besides *grauzone*, a transcriptional activator of *cortex*, no other genes have been identified thus far that function in the same pathway as *cortex*. In this study, a deficiency screen was conducted to identify genes that have a strong genetic interaction with *cortex*. Interactions were detected by examining progression through meiosis and embryogenesis in eggs laid by *grauzone* mutants with various chromosomal segments deleted. *Grauzone* mutants were used to examine alterations to the *cortex* phenotype because *grauzone* mutants exhibit an intermediate *cortex* phenotype by only transcribing low levels of *cortex*. Any genes in the deleted regions that normally interact with *cortex* would cause a visible enhancement or suppression of the *cortex* phenotype. From this screen, it was found that at least one gene between cytological breakpoints 85A1 and 85C2 on the right arm of the third chromosome suppresses the *cortex* phenotype. Fully characterizing the *cortex* suppressors located in this region would elucidate the mechanistic pathway by which *cortex* regulates meiotic completion.

Faculty Supervisor: Terry L. Orr-Weaver
Graduate Student Mentor: Jillian Pesin

Validating the Downregulation of Protein-coding mRNAs by Small Silencing RNAs

Jerry Trejo, Ramya Rajagopalan, David P. Bartel

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Small silencing RNAs are post-transcriptional regulators that affect gene expression. They range in size from approximately 20-25 nucleotides in length, and have a large role in the sequential and orderly expression of genes necessary for proper development. Several small silencing RNAs have been recently identified in *Arabidopsis thaliana*,[†] but thorough characterization of their targets has not been previously explored. To assess the biological significance of this group of novel silencing RNAs (which include miRNAs and tasiRNAs), we performed a comprehensive search of the *A. thaliana* genome and identified a wide range of candidate targets. We subsequently employed a PCR-based validation assay to detect the cleavage products of the computationally predicted targets in cDNA libraries made from RNA extracts of tissues harvested from the organism. In total, four miRNA cleavage targets were validated in cDNA libraries made from silique tissue: *CMT3*, *AGL16*, *MYB113*, and *TAS4*. *CMT3* encodes for a cytosine methyltransferase, while *AGL16*, and *MYB113* encode for transcription factors. Interestingly, the *TAS4* target is a newly predicted tasiRNA locus, and one of the tasiRNAs thought to originate from that locus, tasiR81(-), was found to direct the cleavage of PAP2, a regulator of anthocyanid and phenylpropanoid genes. In addition, the isolation of an intronic cleavage product originating from the *DCL1* pre-mRNA (*DCL1* is necessary for miRNA maturation) establishes a model of the homeostatic self-regulation of this critical component of the miRNA biogenesis pathway.

[†] Rajagopalan, R., Vaucheret, H., Trejo, J., and Bartel, D.P. (2006). A diverse and evolutionarily fluid set of miRNAs in Arabidopsis thaliana. *Genes Dev.* 20 (24), 3407-3425.

Faculty Supervisor: David P. Bartel
Graduate Student Mentor: Ramya Rajagopalan

Evidence for Immortal Strand Cosegregation in Human Liver Adult Stem Cells

Laura Jacox, James Sherley

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Technology, Cambridge, MA 02139, USA

Cairns hypothesized that asymmetrically dividing adult stem cells (ASCs) selectively segregate template chromosomes to the ASC daughter to prevent the accumulation of DNA replication errors. By labeling cells with BrdU and allowing them to grow for several days before cytochalasin D treatment, we could determine whether BrdU labeled chromosomes were equally distributed between daughter nuclei or whether they were selectively segregated to one. We found evidence in support of Cairns’ “immortal strand hypothesis” as there were binucleates that retained label in only one nucleus. Our results provide the first evidence of immortal strand cosegregation in humans and offer greater understanding of how ASCs divide *in vivo*.

Faculty Supervisor: James Sherley

The Role of the Basement Membrane in Shaping the Zebrafish Brain

Heidi Holley, Jennifer Gutzman, Hazel Sive

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Morphogenesis (“generation of shape”) of the vertebrate brain begins early in development, and is required for normal brain structure and function. The mechanisms underlying brain morphogenesis are complex and likely to be evolutionarily conserved. The neuroepithelium that comprises the neural tube (the developing brain) is tightly associated with the basement membrane, a meshwork of extracellular matrix (ECM) proteins that provides a foundation for cell adhesion and organization during tissue morphogenesis. Using zebrafish as a model, we investigated the roles of the laminin-rich basement membrane in shaping the brain by studying the zebrafish laminin mutants, *sleepy* and *grumpy*. We found that laminin is necessary for the formation of the sharp constriction on the outside of the neural tube at the midbrain-hindbrain boundary (MHB). The MHB constriction is required for normal brain bending and packing in the skull, and delineates the future cerebellum from the tectum. Immunohistochemistry for laminin revealed that, in contrast to the sharp MHB bend in wild type embryos, in laminin mutants, this region is only gently curved. No abnormalities in expression of patterning genes or differences in the amount of cell proliferation or apoptosis at the MHB were observed between wild type and laminin mutant embryos, suggesting that these processes are not important to form the MHB constriction. Confocal imaging suggests that cell shape changes at the MHB may enable the neuroepithelium to fold sharply in wild type embryos. We propose that the MHB constriction forms via a two step process, whereby a laminin-independent step leads to an initial, shallow bend and that a subsequent laminin-dependent step is required to sharpen the constriction. Understanding the mechanism by which laminin helps shape the brain is likely to provide insight into neurodevelopmental disorders associated with abnormal brain structure.

Faculty Supervisor: Hazel Sive
Postdoc Mentor: Jennifer Gutzman

The Role of Hepatic SR-BI in Cholesterol Metabolism and Atherosclerosis Protection

Liz Gillenwater, Ayce Yesilaltay, Monty Krieger

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Technology, Cambridge, MA 02139, USA

Epidemiological studies show a strong inverse correlation between high density lipoprotein (HDL) plasma levels and atherosclerosis. Scavenger receptor class B type I (SR-BI) is a membrane bound receptor that mediates many of the atheroprotective effects of HDL, including the process of reverse cholesterol transport in which cholesterol is moved from the peripheral cells of atherosclerotic plaques to hepatic and steroidogenic tissues via HDL. In order to determine the role of hepatic SR-BI in cholesterol metabolism and atherosclerosis protection, we expressed liver-specific SR-BI transgenes in SR-BI/ApoE double knockout (dKO) mice. Expression of near physiological levels of hepatic SR-BI (~0.7x wt, lowTg) does not significantly alter total plasma cholesterol compared to dKO mice, but does result in a significant reduction in the free to total plasma cholesterol ratio (FC:TC) to levels near the FC:TC ratio of ApoE KO mice. In contrast, over-expression of hepatic SR-BI (40xwt, highTg) results in a significant decrease in both total cholesterol and the FC:TC ratio. Consistent with the respective plasma cholesterol levels of dKO, lowTg, and highTg mice, the lipoprotein profiles of the lowTg mice and dKO mice are similar, while the lipoprotein profiles of the highTg mice show reduced cholesterol levels in lipoproteins of all sizes compared to the dKO mice. Preliminary analysis of coronary arteries in the hearts of the lowTg and highTg mice indicates very low levels of damage, as observed by lipid staining of the sections, in comparison to the hearts of dKO mice. Therefore, hepatic SR-BI accounts for a majority of the atherosclerosis protection ascribed to SR-BI, which is at least partially due to the role hepatic SR-BI plays in regulating the free-cholesterol content of lipoproteins.

Faculty Supervisor: Monty Krieger
Postdoc Mentor: Ayce Yesilaltay

Loss of Dicer1 Increases Transformation and Affects the Growth Rate Of K-rasG12D Mouse Embryonic Fibroblasts

Ryan Pester, Madhu Kumar, Tyler Jacks

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A recent paper by Jun Lu et al. (Nature, 2005) described a global reduction of miRNAs in human tumor as compared with normal tissue. This raises the question of whether the loss of miRNAs plays a functional role in tumorigenesis. Dicer1 is an RNAaseIII-like enzyme which is involved in the processing of pre-miRNA to mature miRNAs. By knocking out Dicer1, you can affect an overall loss of miRNAs to examine its effects on tumorigenesis in different cancer models. We tested Dicer1’s role in tumorigenesis by using the Dicer1 conditional knockout allele (Dicer flox) in conjunction with the lox-stop-lox (LSL) K-rasG12D allele. This allele contains an oncogenic form of K-ras placed behind a transcriptional stop cassette flanked by loxP sites. When cre-recombinase is added, the stop cassette is removed and oncogenic K-ras is expressed. In mice, intranasal infection with Adenovirus expressing cre-recombinase has been shown to produce non-small cell lung cancer with complete penetrance and a definite time course. This provides us with a well-defined cancer model with which to test Dicer1 loss of function. We tested the loss of Dicer1 in vitro by generating LSL-K-rasG12D MEF lines with different numbers of the Dicer conditional knockout alleles (i.e. Dicer +/+, +/-flox, and flox/flox). Loss of Dicer increased the transformation potential of K-rasG12D MEFs in soft agar assays. We also expressed Myc and E1A ectopically in the cells to observe whether Dicer deletion cooperates with these oncogenes. Growth curves indicated that loss of Dicer in conjunction with Myc bestows a greater growth rate while loss of Dicer in conjunction with E1A decreases the growth rate in K-rasG12D MEFs.

Faculty Supervisor: Tyler Jacks
Graduate Student Mentor: Madhu Kumar

RNAi-based Therapeutic Strategies in a Fig-Ros Model of Glioblastoma Multiforme

Rishi Puram, Alain Charest, Phillip Sharp

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Institute of Technology, Cambridge, MA 02139, USA

Aberrant expression of the receptor tyrosine kinase (RTK), Fig-Ros, is a well-characterized oncogenic event in high-grade astrocytomas, including glioblastoma multiforme (GBM). Ectopic expression of Fig-Ros leads to the activation of a well-defined intracellular signaling axis that includes SH2 domain-containing Phosphatase 2 (Shp-2), Phosphatidylinositol 3-Kinase (PI3K), and Mammalian Target of Rapamycin (mTOR) to promote oncogenic transformation in both *in vitro* and *in vivo* systems. We sought to determine the effect of RNA interference-mediated silencing of Fig-Ros and molecules in its signaling axis, including the Shp-2 tyrosine phosphatase, on the *in vitro* growth rates of Fig-Ros-derived GBM cell lines and on their ability to initiate tumors *in vivo*. We generated GBM cell lines stably expressing short-hairpin RNA (shRNA) sequences targeting the Shp-2 or Fig-Ros transcripts and subsequently identified cell lines with significant protein suppression. While we did not observe reduced growth of cells with down-regulated Fig-Ros *in vitro*, our initial results indicate that these cells have lost the ability to initiate tumors upon subcutaneous xenograft injection in nude mice. We are currently applying the same experimental approach to investigate the signaling networks of other oncogenic RTKs in GBM, including the epidermal growth factor receptor (EGFR) and the constitutive, ligand-independent variant, EGFRvIII. Beyond examining the roles of RTK signal transduction in solid tumor biology, we also identified a number of effective lipid-derived nanoparticles for the delivery of small-interfering RNAs (siRNA) to GBM cells *in vitro*. Our results offer insights into identifying suitable clinical targets to reverse the course of neoplastic transformation in GBM and present a number of potentially effective lipid-based vectors for the delivery of siRNA in *in vivo* therapeutic systems.

Faculty Supervisor: Phillip Sharp
Postdoc Mentor: Alain Charest

Chromatin Remodeling by Z-DNA

Shuai Chen, Ben Wong, Alexander Rich

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DNA is normally found in the B conformation, but repetition of CG dinucleotides can result in formation of Z-DNA. Z-DNA is left-handed, negative supercoiled and a more rigid conformation compared to B-DNA. Z-DNA in upstream regulatory regions of genes has been shown to increase transcription. Examples of Z-DNA gene activation in humans include c-myc, HO-1 and CSF1. We wanted to elucidate the mechanism by which Z-DNA increases transcription. Using beta-galactosidase activity assays in yeast *Saccharomyces cerevisiae*, we found the extent of this enhancement to be promoter specific and dependent on Z-DNA location relative to the TATA box. It is not affected by changes in helical phasing relative to the TATA box and the transcriptional enhancement does not follow a linear pattern. Using a nucleosome scanning assay, we determined that Z-DNA enhances transcription through regulation of nucleosome location. The rigid structure of Z-DNA along with its left-handed structure prevents nucleosomes from forming and is found in the linker DNA between two nucleosomes. The Z-DNA in chromatin demarcates nucleosome boundaries to expose the promoter region at the DNA-entry and dyad positions on the nucleosome. This nucleosome-positioning by Z-DNA mediates higher accessibility to the TATA box and therefore enhances transcription. Our findings suggest Z-DNA's role in the genomic code of nucleosome positioning *in vivo*. Z-DNA transcriptional control might be involved in diseases such as lupus erythematosus, rheumatoid arthritis, and cancer metastasis and further research needs to be performed to fully understand Z-DNA's role in the etiology of these diseases.

Faculty Supervisor: Alexander Rich
Postdoc Mentor: Ben Wong

Establishment of Stable CD8+ T cell Clonal Lines that Ectopically Express microRNAs

Stephanie Wang, Dr. Beiyan Zhou, Harvey Lodish

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MicroRNAs (miRNAs) are a family of non-coding RNAs that are about 22 nucleotides long. miRNAs have been found in both plants and animals, and are crucial for many biological processes, including that of hematopoiesis. Based on our microarray results of global miRNA expression levels in primary and clonal T cells, we chose to focus on four miRNAs for further analysis of their regulatory roles in T cell activation and function. We have established 8 stable CD8+ T cell clonal lines that ectopically express these four miRNAs. To do so, we infected two CD8+ T cell clonal lines with a retrovirus containing the specific hairpin and flanking sequences of each miRNA of interest. RT-PCR analysis of both the infected and uninfected T cells indicated that the levels of the exogenously introduced miRNAs were between 11 - 66 fold higher in most of the infected T cells as compared to the original uninfected T cell clones. These newly generated CD8+ T cell clones that express high levels of ectopically-introduced miRNAs will thus serve as a model to visualize, on a magnified scale, the effects of the miRNAs on mature T cell function. To further investigate this topic, we measured cytokine secretion in both parental and derived cell clones using quantitative RT-PCR. We will also measure the actual protein levels of these cytokines in the cell lines by western blot analysis, and we will study the cytolytic activity of CD8+ T cells by measuring the lactate dehydrogenase activity released from damaged target cells. In addition, we will quantitate the RNA expression levels (in our cell lines) of certain predicted target genes that code for proteins involved in the function of mature T cells. These studies will allow us to gain a better understanding of the mechanism of miRNA regulation in T cell function.

Faculty Supervisor: Harvey Lodish
Postdoc Mentor: Beiyan Zhou