

2011

4th Annual Biological Sciences Symposium, April 13, 2011

Department of Biological Sciences, Seton Hall University

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Department of Biological Sciences, Seton Hall University, "4th Annual Biological Sciences Symposium, April 13, 2011" (2011).
Biological Sciences Student Work. 2.
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Graduate - Research

1. Vanillate-based Promoter System for Heavy Metal Gene Regulation in *Synechococcus* sp. IU 625. Robert Newby, Jr. & Tin-Chun Chu

Molecular mechanism of the cyanobacterial heavy metal tolerance remains poorly understood. We have investigated the physiological effect of freshwater cyanobacterium under many EPA targeted heavy metals stress in previous studies. Harmful overgrowth of cyanobacteria in freshwater presents a challenge to even the most developed nations. We use a unicellular cyanobacterium *Synechococcus* sp. IU 625 as a model organism to understand and better characterize these interactions. Vanillate is a phenolic source carbon found in freshwater due to the saprophytic digestion of plant lignin. We are proposing the use of a vanillate inducible vector based system to measure the effect of the isolated heavy metal response gene metallothionein. Metallothionein is a cysteine rich protein whose primary purpose regulation of intracellular zinc levels. Previous experiments have shown metallothionein can also respond to other divalent metal cations. The overall goal of this project is to measure the importance of metallothionein to *Synechococcus* sp. IU 625, and have a better understanding of the role it might play in allowing cyanobacteria to cause algal blooms in heavy metal contaminated freshwater.

2. Cyanobacteria Detection in Northern New Jersey Freshwater Lakes. Matthew J. Rienzo, Michelle Reed & Tin-Chun Chu

Eutrophication results in excessive cyanobacterial growth in these urbanized freshwater lakes. Excessive growth of cyanobacteria leads to the formation of dense algal blooms, which ultimately disrupt aquatic life and vegetation in more ways than one. In this study, water samples from several sites along a freshwater lake in Branchville, New Jersey were collected. The six samples were then filtered through a coarse filter with a pore size of 3.0 μm , and a fine filter with a pore size of 0.45 μm . The fine filters were then cut and processed with 5% chelex-100 for DNA extraction. General primers for phytoplankton and specific primers were used in polymerase chain reaction (PCR) assays for detection. The primer sets used in this study are Syn7942_16sRTF-Syn7942_16sRTR, rpsL_RTf-rpsL_RTR, PSf-Ur, Uf-PSr, CYA106_16sF-CYA781_16sR, CYA359_16sF-CYA781_16sR, and Syn7942_cpcF-Syn7942_cpcR, PC β F, PC α R, Micr184F, and Micr431R. Several species of cyanobacteria have been identified. Microscopic observations have been carried out to visualize the morphology of phytoplanktons.

3. Detailed analysis of putative sequences of three closely-related sperm DNA binding proteins in 12 species of *Drosophila*. Zain A. Alvi, Tin-Chun Chu, & Angela V. Klaus

Current evolutionary theory states that protamines evolved from histone linker-like proteins. The current study is aimed at analyzing putative protein sequences of the protamines of 12 *Drosophila* species based upon the reference sequences of two protamines (MST35Ba and MST35Bb) and reference sequence of one histone linker-like protein (MST77F) found in *Drosophila melanogaster*'s sperm nuclei. The analysis was initially conducted using the basic

local alignment search tool (BLAST) which utilizes a conservative algorithm to compare primary biological sequence information. The best matches from each *Drosophila* species were aligned using CLUSTALW, a multiple sequence alignment tool that uses an algorithm to create a phylogenetic tree. The algorithm in ClustalW is specifically designed to align species based upon the global alignment method. In contrast a local alignment algorithm based application called T-Coffee was used to find a conserved region amongst 12 *Drosophila* species. The two protamines, H1 liker like protein, and conserved domains in MST35Ba and MST35Bb were then analyzed with BindN – RF (Random Forests) and BindN+'s (SVM based) for all potential DNA binding sites. Additionally, DNA Binder, a SVM score based DNA Binding predictor, was used to verify MST35Ba, MST35Bb, MST77F, and the conserved regions to be actual DNA binding proteins or regions. The convergence of these results for T-Coffee, DNABinder, BindN-RF and BindN+ allowed for the determination of putative DNA binding domains in MST35Ba and MST35Bb. UCL's PSIPRED was used to predict secondary structure based upon the amino acid sequences for MST35Ba, MST35Bb, and MST77F. The protein functional domains for MST35Ba, MST35Bb, and MST77F were found through Domain Annotation -InterProScan on Swiss-MODEL Workspace. Also this tool was used to predict the disorder in the secondary structure. Lastly Imperial College London's Phyre tool was used once again to predict secondary structure and the disorder. The recognized fold regions with description concerning protein binding regions, consensus functional sites mapped onto these 3D models, superfamily and family descriptions were obtained through Phyre. Additionally, we have designed primers to isolate and sequence MST35Ba, MST35Bb, and MST77F regions in the genomes of *D. simulans* (*D. melanogaster*'s closest relative), *D. pseudoobscura* (a species for which our lab has developed an *in vitro* system for studying spermatogenesis). We are also analyzing chromatin condensation patterns during nuclear transformation in *Drosophila* sperm nuclei. Our hypothesis is that the type of protamines present in the sperm nucleus will affect the pattern of chromatin condensation, which in turn will affect the species-specific shape of the sperm nucleus.

4. Localization of the stem cell niche in the testes of *Drosophila pseudoobscura*. Michael W. Beaury & Angela V. Klaus

In the apical end of the *Drosophila melanogaster* testes, the stem cell niche is characterized by a group of tightly compacted stem cells (also known as the hub) that are responsible for the division and proliferation of germ-line stem cells and somatic cyst cells that are in contact of this hub. After division, the daughter cells of the germ-line stem cells will further divide into spermatogonia enveloped in the daughter cyst cells. These cysts are pushed down the testes toward the basal end, until numerous sperm cells are developed. This spermatogenic process has been well-characterized in *Drosophila melanogaster*; however it has not yet been characterized in the *obscura* group. Using immunochemistry and confocal microscopy techniques, our goal is to determine the characteristics of the apical end of *Drosophila pseudoobscura* testes and localize the stem cell niche.

5. Oxidative stress induces degeneration of specific cyst cells in cultured spermatogenic cysts in *Drosophila pseudoobscura*. Robert W. Yates & Angela V. Klaus

Cellular glutathione (GSH) is a known reducing agent (antioxidant) against reactive oxygen species (ROS). Recent evidence from our laboratory suggests that the survivability of

Drosophila pseudoobscura fly spermatogenic cysts in *in vitro* culture can be improved by adding exogenous GSH to the culture media. In the current work, we analyzed the effect that diminished endogenous GSH expression has on spermatogenic cyst survival *in vitro*. Buthionine sulfoximine (BSO) is a known inhibitor of intracellular GSH production. By adding BSO to *D. pseudoobscura* spermatogenic cyst cultures and measuring the survivability of different cyst types, we determined that early spermatogenic cysts (spermatogonia and primary spermatocytes) are extremely susceptible to degradation due to oxidative stress. Additionally, in later stage elongating cysts, we noted that the cyst cell that surrounds transforming sperm nuclei is also highly susceptible to degradation due to oxidative stress. This indicates that the head cyst cell is more metabolically active than the corresponding tail cyst cell.

6. Mechanism for nuclear migration in developing sperm in *Drosophila pseudoobscura*. Benjamin S. Reimer, Crystal Pristell & Angela V. Klaus

Drosophila is a model system for studying the differentiation of spermatogonia into functional sperm. Spermatogenesis in *Drosophila* involves large-scale cellular restructuring before the sperm are functional. In *Drosophila pseudoobscura* sperm develop within an encapsulating cyst. Within each cyst, a precursor spermatogonium undergoes 5 rounds of mitotic expansion, followed by two meiotic divisions to produce 128 round spermatids. A key event prior to elongation of each individual spermatid into a spermatozoan involves migration of each of the 128 nuclei to one side of the encapsulating cyst. The mechanism for this nuclear migration is unknown. However, it is known that F-actin is involved in organizing sperm nuclei during a much later post-meiotic stage of spermatogenesis. The purpose of the current work was to determine if F-actin plays a role in nuclear migration.

7. The exploration to develop a universal influenza virus vaccine. Malum J Mambula & Luzhou Xing

Influenza viruses infect a wide variety of species including human, pigs and birds. The most concern is the expected emergence of a new influenza pandemic. Hemagglutinin (HA) and Neuraminidase (NA) are two important viral surface proteins which induce immune response in the host. The accumulation of random point mutations in genome at sites of HA or NA causes antigenic drift, however antigenic shift occurs as a result of genetic reassortment between two species pools when a new virus emerges. Both antigenic shift and drift pose problems for vaccine production. Annual administration of the influenza vaccine is required to maintain immunity against the ever changing influenza strains. In this library thesis research, we propose to construct and express a fusion protein as a universal vaccine, which contains one conserved domain of HA and a fragment of M2 protein. The M2 is a transmembrane ion channel protein on the surface of influenza virus possessing highly conserved amino acid sequence. The rational immune responses and protection induced by this vaccine were discussed.

8. Modification of the infant gut microbiome using probiotics to modulate health.

Frank Kirchner & Heping Zhou

Microbial colonization of the infant gut is initiated from the onset of birth and is influenced by the environment in which the neonate is first exposed. Antibiotic exposure, diet and specifically mode of delivery, i.e. vaginal or cesarean birth, have dramatic effects on the developing enteric microbial ecology. The composition and diversity of the developing enteric microbial ecology has been implicated in both health and disease. Potential shifts in the enteric microflora that favor pathogenesis over homeostasis have been associated to the development of various autoimmune and chronic inflammatory diseases such as eczema, atopic dermatitis and infantile colitis. Probiotic intervention has gained interest as a viable therapy for modulating microbial dysbiosis in the developing infant gut to restore and maintain health. This proposed Randomized, Double-Blind, Placebo-Controlled Trial will test the effect of an intervention with “maternal” derived probiotics on the developing neonatal gut of both vaginal and cesarean born infants. Fecal samples from enrolled neonates provided with either placebo or “maternal” derived probiotic therapy will be analyzed for microbial composition via 454 GS FLX pyrosequencing of the V4 hypervariable region of 16S rRNA genes. It is hypothesized that probiotic intervention proposed in this study would dramatically impact the developing naïve infant gut of both vaginal and cesarean birthed neonates. This intentional microbial exposure will provide the scaffold for increased “healthy” microbial enteric colonization. The observed composition and diversity of the vaginal and cesarean born subjects provided the probiotic therapy would resemble an “adult-like” profile at an earlier time within the first year of life and could potentially facilitate health not only during childhood, but throughout adulthood as well.

9. The effects of cadmium and lead on the hemoglobin protein and ALAD enzyme of *Chironomus riparius*. Stefanie K. Geronimo & Carolyn S. Bentivegna

10. Bile PAH determination on *Brevoortia tyrannus* and *Brevoortia patronus* using scanning fluorescence spectrophotometry (SFS). Becky Hawke & Carolyn S. Bentivegna

11. Molecular and toxicological analysis of RNAi suppression on hemoglobin protein expression in chironomids. Jun-taek Oh, Stefanie Geronimo, Anthony Gerardi & Carolyn S. Bentivegna

12. PAH components in oils. Kristen A. Wirasnik & Carolyn S. Bentivegna

13. Investigation of potential signal transduction pathways mediating DFO effect. Lawrence Rasmussen, Adrienne Galang, Alyda Stabile & Jane Ko

14. Study of the protein-protein interacting domain using two hybrid system. Pranjal Nahar, Alyda Stabile, Adrienne Galang, Hamidah Sultan & Jane Ko

Undergraduate – Research

1. Synergistic Antimicrobial Effect of Polyphenolic Compounds and Antiseptics.

Jennifer L. Todd & Tin-Chun Chu

The polyphenols present in both green and black teas contribute to the observed antimicrobial characteristics and health benefits. Theaflavin and its derivatives are the major polyphenolic compounds in black tea. The theaflavins included in this study were theaflavin (TF-1), theaflavin-3-monogallate (TF-2A), theaflavin-3'-monogallate (TF-2B), and theaflavin-3,3'-digallate (TF-3). In addition to those three different theaflavins, black tea powder, black tea crude extract, and oligonol (lychee fruit extract) were tested. Serial dilutions (1.0, 2.5, 5.0, 7.5, and 10.0g/L) of all tea compounds and oligonol were made in order to determine the minimum inhibition concentration. The antimicrobial abilities of the compounds were determined through the zone of inhibition as well as bacterial growth curves. Minimum inhibitory concentrations were determined for each compound. The tea compounds and oligonol were added to various antiseptics (Listerine, Crest, Act, PocketBac, Oxisoft, Germ-X, and Purell) and tested against *E. coli*, *S. epidermidis*, *P. aeruginosa*, *B. megaterium*, *S. thermophilus*, and *M. smegmatis*. The results suggested polyphenolic compounds have synergistic effects against various bacteria. TF1, TF3, and black tea crude extract were also combined with various antibiotics (B10, D30, GM10, S10, CF30, PB300, P10, TE30, RA5, AM10, E15, and C30) against *S. epidermidis* and *E. coli*, but no synergistic effect observed.

2. The Freshwater Cyanophage AS-1 Genome Project: DNA Purification, Sequencing, and Mapping. Lauren M. Strawn, Jonathan Jimenez & Tin-Chun Chu

Freshwater Cyanophage AS-1 is the virus that infects *Synechococcus* sp. IU 625 (SIU 625), formerly known as *Anacystis nidulans*. Cyanophage has been suggested as a good environmental indicator due to its natural ability to control growth of cyanobacteria, a common contributor to harmful algal blooms. AS-1 DNA was previously sequenced into sixty-six contigs. Several different dilutions of titers were made and five different methods of DNA extraction were compared to determine the most effective method for the AS-1 cyanophage. Based on Blastx searches performed on all contigs, seven different contigs were chosen to analyze and eleven primers were created using NCBI Primer-BLAST and PrimerQuest™ PCR products were analyzed by electrophoresis gels and sequencing. The sequencing results were analyzed using FinchTV and Blastn to generate consensus sequences for the contigs. Based on these consensus sequences, some contigs were found to have possible overlap. A relative map of some contig positions has been created. The results obtain has helped us further map and understand the AS-1 genome.

3. Effects of high fructose on cultured chick cardiomyocytes. Theodore R. DaCosta, Zain A. Alvi & Angela V. Klaus

Fructose is a six-carbon polyhydroxyketone, and an isomer of glucose, having the same molecular formula (C₆H₁₂O₆) but different structure. Fructose is a monosaccharide that can be used by the body for energy. When fructose first became popular, it was seen as a cheaper and sweeter substitute for sucrose and has since been used in many foods and drinks in the form of

high fructose corn syrup. Over the past three decades fructose consumption has increased 25%. Studies show that fructose can only be metabolized in the liver; however, diets high in fructose show adverse effects on organs other than the liver, including the heart. Fructose is passively transported across membranes via its primary transporter, GLUT5. Using embryonic chick cardiomyocytes, we propose to study the ability of heart cells to take up and metabolize fructose, looking for the effects it has on the heart cells. The cells will first be tested for expression of GLUT5 (fructose transporter) by indirect immunofluorescence staining. After looking for the expression of GLUT5, the ability for the cells to metabolize fructose will then be studied by testing cell viability using fructose as the cellular energy source.

4. Three-dimensional organization of nuclei within developing spermatogenic cysts in *Drosophila pseudoobscura*. Crystal L. Pristell & Angela V. Klaus

Previous work in our laboratory was aimed at the development of an in vitro system for culturing *Drosophila* sperm cells. The current work is aimed at analyzing spermatogenic cyst morphology so that we can accurately characterize cyst maturation in our in vitro culture system, as well as determining the three-dimensional organization of developing sperm nuclei within cysts. It is currently unknown how transforming nuclei are arranged with respect to each in three dimensions. Sperm precursor cells develop within cysts and eventually mature to produce motile, elongate sperm cells. Germline stem cells are maintained in the stem cell niche in the apical end of the testis. Germline stem cells differentiate and become encapsulated in a cyst. After encapsulation, the germline cell (called a “gonialblast”) undergoes a series of divisions that increase the number of sperm precursors within the cyst. In *D. pseudoobscura*, there are five mitotic divisions, followed by the two meiotic divisions, resulting in 124 haploid cells ultimately being produced. In the current work, we characterized cyst morphology using phase contrast and wide-field fluorescence microscopy. Additionally, we analyzed spermatogenic cell arrangements in cysts undergoing nuclear transformation using three-dimensional imaging via confocal laser scanning microscopy.

5. Scanning electron microscopic characterization of *Drosophila* sperm morphology. Jennifer Goonetilleke, Matthew Emery, & Angela V. Klaus

Drosophila pseudoobscura belongs to the obscura species group of flies in the genus *Drosophila*. The obscura group is characterized by males with ellipsoid testes and by the presence of two sperm types produced within the testes: parasperm and eusperm. *Drosophila melanogaster* is the most widely used member of the *Drosophila* family in scientific research. Unlike its cousin in the obscura group *D. melanogaster* only has one type of sperm (eusperm) which is encased in its long tubular testes. In the current work, we report sperm morphology characterization of *D. melanogaster* and *D. pseudoobscura* using scanning electron microscopy (SEM). To the best of our knowledge, this is the first SEM study on *Drosophila* sperm morphology.

6. Protective effects of estrogen against hypoxia in chick cardiomyocytes. Lauren J. Clark, Zain A. Alvi & Angela V. Klaus

As women age, the cyclic biological production and fluctuation of 17-beta-estradiol significantly decreases during pre- and post-menopausal phases thus resulting in increased chances of cardiac

and cardiac-related illnesses. Oxygen is a major determinant of cardiac gene expression and oxygen deprivation can jeopardize cardiac viability during the physiological process of hypoxia/re-oxygenation thus activating reactive oxygen species (ROS). However, estrogen is believed to act as a cyto-protectant against oxidative injury in specific signal transduction pathways. However, there is little known about the mechanism, specifically, in which estrogen directly protects cardiomyocytes. In a study conducted in adult female rats, estrogen coupled with increased heat shock protein (HSP) 72 was found to protect hypoxia-reoxygenation in cardiomyocytes. We propose to connect 17-beta-estradiol to increased HSP108 which is a common heat shock protein expressed in many tissues of the chick and is responsive to hormones. Thus the goal of the current work is to determine if the chick cardiomyocyte model system can be used as a viable model for cardiac myopathy. Cardiomyocytes will be grown via primary cell culture. We will first test for expression of the estrogen receptor ER-alpha via Western Blot assay or indirect immunofluorescence labeling. Following this, hypoxia-induced chick cardiomyocytes will be exposed to different concentrations of estrogen to see if hypoxia symptoms persist, decrease, or disappear; in addition to testing whether the physiological effects of hypoxia are irreversible *in vitro*. This will give insight into the magnitude of effects that estrogen has on the physiological systems of vertebrates. We hypothesize that as the amount of estrogen exposure to hypoxia-induced chick cardiomyocytes increases, the more these cells will return back to their normal homeostatic physiological state.

7. A method for sperm nucleus isolation in *Drosophila pseudoobscura*. Diasia S. Brooks & Angela V. Klaus

Sperm DNA is bound by sperm-specific DNA binding proteins called protamines. Protamines are small, highly basic proteins that assist with DNA compaction within the sperm nucleus during the post-meiotic stage of spermatogenesis called spermiogenesis. During spermiogenesis, the sperm nucleus transforms from a spherical form to a highly elongated needle-like form in *Drosophila*. In the current work, we describe a method for mechanically dissociating sperm heads from sperm tails (using sonication) in *Drosophila pseudoobscura* so that we can isolate and characterize the sperm DNA binding proteins for this species. We chose to develop a mechanical isolation method rather than chemical isolation as previous work in our lab has shown that *Drosophila* sperm heads are highly sensitive to the anionic detergent SDS. However, in future work, we will test the cationic detergent CTAB as a chemical agent to disrupt sperm tails.

8. Bioavailability of Louisiana source oil in fundulus fish as measured by scanning fluorescence spectrophotometry. Vaishali K. Kothari & Carolyn S. Bentivegna

9. Study of the cellular distribution of a PCBP interacting protein using confocal microscopy. Faith Ikalina, Amanda Hunkele, Pranjal Nahar & Jane Ko

10. Setup of microarray technology to examine gene expression. Hader E. Elashal, Patrick W. Fedick, Viren Jadeja & Heping Zhou

Graduate – Microbial Physiology Lab

- 1. The Effect of Zinc and Copper Stress on Freshwater Cyanobacteria .** Barbara Fafara, Victoria Floriani, Robert Newby Jr., Zainab Abdillatif, Rebecca Hawke & Kari Wiedinger
- 2. The Effect of Nickel and Cadmium Stress on Freshwater Cyanobacteria.** Vanessa Ballentine, Johanna Park, Maher Youssif, Daniel Acosta, Hitaishi Dussa, Stefanie Geronimo & Kristen Wirasnik

Undergraduate – Senior Seminar

- 1. Ankylosing spondylitis pathogenesis: a Case of identity theft.** Brittany L. Hervey & Stephen P. Stracquatano
- 2. A home-use, enzyme-based E-DNA biosensor for the early detection of HIV Virus.** Mira Yazigi & Evelyn J. Brito
- 3. Synthesizing functional truncated dystrophin via adenovirus and lentivirus plasmid vectors.** Matthew Albert & Chloe Morales
- 4. HPV awareness and vaccination determinates in comparative minority versus majority youth populations.** Derrick D. Blackburn & Anju R. Kaimulayil
- 5. Fatty acid induced activation of brown adipose tissue.** Sarah J Osmun, Michael A Maiorelli, & Dave Eliassaint
- 6. Effect of sleep on weight gain and thyroid function.** Tamika R. Carty & Jaelyn E. Douglas
- 7. The selective destruction of cancer cells through the use of programmed oncolytic viruses.** Matthew P. Emery & Christopher DiPietro
- 8. Selection and evaluation of anthrax vaccination.** Amisha Tailor & Saul Rodriguez
- 9. Understanding the effects of Apolipoprotein E gene polymorphisms on concussions in college athletes.** Eric P. Morgenroth & Hassan M. Aly