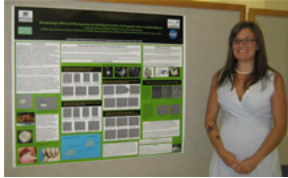


Complex Biological Systems Summer Undergraduate Research Program Summer 2008 Cohort Research Abstracts



❖ Lindsay Klouser

Montana State University - Bozeman

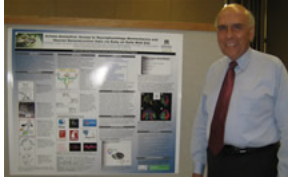
Mentor: Sheila Nielsen-Preiss, Cell Biology & Neuroscience

Research Abstract:

Candida albicans is one of the most common human fungal pathogens that exist. It is a common component found in the gastrointestinal tract. In patients with compromised immunity, *C. albicans* can cause opportunistic and systemically life-threatening infections. Some of the most common infections include thrush, yeast infections and several types of candidiasis. Under certain conditions, *C. albicans* can change morphologically from a budding yeast to a filamentous form (ex. germ tubes, hyphae, pseudohyphae). These changes occur from environmental stressors and can contribute to how virulent the pathogen becomes inside the host. Normal yeast cells disseminate through the blood stream, while filaments invade tissue. It is hypothesized that the filamentous form of the yeast is required for infection of host tissues.

Recent studies show some bacteria become increasingly virulent and/or resistant to antibiotics in long-term spaceflight conditions. In addition, astronauts that have experienced space travel for long periods of time have compromised immune systems, making them more susceptible to illness and infection.

Early studies conducted at the Hyman lab on the yeast *Saccharomyces cerevisiae* show changes in morphology and gene expression following exposure to SMG (simulated microgravity). *S. cerevisiae* is a good model system to study the yeast response to these altered environmental conditions, but we have become interested in the response of pathogenic yeast to these environmental stresses. Our recent studies show that the effects of SMG on the opportunistic pathogen *C. albicans* were comparable to that observed in *S. cerevisiae*, with changes in morphology and gene expression.



❖ **Angelo Kontgas**

Utah State University

Mentor: Gwen Jacobs, Cell Biology & Neuroscience

Research Abstract:

The cercal sensory system of *Acheta domestica*(*Ad*), the European house cricket, has been under investigation for some time.^{3,6} Two repositories of data from this research: Neurophysiology_Biomechanics and Neuron_Reconstruction, currently accessed via the NeuroSys System,⁷ will be ported to an improved internet web page. The web page is being developed with Ruby on Rails technology. Herein is an overview of the *Ad* science and web page development progress.



❖ **Bridgett McNulty**

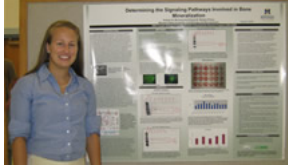
Montana State University - Bozeman

Mentor: Roger Bradley, Cell Biology & Neuroscience

Research Abstract:

As the central nervous system forms in vertebrates, neural crest cells migrate throughout the embryo to give rise to the majority of the peripheral Nervous System (PNS). A major theory regarding the specificity of neural crest cell migration patterns centers on cadherins, proposing that homophilic binding between cadherins on both migratory neural crest cells and target stationary cells is behind neural crest cell fate. This project focuses on a subclass of cadherins known as protocadherins, specifically chick protocadherin-1(cPcdh-1). Little is known concerning the cytoplasmic proteins that interact with protocadherins. Consequently, the main objective of this project centers around isolating and identifying binding partners of cPcdh-1.

A fusion protein construct consisting of glutathione s-transferase (GST) has been prepared containing the cytoplasmic domain of cPcdh-1. The GST fusion construct has successfully been expressed in bacteria in small and large scale and the resultant fusion proteins purified by affinity chromatography using glutathione agarose. Bound proteins were analyzed by SDS-page. The fusion construct was immobilized on GST beads and incubated with embryo extracts to isolate possible binding partners. 2-D gel analysis followed by mass spectrometry will allow us to identify and characterize these binding partners.



❖ Katelyn Michaud

University of Maine - Farmington

Mentor: Sheila Nielsen-Preiss, Cell Biology & Neuroscience

Research Abstract:

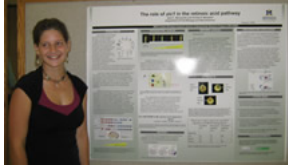
Millions of people suffer from diseases that contain a component of dysregulated cellular mineralization each year. These diseases include mineralization defects, such as the increased mineral formation with arthritis that affects over 2% of the world population [1, 2] and vascular calcification which is responsible for strokes and heart attacks (the leading cause of death in the US) [3, 4] as well as osteomalacia and osteoporosis, which are both characterized with diminished mineralization in bone tissue [5, 6]. Bone tissue is a specialized form of connective tissue that forms the human skeletal system. On average, the human skeleton replaces itself every ten years. Bone remodeling requires multiple levels of communication from osteoblasts, which produce bone, and osteoclasts, which resorb it [7]. During osteogenesis, osteoblasts mineralize the bone matrix by promoting the nucleation of hydroxyapatite in the interior of membrane-limited matrix vesicles (MVs) and by propagating the hydroxyapatite onto a collagenous extracellular matrix called osteoid [8]. Tissue-nonspecific alkaline phosphatase (ALP) plays an important role in bone mineralization by catalyzing the hydrolysis of phosphomonoesters at an alkaline pH and by breaking down pyrophosphate, a potent inhibitor of calcium phosphate deposition at the extracellular level [8]. Mineralization is a complex process requiring the synthesis and localization of several crucial proteins that require temporal and spatial regulation during differentiation and ultimately mineralization.

Mitogen activated protein (MAP) kinases play pivotal roles in cellular responses to cytokines, environmental stress and growth factors. They are divided into 4 classes: extracellular signal-regulated kinases (Erks), C-Jun N-terminal kinase (Jnk), p38 MAPKs, and ERK5 [9]. MAP kinases have also been associated with osteoblastic proliferation and differentiation. Previous studies indicate that Erk activity is required for proliferation, migration, differentiation and mineralization in primary human osteoblasts [10]. Other studies, with the use of inhibitors, showed that Erk activity is required for MC3T3-E1 proliferation, but not differentiation [11]. In contrast, other studies in MC3T3-E1 cells indicate that transforming growth factor β inhibition of ALP activity and mineralization is mediated by Erk activity [12]. In this study, the Erk inhibitors (PD98059 and U0126) resulted in an increase in ALP activity and mineralization. In yet another study in MC3T3-E1 cells, p38 MAPK was suggested to play an important role in osteoblast differentiation. When p38 MAPK was inhibited with a specific inhibitor, the expression of differentiation markers, such as ALP activity and mineral deposition were significantly reduced. Inhibition of Erk with specific inhibitors did not

significantly affect osteoblastic differentiation. Thus, the study concluded that p38 MAPK is required, but Erk is not necessary for osteoblastic differentiation [9]. Previous research on the requirement of Erk in osteoblastic differentiation and mineralization is contradictory. *We propose that Erk is required for osteoblastic proliferation and differentiation, but not for mineralization.*

Annexin II (AnxA2) has recently been identified as a protein potentially important in cellular mineralization. AnxA2 is a member of the annexin family, which is characterized by its annexin repeat domains and Ca^{+2} and lipid-binding properties. It was originally identified as a src-kinase substrate and an inhibitor of PLA2 activity [7]. AnxA2 is found in a variety of cell types at different subcellular localization and has been recently localized to the cytoplasm in osteoblastic cells [7]. In recent studies, AnxA2 along with ALP has been localized in lipid rafts (membrane microdomains). The lipid rafts containing AnxA2 appear to be important for ALP activity and may facilitate the osteoblastic mineralization process [7]. AnxA2 exists as three major species: a monomer, a heterodimer or a heterotetramer with its S100-family binding protein, p11 [13, 14]. *We propose that AnxA2 only inhibits cPLA2 activity when in the heterotrimeric form with p11 and p11 alone is a potent inhibitor of cPLA2.*

**References available upon request.*



❖ **Sara Muscarelli**

Montana State University - Bozeman

Mentor: Christa Merzdorf, Cell Biology & Neuroscience

Research Abstract:

Neurulation is the process by which an embryo begins to develop a nervous system. During this process, cells of the ectoderm form the neural plate, epidermis, and neural crest. Neurulation is regulated in part by genes called transcription factors, which control the expression of other genes by binding to their promoters. The Zic family of zinc finger transcription factors is highly influential in maintaining normal neural development in vertebrates.

Our goal is to understand the role that the Zic1 transcription factor plays during early neural development. *Xenopus laevis* serves as the primary model organism.

A genomic screen looking for direct targets of Zic1 has recently been carried out by the Merzdorf lab. A comprehensive list of potential genes was generated and verified using PCR and in situ hybridization. Three of the direct targets, *CRABP-II*, *cpl-1*, and *SRC-3* are involved in the regulation of the retinoic acid (RA) pathway. This project focuses on the direct target *CRABP-II*.

Retinoic acid plays an important role in the normal development of the hindbrain and spinal cord. Retinoic acid forms an extracellular gradient that controls expression of the Hox transcription factors. *hox* genes regulate the anterior-posterior development of the hindbrain and spinal cord. In vertebrates, *hox* gene clusters are present on four different chromosomes. The *hox* genes are ordered on the individual chromosomes in a manner colinear with their expression in the embryo. *hox* genes expressed anteriorly require low levels of RA to be expressed while *hox* genes expressed posteriorly require high levels of RA to be expressed.

CRABP-II binds retinoic acid in the cytoplasm, where it controls the availability of the molecule in addition to providing it with protection. It also carries retinoic acid to the nucleus where it will bind to its nuclear receptor RAR (retinoic acid receptor).

Zic1 regulates the expression of its direct gene target, *CRABP-II*, that is involved in the retinoic acid pathway. Thus, I am testing the hypothesis that Zic1 acts through *CRABP-II* to induce retinoic acid responsive *hox* genes (see Figure 5). Since Zic1 is active primarily in the developing hindbrain, the *hox* genes that will be analyzed are expressed in the hindbrain and include *hoxA1*, *hoxB1*, *hoxB3*, and *hoxD1*.



❖ **Seanna Pieper-Jordan¹ & Cinnamon Spear²**

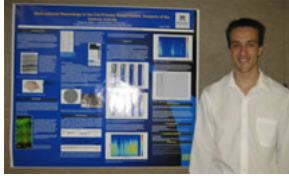
¹Kamehameha Schools – Honolulu (enrolled in Yale University – Fall 2008) ²Dartmouth College

Mentor: Mark Burr, Center for Biofilm Engineering

Research Abstract:

The development of bacterial communities, the agents of biocorrosion, pathogenic growth and deterioration of water quality, in piping networks is recognized as a common risk to drinking water distribution. An estimation of 99.9% of bacteria grow in biofilms, which offer a protective matrix of extracellular polymeric substances produced by sessile cells, which have attached to surfaces, such as pipes, where higher concentrations of nutrients accumulate. In biofilm development, dissolved organic matter, including humic substances, are important food sources for bacteria and are readily utilized by biofilms. Previous studies have shown that carbon concentration and carbon source play an important role in, not only the growth of biofilms, but also the bacterial composition they possess. In one study, denaturing gradient gel electrophoresis (DGGE) profiles showed a difference in the bacterial species of biofilms grown in 0.04 millimolar carbon to those grown in 2.53 millimolar carbon¹. Community differences were also seen between biofilms fed two types of detritus² and biofilms collected in glacial and nonglacial streams³.

The following research analyzes the influence of various humic substances on developing and established biofilms. By comparing the diversity of bacterial communities grown in different carbon sources, the investigation may provide insight to the attraction of bacteria to conditioned surfaces, the behavior of biofilm formation and the environmental preference of established biofilms.



❖ **Roberto Rojnic**

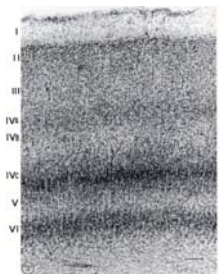
Montana State University

Mentor: Charlie Gray, Cell Biology & Neuroscience

Research Abstract:

Gamma oscillatory activity [30-80Hz] of the local field potential has been found to occur when perceptual information from simple stimuli is being integrated within the primary visual cortex, thus reflecting stimulus properties (Gray 1989, Gray 2000). Studies conducted so far have characterized gamma activity for neurons within different layers of the cortex in response to simple and complex stimuli, however such investigations have not been done for all layers simultaneously.

In the present exploratory investigation the goal was to conduct spectral analysis on the local field potential (LFP), in order to characterize the laminar profile of the gamma activity across all 6 layers (Fig.1) of the primary visual cortex of a cat when stimulated with natural scene and sine-wave grating stimuli (Fig 6).



The figure on the left shows the 6 layers of the striate cortex. Visual input from the eye innervates into the dLGN (dorsal Lateral Geniculate Nucleus located in the lateral- ventral portion of the thalamus), which then connects into layer IV of the primary visual cortex. Once in layer IV, visual input is relayed to layers 2 and 3, which connect to other layers of the striate cortex as well as to different cortical areas beyond the primary visual cortex.

Figure 1. Layers of the monkey visual cortex. Source: Bear, 2006.

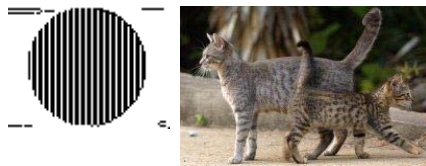
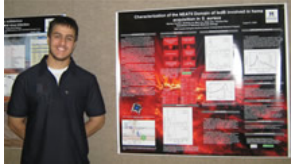


Figure 6. Example of natural scene and grating stimuli. On the left, sine-wave grating stimuli. On the right a snapshot of the time-varying natural scene stimuli.



❖ Mohsen Soofian

University of Washington

Mentor: Ben Lei, Veterinary Molecular Biology

Research Abstract:

Staphylococcus aureus is a spherical, Gram-positive bacterium that makes yellow colonies. It is facultatively anaerobic, meaning it can perform aerobic respiration but also switch to fermentation if necessary. They can be found on the skin and inside of the noses of humans. Also, about 20-30% of all people are staph carriers in this manner. *S. aureus* causes a variety of diseases, such as pneumonia, meningitis, endocarditis, toxic shock syndrome, and sepsis.

Every year about 500,000 Americans become infected with *S. aureus*. It is the leading nosocomial infection in America, which has a massive economical impact on our society. Furthermore, humans are not the only targets of *S. aureus*, as livestock can be affected as well. Human and livestock strains are very similar, which increases the chance of mutations that could lead to more virulent strains (such as methicillin- and vancomycin-resistant *S. aureus*).

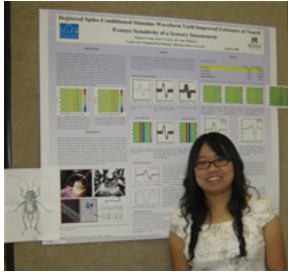
Furthermore, new strains of highly drug-resistant *S. aureus* make current antibiotics increasingly ineffective, which usually work by inhibiting the synthesis of the peptidoglycan layer of the bacterial cell wall. To combat this concerning bacteria, new therapeutic targets must be found.

Remarkable new research suggests starving the bacteria of nutrients as a viable option. A highly critical nutrient for *S. aureus* is iron, which is used in many of its cellular processes, such as the electron transport chain. *S. aureus* needs iron to grow and remain virulent. Hence, if *S. aureus* were denied iron, it would not be able to mount an infection.

S. aureus acquires heme as a preferred iron source. The bacteria lyse erythrocytes using hemolysins, which elicits an outpouring of free hemoglobin. This provides *S. aureus* with a highly rich source of heme. Hemoglobin loses its heme to iron-regulated surface determinant protein B (IsdB), where the heme is transported inside of cells by the additional Isd proteins (Fig. 2).

Previous research has documented the ability of full-length IsdB to transfer heme to IsdA and IsdC, and also to accept heme from methemoglobin (metHb). Interestingly, IsdB has two domains: NEAT 1 and NEAT 2. The roles of these domains in heme binding and transfer are not known.

Our objective is to determine whether the NEAT 2 domain binds heme, efficiently acquires heme from metHb, and rapidly transfers heme to IsdA and IsdC. To achieve the objective, first we will need to isolate the IsdB NEAT 2 protein and then examine the heme transfer reactions involving the NEAT 2 domain. Our results show that IsdB NEAT 2 transfers heme to IsdC and IsdA, but cannot efficiently uptake heme from metHb.



❖ **Lingyao Yang**

Salem College

Mentor: Alex Dimitrov, Cell Biology & Neuroscience

Research Abstract:

What is the meaning associated with a single action potential in a neural spike train? One general approach toward formulating this question involves estimating the average stimulus waveform preceding spikes in a spike train. Many different algorithms have been used to obtain such estimates, ranging from spike-triggered averaging of stimuli (Watt et al., 1975) to correlation-based extraction of 'stimulus-reconstruction' kernels (Bryant et al., 1976) or spatio-temporal receptive fields (Theunissen et al., 2001). We demonstrate that all of those approaches yield distorted estimates of the mean stimulus waveform since they aligned the waveform segments to one another based on the precise time of spike occurrence and ignored the intrinsic 'jitter' in the stimulus-to-spike latency. Therefore, we present an algorithm that takes the 'jitter' into account and enables the partial disentanglement of the questions addressed in the studies of neural coding, called 'Dejittered Estimate'. We expect the 'Dejittered Estimate' to provide a better estimate of the mean waveform eliciting a spike than other standard algorithms and improves the feature estimates (Dimitrov et al., 2005)