Contents

2 Shuo Han
A Game of Switches: Epigenetic Regulation of Variegating Cell Wall Genes in Yeast

3 Scott Chilton
Expression of the Duplicated pstS Phosphate Transport Gene in Prochlorococcus sp. MIT9301

4 Samir Zaidi
Oct-4 Expression Is Dispensable for Somatic Stem Cell Self Renewal and Tissue Maintenance

5 Lauren McLendon
Role of Fibronectins Containing EIIIA and EIIIB in Growth Factor Signaling

6 Jonathan Fu
Differential Cleavage Patterns of FGF Ligands Neuregulin, TGF-α, and HB-EGF

7 Hanna Kuznetsov
The Role of Osteopontin in Systemic Instigation of Indolent Tumor Growth

8 Dan Yuan
Non-homologous End-joining in Sinorhizobium meliloti

9 Colleen Mosley
Variability of Synaptic Homeostasis in Hyperexcitable Neurons in vivo

10 Andrew Glazer
Planarians as a Model for the Study of Regeneration and Behavior

11 André Green
Conserved Secondary Elements of the Dengue Virus 3' UTR are Sufficient to Confer Significant Partial Viral Translation

12 Alia Carter
Investigation of the Advantage of the Apc^{Min}, Apc^{Ae14}, and Apc^{null} Alleles to Tumor Formation in Colorectal Mouse Models

A Game of Switches: Epigenetic Regulation of Variegating Cell Wall Genes in Yeast

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Many cell wall genes in *Saccharomyces cerevisiae* variegate by generating heterogeneous cell-surface protein expression from a genetically identical population. This cell-surface variation is regulated by the binding of the trans-acting factors, such as histone acetyltransferases (HATs) and histone deacetylases (HDACs), to histone proteins, which alter their interaction with the gene promoters. All variegating genes tested here are non-essential. Each gene had been replaced at its normal chromosome site with either a *GFP* or a *URA3* construct driven by its normal promoter to report visually the gene's activity. This study investigated the phenotypic consequences of knocking out each of eleven potential trans-acting factors (HATs and HDACs) on each of eight variegating yeast cell wall genes. The cell wall genes tested here are *FIT2*, *FIT3*, *AGA1*, *SAG1*, *DSE1*, *DSE2*, *FIG1*, and *FIG2*. The potential trans-acting factors examined are *GCN5*, *SAS2*, *SPT10*, *HOS1*, *HOS3*, *HST3*, *HST4*, *SFT3*, *SIR2*, *SIR3*, and *SIR4*. We identified several trans-acting factors, the on and off switches, that affected the switching of each of the eight variegating cell wall genes. Many of the switches found behaved as reported in the literature, whereas others behaved differently. The inconsistency between our results and those previously reported in literature indicates that the interaction between the switches and the promoters is not always direct. Furthermore, our findings suggest that epigenetic regulation, more specifically, the mechanism of histone modification, generates cell surface variations in yeast. The ability for cells to exhibit a diverse cell surface expression by variegating nonessential genes is a heritable characteristic, which may confer benefits to individual cells within a genetically homogenous population.

Faculty Supervisor: Gerald Fink
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Expression of the Duplicated *pstS* Phosphate Transport Gene in *Prochlorococcus* sp. MIT9301

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The marine cyanobacterium *Prochlorococcus* is the most abundant oxygenic phototroph on the planet and as such is believed to play an important role in the biogeochemical cycles of carbon, phosphorous and other elements. *Prochlorococcus* thrives in low nutrient regions of the ocean, and genetically distinct ecotypes have evolved different mechanisms for acquiring dilute nutrients. The availability of phosphorous, in particular, limits growth in many regions of the ocean. Here we examine *Prochlorococcus* strain MIT9301 which has two copies of the *pstS* gene, which encodes a phosphate binding protein involved in phosphate uptake. One copy is located in a genomic island and may have been acquired by gene duplication or horizontal gene transfer. We grew MIT9301 under phosphate-limiting and phosphate-replete conditions and measured gene expression during a 48-hour time course. The two copies of *pstS* were differentially expressed: the island-encoded version was clearly upregulated, while the other was minimally expressed, in response to phosphorous starvation. These results suggest that the two copies might be functionally different, or one may be nonfunctional. Thus gene acquisition and regulatory evolution are both important components of adapting to low nutrient conditions.

Faculty Supervisor: Penny Chisholm
Mentor: Maureen Coleman

Oct-4 Expression Is Dispensable for Somatic Stem Cell Self Renewal and Tissue Maintenance

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Oct-4 is a Pou domain-containing transcription factor known to regulate pluripotency of the inner cell mass of mammalian blastocysts and embryonic stem cells. After implantation, Oct-4 is inactivated through a series of repressive epigenetic modifications. Recent publications have however suggested that Oct-4 also maintains pluripotency in somatic stem cells of adults. To investigate whether Oct-4 is a regulator of somatic stem cells, we employed a Cre-lox recombination approach to efficiently inactivate the Oct-4 gene in a number of somatic tissues, including the intestinal epithelium, hair follicles, brain, liver and bone marrow hematopoietic and mesenchymal stem cells. We found no evidence of abnormal homeostasis or regenerative capacity in tissues lacking Oct-4.This study thus provides compelling evidence that Oct-4 is dispensable for somatic cell function and regeneration in the adult mammals, and that the pluripotency regulatory network functioning in embryonic stem cells does not exist in the stem cells of somatic tissues.

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Role of Fibronectins Containing EIIIA and EIIIB in Growth Factor Signaling

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Fibronectin is an extracellular matrix protein that exists in several alternatively spliced isoforms. The fibronectin splice variants containing the exons EIIIA and EIIIB are upregulated during tumorigenesis, wound healing, and angiogenesis[1]. Because fibronectin receptors are critical components of growth factor signaling, we explored the potential roles of EIIIA/EIIIB containing fibronectins during signal transduction. To this end, we compared the phosphotyrosine profiles of fibronectin deficient fibroblasts plated on recombinant fibronectin fragments containing or lacking EIIIA/B stimulated with 5% bovine serum. Analysis of overall phosphotyrosine profiles using the monoclonal phosphotyrosine antibody 4G10 identified a band of approximately 46 kDa that was more prominently activated when cells were stimulated on EIIIA/B containing fragments. By using antibodies specific for activated forms of several MAPK family members, we determined that this band was the Jun N-terminal kinase, JNK. Further analysis demonstrated that inclusion of the EIIIA, rather than the EIIIB exon, is responsible for the differential activation of JNK in response to serum stimulation. We are currently working on identifying the growth factor and downstream components of the signaling pathway responsible for differentially activating JNK on EIIIA containing fibronectin isoforms. Elucidation of the pathways involved in differential responses to growth factor stimulation mediated by these variant extracellular matrix components will provide significant insights into numerous developmental and pathological processes.

Faculty Supervisor: Richard Hynes
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Differential Cleavage Patterns of EGF Ligands Neuregulin, TGF-α, and HB-EGF

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Metalloprotease (MMP) cleavage of type 1 transmembrane proteins is linked to the regulation of several signaling pathways and clinically relevant diseases, including cancer, neurodegenerative disease, inflammation, and cardiac hypertrophy. While inhibition of metalloproteases has had promising therapeutic effects in rodent disease models, our lack of understanding of regulatory mechanisms makes such therapies nonspecific, leading to detrimental side effects. Here we introduce a FACS-based assay that can monitor intracellular and extracellular shedding of model EGF transmembrane ligands: HB-EGF, Neuregulin, and TGF-α. Initial results show that these ligands respond to physiological stimuli, osmotic stress, phorbol ester, and G-protein activation, to different degrees and with varying kinetics. Neuregulin and HB-EGF are cleaved quickly in response to osmotic stress and phorbol ester but not to G-protein activation; meanwhile, TGF-α has a minimal response to all three stimuli. Our findings demonstrate that EGF ligands are differentially regulated by known stimuli, suggesting a future in specific inhibition therapies for metalloprotease-related pathologies.

Faculty Supervisor: Harvey Lodish
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The Role of Osteopontin in Systemic Instigation of Indolent Tumor Growth

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Tumors are not aggressively growing islands in an organism; instead, they interact with their host environments to obtain nutrients and recruit an extracellular matrix, blood vessels, and other normal cells to form the tumor stroma. A rich source of some of these supportive cells is bone marrow. To study the nature of this systemic interaction between tumor and bone marrow, an in vivo binary tumor xenograft model was designed. The right flank of a mouse was subcutaneously injected with "instigator" BPLER aggressive tumor cells, and the left flank was injected with "responder" HMLER indolent tumor cells. While responders injected contralaterally with controls remained indolent, those injected with instigators were activated to grow aggressively. Instigators activate the host bone marrow by a primary signal; the activated bone marrow then activates the responder. This primary signal was found to be osteopontin (OPN). Osteopontin is necessary for systemic instigation; when it was inhibited with shRNA, responders could not be instigated to grow. In order to test whether OPN was sufficient for this systemic instigation, I cloned the hOPN gene into the pBabe Hygro plasmid. We then transfected human 293T cells with this recombinant plasmid and plasmids carrying retroviral components. Non instigator PC3 cells were infected with the assembled retrovirus now carrying the hOPN gene. These cells were then injected into the right flank of a mouse contralaterally to responders. The responder tumors grew poorly, suggesting that while OPN is necessary for systemic instigation of indolent tumor growth, it is not sufficient. Future studies will search for other necessary components of the primary instigating signal, as well as determine the signal's timing. Results of these studies will further our understanding of interactions aiding tumor growth and metastasis, and will potentially lead to the development of new cancer drugs.

Faculty Supervisor: Robert A. Weinberg
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Non-homologous End-joining in *Sinorhizobium meliloti*

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Non-homologous end joining (NHEJ) is the main repair pathway for DNA double-stranded breaks (DSBs) in higher eukaryotes. Recently, an evolutionarily conserved pathway for NHEJ has been discovered in certain bacteria. The bacterial NHEJ is a two-component system comprised of DNA end-binding Ku protein and LigD, a multifunctional repair enzyme. Surprisingly, *Sinorhizobium meliloti* carries four genes encoding Ku homologs, which we have designated *sku1, sku2, sku3* and *sku4*. To study the role of NHEJ in vivo, we constructed deletions of each gene. Of the deletion mutants, *sku2* is the most strongly sensitized to ionizing radiation (IR), a treatment that induces DSBs. Reporter fusion analysis revealed that *sku2* is strongly expressed in stationary phase and during chronic infection of host plants. To visualize subcellular localization of Ku proteins in living cells, we fused Sku2 to YFP. IR treatment induced foci formation of Sku2-YFP in a dosage-dependent manner, suggesting that Sku2 localizes to DSBs in vivo. Interestingly, knockouts of unknown genes located downstream of *sku2* also confer sensitivity to IR. We are currently investigating the possible roles of these genes in NHEJ.

Faculty Supervisor: Graham Walker
Postdoc Mentor: Hajime Kobayashi

Variability of Synaptic Homeostasis in Hyperexcitable Neurons in vivo

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Neuronal homeostasis is a term that refers to the compensatory mechanisms by which a neuron changes its activity after changes in its environment or electrical properties in order to bring its activity back to a normal level. Mechanisms to maintain homeostasis include changing the firing properties of a neuron, altering amounts of excitation or inhibition in a neural circuit, and changing synapse strength or number. Using a genetic method, we changed the intrinsic excitability of individual new neurons and were able to study the compensatory mechanisms displayed in vivo in the rat olfactory bulb (OB). By introducing a prokaryotic voltage-gated sodium channel called NaChBac into immature granule cells (GC) in the OB, we examined how increased excitability affects synapse formation. NaChBac has a more negative activation potential and more positive inactivation potential than fast inactivating sodium conductances in neurons, so it causes the GCs to have more frequent and longer action potentials, and thereby increases their excitability. We examined synapse density in NaChBac versus control GCs to determine whether compensatory processes in these GCs would bring their excitability back to a more normal level in adult and postnatal animals. Adult-generated GCs integrate into a mature, functional circuit, while postnatal-generated GCs integrate into a developing circuit. We found that hyperexcitability did not affect synapse formation in the adult-generated GCs, but that there was a reduction in synapse density in postnatal-generated GCs. We also observed that whereas genetically-manipulated postnatal-generated GCs showed a reduction in excitatory postsynaptic currents, the same manipulation in adult-generated GCs did not show any change in these currents. These observations reveal that the homeostatic compensatory mechanisms active in postnatal- and adult-generated GCs operate in different ways.

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Planarians as a Model for the Study of Regeneration and Behavior

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S. mediteranea is a free-living, planarian flatworm able to regenerate any region of its body following wounding. Planarians are triploblastic and contain differentiated structures including a brain, sensory neurons, musculature, epidermis, a pharynx, and a digestive system. Planarians accomplish regeneration by the expansion of a population of adult stem cells called neoblasts, which can differentiate into essentially all adult tissues. Recently, new techniques have enabled genetic manipulation of planarians. A sequenced genome and the use of *in situ* hybridizations and RNA interference have allowed for the study and perturbation of planarian genes. These techniques now allow the exploration of planarian behavior on a molecular and cellular level. Planarian behavior is of long-standing interest because planarians can regenerate parts or the entirety of their nervous system. How does neuronal regeneration impact and restore behavior?

In order to establish planarians as a model organism for the study of behavior, a number of planarian behavioral paradigms were established. Methods were developed for the exposure of planarians to negative stimuli (shock, touch, light, chemical exposure), neutral stimuli (odors, textures), and positive stimuli (food, dark). Using these stimuli, a suite of planarian behaviors were explored. Planarians robustly display negative phototaxis, positive and negative chemotaxis, feeding responses, and avoidance responses to negative stimuli. Attempts were also made to modify these innate behaviors by stimulating the production of associative memories. Memory assays consisted of two periods: a training period and a testing period. During the training period, worms were exposed to paired stimuli, such as a neutral stimulus and a negative stimulus. After training, the worms were tested in various assays to determine if the training altered their innate behavior in the presence of the neutral stimulus. Using the scheme outlined above, a number of promising protocols for planarian learning were developed. While the results are still under investigation, the work will hopefully enable the utilization of molecular techniques for the study of behavior and regeneration.

Faculty Supervisor: Peter Reddien

Conserved Secondary Elements of the Dengue Virus 3' UTR are Sufficient to Confer Significant Partial Viral Translation

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Dengue virus (DENV), the prototypical member of the genus Flavivirus in the Flaviviridae family, is a positive-sense, single-stranded RNA virus that efficiently replicates in its host despite lacking a 3' polyadenylated tail. A number of reports indicate that conserved secondary structures and sequences within the 3' UTR play a role in enhancing DENV translation. We employ an in vitro transcribed RNA reporter transfection method to further characterize these structures of the DENV 3' UTR. Reporters were constructed with individual or pairs of conserved structural elements of the 3' UTR in order to determine the sufficiency of these structures for viral translation. Although no single structure conferred full viral translation, one pair of structures shows translation levels exceeding 50% of the full 3' UTR: the combination of CS1, a 10-nt cyclization sequence, and DB1, a bi-lobed stem loop. Additionally, DB2, a second bi-lobed stem loop similar to DB1, and the proximal and medial portions of the upstream variable region contribute considerably to translation. This work is the first to address the sufficiency of specific elements of the 3' UTR in the translation mechanism and suggests that certain elements may act as functional modules that enhance viral translation in cooperation with other viral or host factors.

Faculty Supervisor: Lee Gehrke
Mentor: Glover (Trei) Martin

Investigation of the Advantage of the Apc^{Min}, $Apc^{\Delta e14}$, and Apc^{null} Alleles to Tumor Formation in Colorectal Mouse Models

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Inactivation of the Adenomatous Polyposis Coli (APC) gene is responsible for the majority of sporadic colorectal cancers and the hereditary autosomal dominant disease, Familial Adenomatous Polyposis (FAP). FAP is characterized by an accumulation of benign colonic adenomas that progress to malignancy if left untreated. The most prevalent form of APC inactivation occurs through mutations resulting in various truncated protein products that are each associated with a particular phenotype of FAP. To investigate if truncated Apc is advantageous to intestinal tumor formation, we compared the two different truncation alleles, Apc^{Min} and $Apc^{\Delta e14}$, (truncated at codon 850 and codon 580, respectively) and the null allele, $Apc^{Null/+}$ in colorectal mouse models. Differences between each genotype were measured in tumor burden and tumor volume. $Apc^{\Delta e14/+}$ mice had a greater tumor burden than $Apc^{Min/+}$ mice in the proximal small intestine at 4 months of age. Similarly, $Apc^{Null/+}$ mice at 3 months of age exhibited a greater tumor burden in the proximal small intestine compared to age-matched $Apc^{Min/+}$ mice. However, no differences in tumor volume were found across each genotype of similarly age-matched mice. These data suggest that while a null genotype may be more beneficial to tumor development, the truncated Apc protein of the $Apc^{\Delta e14/+}$ is more advantageous to tumorigenesis than the $Apc^{Min/+}$ protein product. Our results lay the initial groundwork for studying the difference in tumor phenotypes associated with the myriad of Apc truncations, which would prove useful in understanding and treating colorectal cancers.

Faculty Supervisor: Tyler Jacks
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