

Session A – Biology (Alphabetical Order)

Determining New Early Transcription Factors Involved in FoxY Expression

Evelyn Chou

Mentors: Eric Davidson and Joel Smith

The study of gene regulatory networks in the developing sea urchin is a crucial step in understanding the mechanisms underlying embryogenesis. To further explore these mechanisms, a set of nine transcription factors in the *S. purpuratus* genome was characterized using quantitative PCR analysis. These gene expression time courses will be used to detect potential upstream or downstream transcription factors and to construct the gene regulatory network involved in regulating FoxY expression. Additionally, FoxY BAC-GFP injections coupled with morpholino knockdown of Notch resulted in a loss of FoxY expression in the bottle cells. Prior research shows that early expression of FoxY in sea urchins begins in the small micromeres and migrates to the bottle cells during initial invagination. We therefore hypothesize that Notch is expressed upstream of FoxY and mediates its expression in the bottle cells.

Endoplasmic Reticulum Exit Site (ERES) and Upregulated Nicotinic Receptor Dynamics

Pallavi Gunalan

Mentors: Henry A. Lester, Rahul Srinivasan, and Rigo Pantoja

The mechanism of cargo movement and endoplasmic reticulum exit site (ERES) formation has been studied, but never in conjunction with nicotine and cytosine-induced receptor upregulation. The Lester lab has studied neuronal nicotinic acetylcholine receptors (nAChRs), cationic channels found in the brain, using total internal reflection fluorescence microscopy (TIRFM) and Förster resonance energy transfer (FRET). The characteristic diffusive movements followed by seemingly directed transport of the puncta in these images implicate microtubules and associated motor proteins in assisting cargo transport from the ER to the Golgi. Using the microtubule disrupter nocodazole, and the Golgi disrupter Brefeldin A (BFA), we hope to understand the role of microtubules in transporting nAChRs after inducing upregulation using cytosine and nicotine.

Characterizing Host-Seeking and Chemotaxis Behavior in Insect-Parasitic Nematodes

Annie V Hong

Mentors: Paul Sternberg and Elissa Hallem

Insect-parasitic nematodes of the genera *Heterorhabditis* and *Steinernema* are currently used as biocontrol agents for many agricultural pests. Two foraging behaviors have been categorized. Cruiser foragers, like *H. bacteriophora*, move constantly in search for hosts while ambushers, like *S. carpocapsae*, are stationary until a host enters their proximity. We hypothesized that the different foraging behaviors of insect-parasitic nematodes may lead to distinct chemotaxis behavior. We have developed assay protocols to test a diverse odorant panel that includes constituent chemicals of plants and common insect hosts. We are currently identifying odors that elicit strong attractive and repulsive responses in parasitic worms. Once odors fitting the criteria are found, the corresponding neurons can be identified and their function investigated. Overall, our results are a necessary foundation to identifying the signaling pathways and neural circuitry that mediate odor response.

Analysis of the *Cis*-Regulatory Modules Governing Expression of *FoxA* in *Strongylocentrotus purpuratus*

Sharon R. Kuo

Mentors: Eric H. Davidson and Smadar Ben-Tabou de-Leon

The proper development of organisms is regulated by the gene regulatory networks that control the spatial, temporal, and quantitative expression of genes. In *S. purpuratus*, the transcription factor FoxA is an integral part of controlling endomesoderm development; FoxA promotes endodermal fate and prevents mesodermal fate in endodermal cells. The expression of a gene is governed by its *cis*-regulatory modules, and for the gene *foxa* to be expressed with spatial and temporal precision, several positive and negative inputs must be present. In order to determine the inputs necessary for *foxa* expression, reporter constructs are created to determine the location of the binding sites of the inputs. Data on expression levels is measured using qPCR and analyzed to determine the effect of each transcription factor on the expression of *foxa*. We have identified three direct activators of *foxa*: Brachyury, Hox 11/13, and GataE. Hox 11/13 initiates *foxa* expression, while Brachyury and GataE increase *foxa*'s expression level as endoderm specification begins. The *cis*-regulatory modules of *foxa* integrate these inputs and the inputs of other transcription factors to execute the accurate spatial, temporal, and quantitative expression of *foxa*.

Expanding the Sea Urchin Ectoderm Gene Regulatory Network

Micah J. Manary

Mentors: Eric Davidson and Enhu Li

Gene regulatory networks are a powerful way to understand developmental spatial and temporal gene specification. With the *Strongylocentrotus Purpuratus* endomesoderm network reaching completion, more attention must be given to completing the ectoderm specification network. After perturbing gene expression by injecting gene specific morpholino anti-sense oligonucleotides (down-regulation) and mRNA (up-regulation) into developing embryos, we can measure the effects on downstream genes using quantitative polymerase chain reaction and whole mount in-situ hybridization. The changes in total quantitative or spatiotemporal expression indicate new connections in the gene network that can then be verified by analyzing the gene sequence directly for known target binding sites, then mutating these sites to replicate the phenotype. We have found up-regulative connections between FoxG and the downstream genes Bra and FoxA in the oral ectoderm, as well as new inputs into the signaling molecule VegF3 in the oral and aboral regions, which are continuing to be characterized. The outputs from VegF3 are also beginning to be characterized, though further work is needed. These new connections bring the ectoderm network closer to completion and elucidate the regulation of complex expression patterns.

Caton: Spike Sorting Software for Multi-Site Probes

John Schulman

Mentor: Gyorgy Buzsaki

We present a method of doing spike sorting on electrophysiological data recorded from multi-site probes and a software package, called Caton, that implements it. We use the spatial layout of the recording sites in both detection and clustering. In the detection step, we look for sets of super-threshold samples that are contiguous in space and time. This allows us to detect simultaneous spikes in different parts of the probe. In the clustering step, we use maximum-likelihood EM algorithm with a mixture of Gaussians model, but we modify the covariance matrix estimation. The full-covariance-matrix mixture-of-Gaussians model gives poor results on high-dimensional data because of finite-sample issues. However, we make a conditional independence assumption that greatly reduces the number of parameters of the clusters, and we do maximum likelihood estimation using this restricted model. This allows the algorithm to maintain good performance on high-dimensional data. The program has been tested on data with up to 32 channels.

Detecting Multiple Oligomerization States by Multidimensional Analysis of FRET Images

Kim Scott

Mentor: Henry Lester

Fluorescence resonance energy transfer (FRET) microscopy is commonly used to measure distances between fluorophores or to qualitatively confirm interaction of proteins. Whereas multiple FRETing oligomerization states may be characterized clearly using single-molecule techniques, cellular applications suffer from wide and immeasurable variability in concentration of each species across pixels. Several examples are presented to demonstrate the inapplicability of Gaussian mixture model fitting to normalized FRET distributions to the problem of identifying populations of pixels of similar oligomerization state ratios. Instead, a direct clustering approach is developed in which each pixel is considered as a (FRET, acceptor, donor) triplet and assigned a probability of belonging to each of several clusters. The probabilities may be used to create an informative colored map of the cell indicating the locations of the various oligomer types.

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Skeletogenic gene studies in representative cidaroid *E. tribuloides*

Esther Shyu

Mentors: Eric Davidson and Joel Smith

Major morphological differences among species may be the result of evolutionary divergences in gene expression; divergent developmental programs between echinoderms provide ready opportunities for investigating such occurrences. While true sea urchins (euechinoids) specify larval skeletons during early embryonic stages, other echinoderms do not: pencil sea urchins (cidaroids), euechinoids' closest living relatives, only develop larval skeletons at the end of embryogenesis, suggesting precocious larval skeleton specification is an evolutionarily novel adaptation. By comparing embryonic expression patterns and control mechanisms of key skeletogenic genes, such as *alx1* and *veg3*, in euechinoids and cidaroids, we can determine networks controlling echinoderm larval skeleton formation and mechanisms of evolutionary divergence. However, though well-studied in euechinoids, genes involved in larval skeleton development are not characterized in cidaroids. We identified putative skeletogenic gene sequences from cidaroid *Eucidaris tribuloides* cDNA via methods including degenerate PCR and large-scale RNA sequencing. Comparing the temporal and spatial expression profiles of these genes, as determined with quantitative PCR and whole mount in situ hybridization, to those of euechinoid counterparts will provide insight concerning evolutionary changes that have influenced echinoderm skeletogenic gene regulation differences and subsequent patterns of larval skeleton formation. These findings may ultimately have important implications for understanding body part evolution.

The Regulatory Role of the Wnt Signaling Pathway in *Strongylocentrotus purpuratus* Endoderm Specification

Natnaree Siriwon

Mentors: Eric H. Davidson and Isabelle Peter

To solve the signaling interactions between endoderm precursor cells in *Strongylocentrotus purpuratus*, we investigated the role of Wnt signaling pathways in the regulation of endoderm specification genes that are downstream of the Tcf transcription factor. First, QPCR analysis of the Wnt gene expressions revealed that five Wnt genes, out of thirteen that were reported (Croce et al., 2006), were expressed in the 18h-24h-blastula –these five Wnt genes are Wnt-1, -4, -5, -8 and -16. Moreover, QPCR analysis of genes encoding frizzled related proteins, receptor of Wnt ligands, showed that all of the reported Frizzleds (FRP-1, -4, -5/8, -9/10 and SFRP-1/5, -3/4) were expressed throughout the time course and also in the 18h-24h-blastula. FoxA and Hox11/13b are the genes downstream of the Tcf transcription factor in veg2 lineage endodermal cells and veg1 lineage endodermal cells, respectively. However, morpholino anti-

sense perturbation assay of Notch and Hox11/13b dislocates the expression of these genes in the 24h mesenchyme embryo. FoxA was affected by the perturbation of Notch; hence, in Notch perturbation, FoxA's expression shifted to veg2 lineage mesodermal cells. Hox11/13b's expression in veg1 lineage endodermal cells were prevented when Hox11/13b itself was perturbed, so we found that the expression of Hox11/13b is still confined in the veg2 lineage endodermal cells instead of expanding to the veg1 region. In order to identify the responsible signals, we did a QPCR analysis to monitor the change in expression of Wnt and frizzled genes in Hox11/13b, Notch and Delta perturbations. The samples are 18h, 21h and 24h embryos injected with Hox11/13b, Notch or Delta morpholino anti-sense. From five Wnt genes expressed in the 24h embryo, only Wnt-16's expression showed a significant decrease in Hox11/13b MASO embryos. The expression of SFRP-3/4 also showed a significant decrease, of three folds and above, at 24h in two repeats and slight decrease, two fold, in the other repeats. Other genes, however, were not repeatedly affected by the perturbation. In the mean time, we are creating whole mount in situ hybridization assays of 18h, 24h and 48h embryos detecting the spatial location of Wnt-5, -8, and -16, SFRP-3/4 and -1/5 and FRP9/10's expression.

Polysaccharide A from *Bacteroides fragilis* suppresses the induction of inflammatory cytokines in response to LPS

Gloria Tran

Mentor: Sarkis Mazmanian and June Round

Bacteroides fragilis, a commensal bacteria in the mammalian gut produces polysaccharide A (PSA), which directs the maturation of the immune system as well as protects from intestinal inflammation and experimental colitis. Prompted by preliminary data, we wish to investigate the suppressive effects PSA has on systemic inflammation by creating a model of endotoxic shock, a condition that results from severe gram-negative bacterial infections. Innate immune cells such as macrophages and dendritic cells derived from mouse bone marrow are exposed to LPS, a lipopolysaccharide from the cell walls of gram-negative bacteria. These cells are treated with PSA prior to or simultaneously as exposure to LPS and the induction of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) are measured through standard laboratory protein and cell assays. Pre-treatment with PSA reduces the level of IL-6 and TNF produced when the cells are given LPS. When PAM3CSK4, another ligand acting through Toll-like receptor 2 (TLR2), is given instead, cytokine levels are not effected, showing the specificity of PSA in signaling for the decreased induction of TNF and IL-6 through TLR2. Current experiments aim to see whether the presence of TLR2 is necessary for PSA to suppress TNF and IL-6 induction.

Identification of Gene(s) that Mediates Species-Specific Niche Occupying Phenotype During *Bacteroides fragilis* Colonization of the Axenic Mouse Gut

Vivian Yang

Mentors: S. Melanie Lee and Sarkis Mazmanian

The mammalian gastrointestinal tract harbors a complex and diverse microbiota with densities approaching 10^{12} microbes per gram comprising of up to 50% of the fecal mass. Studies have shown that 90% of the human distal gut microbiota belong to 2 of 70 known divisions of the Bacterial Kingdom, *Bacteroides* and *Firmicutes*. The project focuses on the colonization numbers and patterns of *Bacteroides fragilis* and *Bacteroides vulgatus*. Unpublished results from the laboratory of Assistant Professor Sarkis Mazmanian strongly suggest that both *B. fragilis* and *B. vulgatus* colonize distinct, saturable, and species-specific niche within the germfree mouse intestine. The purpose of this project is to identify the gene or genes that are responsible for controlling the species-specific niche occupying phenotype during *B. fragilis* colonization of the axenic mouse. We propose to elucidate the molecular mechanisms of niche-specific gut colonization by *B. fragilis* in germ-free mice by construction of a *B. fragilis* genomic library in the *E. coli* host, conjugation of the *B. fragilis* genomic library from *E. coli* to *B. vulgatus*, and screening of the library for clones that colonize the *B. fragilis*-specific niche in the mouse intestine. We intend to find a clone of *B. vulgatus* that is able to co-colonize a *B. vulgatus* monocolonized mouse intestine. Studies are ongoing. These studies will result in findings that will elucidate the genetic basis of symbiont bacterial colonization in the mammalian intestine.

Study of Spatial Gene Expression in Sea Urchin Larval Development

Erin N. Zampaglione

Mentor: R. Andrew Cameron

The sea urchin has a remarkable life cycle. It develops indirectly, going from an embryo to a larva, then finally to the adult form. The embryonic stage of the sea urchin is well studied; however, the larval stage is not. It is in the larval stage that the rudiment, the part of the larva that will ultimately develop into the adult, forms. Therefore, there are many transcription factors expressed in the larval rudiment that are not expressed in the embryos whatsoever. By using a technique called whole mount in-situ hybridizations, it is possible to determine where in the larva these genes are expressed. Once this spatial information has

been obtained, further research can be performed by perturbing certain genes to determine how they contribute to the developmental process of the larva.

***Steinernema* Jumping Responses to Volatile Stimuli**

Yuanjun Zhang

Mentors: Paul Sternberg and Adler Dillman

Nematodes in the genus, *Steinernema*, are entomopathogenic (insect parasites). These nematodes are often found in the infective juvenile (IJ) stage outside of insect hosts. Certain species in *Steinernema* have the ability to jump in the IJ stage. Past research has indicated that IJs respond by jumping to insect air emitted by the common house cricket *Acheta domesticus* and the greater wax worm, *Galleria mellonella*. Using GC-MS (gas chromatography-mass spectrometry) to analyze air samples taken from crickets and wax worms, we are able to determine the specific volatile compounds emitted by these insects. Certain *Steinernema spp* jump when stimulated by some compounds found in insect air. Varying sensitivity between *Steinernema spp* to volatile compounds suggests that nematodes may have the ability to distinguish different insects and have a preferred host. Comparisons of jumping behavior between different nematode species will increase our understanding of these insect parasites, their host finding behaviors, and the ecological niches they inhabit.