2013 Rhode Island Summer Undergraduate Research Fellowship Conference

Friday, August 2, 2013
8:00 AM

College of Pharmacy
and
Center for Biotechnology & Life Sciences
University of Rhode Island

Supported by

Rhode Island NSF EPSCoR
Experimental Program to Stimulate Competitive Research

RI-INBRE & RI NSF EPSCoR

6TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, AUGUST 2, 2013
COLLEGE OF PHARMACY AND CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES
UNIVERSITY OF RHODE ISLAND
KINGSTON, RI

8:00 – 9:00 AM	**CONTINENTAL BREAKFAST AND POSTER SET-UP**
9:00 – 9:30 AM	**WELCOMING REMARKS**

– DR. DAVID DOOLEY, PRESIDENT, UNIVERSITY OF RHODE ISLAND

– MS. ELIZABETH ROBERTS, LIEUTENANT GOVERNOR, STATE OF RHODE ISLAND

– DR. ZAHIR SHAIKH, RI- INBRE PRINCIPAL INVESTIGATOR & PROGRAM DIRECTOR, UNIVERSITY OF RHODE ISLAND

– DR. JENNIFER SPECKER, RI NSF EPSCOR PRINCIPAL INVESTIGATOR, UNIVERSITY OF RHODE ISLAND

– DR. LISA ZUCCARELLI, CHAIRWOMAN, BIOLOGY & BIOMEDICAL SCIENCES AND CHEMISTRY, SALVE REGINA UNIVERSITY

– DR. DIOSCARIS GARCIA, POST DOCTORAL SCHOLAR, BROWN UNIVERSITY

9:30 – 12:30 PM	**SURF POSTER SESSION**
12:30 PM	**Lunch**

EXHIBITORS	
Cores RI	Located near the Central Stairway on the 1st Floor of the Pharmacy Building
University of Rhode Island **College of Pharmacy** **Graduate Programs in Pharmaceutical Sciences**	Located outside Room 240 on the 2nd Floor of the Pharmacy Building
Brown University **Office of Graduate & Postdoctoral Studies** **Division of Biology & Medicine**	Located in the Main Hallway on the 1st Floor of the Center for Biotechnology & Life Sciences
University of Rhode Island Graduate School	Located in the Main Hallway on the 1st Floor of the Center for Biotechnology & Life Sciences

DEMONSTRATIONS & TOURS	
RI-INBRE Bioinformatics & Centralized Research Core Facilities	Tours available at 10:00 AM, 11:00 AM, and 12:00 PM. Meet at the elevator nearest the Check-in Table in the Lobby on the 1st Floor of the Pharmacy Building.
Making Science Visible in 3D	Meet outside the 3D Facility for Biomedical Sciences (Room 170) at 11:45 AM on the 1st Floor of the Pharmacy Building.
Medicinal Garden Tours	Meet at 11:45 AM near the signs at the Central Staircase on the 1st Floor of the Pharmacy Building.
Patient Simulation Center	Meet at 11:45 AM near the signs at the Central Staircase on the 1st Floor of the Pharmacy Building.

POSTER PRESENTATION SCHEDULE

*** PLEASE NOTE: Posters are to be set up prior to the welcoming remarks and should remain up until 12:30 PM. Posters are to be manned for presentations according to the schedule below.*

Presentation Times	**Research Theme to be Manned**	**Poster List on Page #**
9:30 AM – 10:30 AM	Molecular Biology	19
	Genetics	11
	Marine Sciences	13
10:30 AM – 11:30 AM	Behavioral Sciences	1
	Chemistry	6
	Cell Biology	3
	Environmental Sciences	9
	Microbiology	17

BEHAVIORAL SCIENCES

LOCATED IN THE LOBBY ON THE 1ST FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 10:30 -11:30 AM

EMPIRICAL BAYES ANALYSIS OF RNA-SEQ WITHOUT REPLICATES FOR MULTIPLE CONDITIONS

Xiaoxing Cheng, Zhijin Wu, *Department of Biostatistics*, Brown University, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The recent developments in RNA-sequencing (RNA-seq) technology has led to a rapid increase in transcriptome data in the form of counts, because RNA sequencing in theory get less technological variation than microarrays [Marioni et al., 2008]. Identifying differential expression (DE) remains to be a key task in functional genomic among other applications of sequencing technique. As RNA-seq application extends to non-model organisms and experiments involving a variety of conditions from environmental samples the need for identifying interesting target RNA-seq without replicates is increasing. We present a novel empirical Bayes method for RNA-seq differential expression analysis for multiple samples without replicates. This method is targeted to rescue existing RNA-seq datasets that lack replicates. There have been a number of statistical methods for the detection of DE in RNA-seq data, which mostly focus on statistical significance of DE. We argue that, as transcription is an inherently stochastic phenomenon and organisms respond to a lot of environmental factors, it is possible that many expressed genes have DE, only to a different extent. Thus we focus on estimating the magnitude of DE. In our model, each gene is allowed to have DE, but the magnitude of DE in each treatment group is a random variable. So we assume most genes have small differences, the prior for DE is centered at zero. And Maximum a Posteriori (MAP) estimation for the magnitude of differential expression provided is shrunk towards zero. The estimator we provided gives better false discovery rate (FDR) and better understanding of DE.

SKIN TONE AND CRIME STEREOTYPES AFFECT WHITES' VISUAL ATTENTION TO BLACK FACES

Sathiarith Chau, Brandon DeSimone, Michael Saccoccio, Thomas Malloy, *Department of Psychology*, Rhode Island College, Providence, RI

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Guided by associative network (Eberhardt et al., 2004) and racial phenotype (Maddox, 2004) theories in a face identification paradigm, Whites searched for and identified the perpetrator or victim of a crime from an array of three Black faces varying in skin tone. During identification, eye movements were recorded continuously. In both perpetrator and victim conditions, the darkest toned faces were the first fixated upon. Crime roles (perpetrator or victim) interacted with skin tone and affected visual attention to target faces. In the perpetrator condition, dark and average tone faces attracted more visual attention than light faces, whereas in the victim condition, light faces attracted more visual attention than dark or average faces. Biased visual attention was not followed by discriminatory choice. Direct and continuous measurement of eye movements confirmed that skin tone bias operates at the earliest stage of information processing via a visual attention mechanism.

THE EFFECT OF MATERNAL ECONOMIC STRESS ON PSYCHOLOGICAL CONTROL AND EXTERNALIZING BEHAVIORS IN ADOLESCENTS

Amanda DiPaola, Emily Cook, Kristen Wilkinson, Trisha Kiley, Amanda Welch, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Development of externalizing problems (i.e., delinquent behaviors) is linked to certain types of parenting behaviors during vulnerable periods of adolescence (Beyers, 2003). Similarly, research has found that psychological control, a negative form of parental control, is associated with increased externalizing behaviors (Pettit, 2003). Past research, has not touched upon the precursors of psychological control as a parenting behavior, which may increase externalizing behaviors. One potential precursor is stress that parents might experience, in particular financial stress. Thus, the current study examines the relationship among financial stress, parent's use of psychological control, and adolescents' externalizing problems. Specifically, we hypothesized that maternal caregivers experiencing economic stress will report using higher levels of psychological control. Furthermore, we hypothesized that increased psychological control wil l be associated with increased externalizing behaviors in adolescents. To test these hypotheses, fifty participants (females=64.0%) between the ages of 13 to 17 along with their maternal parent completed in-home surveys. Maternal economic stress was measured by the mom report of economic hardship (Conger & Elder, 1994). Psychological control was measured by the mothers report on the Parent Behavior Inventory-Autonomy Granting subscale (CRPBI, Schludermann, 1970). Externalizing behaviors were measured by the Child Behavior Checklist Youth Self-Report (CBCL, YSR, Achenbach, 1991a).

The IBM SPSS Statistics 20 was used to test a series of multiple regression models. The significant threshold for all models was set at p <. 05. Results showed that higher maternal economic stress was directly associated with increased psychological control, ß=.291, p=. 04. Psychological control was directly associated with externalizing behaviors, ß=.321, p=. 02. Results of this study will help to develop programs for parents that target parenting behaviors that are detrimental to adolescent's development. With an understanding into how economic stress is affecting families we can implement new strategies to help elevate this stress, and correct negative parental control.

FRACTAL ANALYSIS OF THE SPATIAL STRUCTURE OF HUMAN EYE SCAN PATHS

Colleen Marlow, Logan Foust, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The human eye when viewing a scene makes a series of quick movements through the visual field in order to position the regions of interest in a scene on the fovea, the region of the retina of highest acuity. Collectively these movements over time lead to a visual scan path which is a direct behavioral measure of the underlying mechanisms at play in the human visual system. Traditionally the structure of scan paths has been characterized by breaking it into a series of fixations (collections of relatively smaller jumps in eye position) and saccades (relatively larger jumps in eye position) which are defined either by the experimentalist directly or computer software packaged with eye trackers. This method however simplifies the trajectory and can often wash out much of the complex spatial structure inherent in the scan path. Here we have developed a fractal analysis algorithm which takes a more global approach to quantifying the structure of the scan path and is more attuned to quantify the complexity of the structure of the scan path. We show that the spatial structure of human eye scan paths during free viewing are fractal, or scale invariant, and that their complexity can be quantified using the scaling parameter known as the fractal dimension. We propose that this method of quantifying the complex structure of scan paths be used as a means to compare eye movements across individuals and behavioral scenarios and shows promise to aid in our understanding of the human visual system.

ASSESSING WORD COMPREHENSION ACROSS WORD CLASS

Emely Bueno, Amanda Gaskill, Amanda Wallace, Beverly Goldfield, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The data which inform theories of language acquisition are typically measurements of production, ignoring comprehension, which is difficult to assess. From an applied perspective, comprehension is also an important index of developmental status, but standardized instruments are problematic for many young children with a limited behavioral repertoire, including children with autism. We examine language comprehension using the Preferential Looking Task (PLT), which measures change in visual attention. The PLT assesses word comprehension by comparing a child's visual attention recorded by an eye tracker to three images (1 target and 2 distracters) depicted on a computer monitor before (baseline) and after (test) the target image is labeled. Comprehension is defined as an increase in attention to the target image during the test trial compared to baseline presentation. We used the PLT to test word comprehension in normally developing pr eschool children. We also administered the Peabody Picture Vocabulary Test (PPVT), a standardized test which requires children to point to a picture named by the experimenter. We hypothesized that PLT scores would correlate positively with PPVT scores. To date, we have tested word comprehension (21 nouns, 4 adjectives, 8 verbs) in 38 children at ages 3 (n= 7), 4 (n=12), 5 (n= 15) and 6 (n= 4) years of age. Across all ages there was a significant increase in visual attention to the target image from baseline to test. Mean comprehension scores were highest for nouns, followed by verbs and adjectives. As predicted, there was a significant positive correlation ($r= .41$, $p < .05$) between scores on the PLT and the PPVT. These data suggest that the PLT may provide a reliable method for assessing word comprehension in children who lack the behavioral skills necessary for standardized testing.

NEGATIVE LIFE EVENTS AND DEPRESSION'S INFLUENCE ON SOCIAL COMPETENCE

Trisha Kiley, Emily Cook, Kristen Wilkinson, Amanda DiPaola, Amanda Welch, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Past research suggests that higher levels of depressive symptoms were associated with more negative life events and relatively high levels of helplessness in social situations (Nolen et al. 1992).Links between negative life events, depression, and interpersonal behavior deficits have received little attention. Not much is known about the relationship between financial stress in adolescent's homes and their everyday social interactions particularly peer conflict management. Also, little research has been done to examine whether depression reports from adolescents maternal caregivers will partially explain the relationship between financial stress and peer conflict management. Overall this study examines how depression and negative life events effect social competence. Because adolescence is such an important time in developing peer relationships, identifying potential causes of social incompetence is essential to healthy adolescent development. Participants in this study included 50 youths between the ages of 13 to 17 in grades 9th through 11th. Upon analyzing the data using a series of multiple regressions, financial stress reported from moms was associated with adolescent's reports of conflict management in social situations. Mom's reports of depression for youth partially explain the relationship between financial stress and adolescent's social conflict management. Surveys are used to assess measurements. Furthermore, negative life events and social competence have been broken down in three subgroups which include; financial stress, family conflict, youth perceived stress, social initiation, social disclosure and social conflict management. Measures used include the CBCL depression scale, economic hardship and family conflict scales. Finally to measure social competence the interpersonal competence scale is used. This study is ongoing and it is important to use this study to understand these relationships to further understand how certain aspects of depression and negative life events can directly impact how youths social competence is affected.

THE UNIQUE MENTAL REPRESENTATION OF PRETENSE

Alexandra Cribbin, Kathryn Graf, Kelly Murner, Christina Taylor, Jennifer Van Reet,
Department of Psychology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Previous research has shown that children use self-control when representing pretense: after reading a story about pretending, children are slower to respond to a word associated with the real aspect of the story (a real associate), than to both a word associated with the pretend aspect of the story (a pretend associate) and an unassociated word (control). Two experiments were conducted further exploring this effect. Participants for both experiments were preschoolers, 8-10 year olds, and adults. The first experiment was performed to see what is unique about pretense. Participants read 24 short stories involving two objects. Participants then saw a picture of one of the two objects mentioned or an unrelated object and named it into a microphone; reaction time (ms) was measured. Results showed adults and 8-10 year olds reacted quickest to both mentioned associates, while preschoolers reacted similarly to all three associates. Thus, adults and 8-10 year olds activated both objects in the story, but preschoolers did not activate anything, possibly indicating that the task was not engaging enough. The second experiment examined how participants mentally represented pretense after self-control was depleted. First, participants were depleted using a standard computerized Stroop task, in which they were instructed to pay attention to the appearance of a word, not its meaning. The second half of the experiment followed the same procedure as Experiment 1, except the stories contained pretend actions. Results showed that adults reacted quickest to the real associates, 8-10 year olds reacted the same to the real and pretend associates, and preschoolers reacted quickest to the pretend associates. Together, these studies suggest that in order to represent pretense, adults activate reality, preschoolers activate pretense and 8-10 year olds activate both, revealing a possible developmental timeline.

EARLY STRESS AND LATER PSYCHOPATHOLOGY: EFFECT OF JUVENILE EXPOSURE TO PREDATOR ODOR ON ADOLESCENT AND ADULT ANXIETY AND PAIN NOCICEPTION

Kaitlyn Dahlborg, Lauren O'Loughlin, Ryan Post, *Departments of Biology and Psychology*, Providence College, Providence, RI; Michelle Oullette, *Department of Biology*, Providence College, Providence, RI; Christopher Bloom, *Department of Psychology*, Providence College, Providence, RI

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Stress during juvenility and adolescence has been shown to be a reliable precursor to anxiety, stress, and pathology later in life. Clinical researchers have tracked patients with early life trauma and noted generalized anxiety disorder, unipolar depression, and risk-taking behaviors in late adolescence into early adulthood. Animal models provide an opportunity to investigate the neural and developmental processes that underline the relationship between early stress and later psychological disorders. The present model used repeated exposure to trimethylthiazoline (TMT), a component of fox feces, as an unconditioned fear-eliciting predator odor to induce stress in juvenile rats ages postnatal day (PND) 23 through 27. Bolus and open field data were collected during scent exposure—either TMT or a distilled water control—as a measure of acute anxiety. After further physical maturation (PND42) animals were tested using an elevated plus maze (EPM) and Plantar test (Hargreaves method) to assess any lingering effects of the early life stress. To assess how an additional stress later in life, rats (PND43) were then exposed to inescapable shock (.8 mA) and again tested on EPM and Plantar. A final testing period was conducted in the adult rats (PND63) to assess resulting changes in adult behaviors. Results are discussed in the context of the role of early life stressors in the manifestation of human pathologies such as PTSD and non-suicidal self-injury.

EFFECTS OF EARLY EXPERIENCE AND INTER-ALPHA-INHIBITOR PROTEIN ON WORKING MEMORY AND SPATIAL LEARNING IN A RODENT MODEL OF HYPOXIA-ISCHEMIA

Cynthia Gaudet, Keyshla Melendez, Zahra Melendez, Matt Hall, *Department of Biology*, Rhode Island College, Providence, RI; Stephanie Chauvin, Travis Dumais, Steven Threlkeld, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Neonatal cerebral oxygen deprivation and reduced blood flow (hypoxia-ischemia, respectively) induces global brain injury. Neonatal hypoxia-ischemia (HI) reportedly impairs working memory performance in humans, which hinders long-term learning throughout maturity. Further, early behavioral remediation has been suggested to improve cognitive outcome in children with developmental neuropathology. With few treatment options available for infants with HI injury, the aim of this study was to evaluate the effects of an initial 2-dose treatment of anti-inflammatory inter-alpha-inhibitor protein (IAIP) combined with an early task-specific experience, to improve long-term behavioral outcome in a rodent model of HI. Subjects were divided into two experience groups (Juvenile/Adult testing vs. Adult only). Each experience group was subdivided into three treatment conditions (HI+Vehicle, HI+IAIP, and Sham subjects). For the injury groups, complete cauteri zation of the right common carotid artery (ischemia) and 120 minutes of 8% O2 (hypoxia) was induced at postnatal day (P) 7. An eight-arm-radial water maze was used to assess both working and reference memory simultaneously. A win-shift paradigm across multiple trials (4 trials/ day) was assessed performance on the eight-arm-radial water maze task. Data suggest that early experience and anti-inflammatory treatment may improve long-term behavioral performance in a rat model of neonatal hypoxia-ischemia.

THE INTERGENERATIONAL TRANSMISSION OF DEPRESSION

Kristen Wilkinson, Emily Cook, Trisha Kiley, Amanda DiPaola, Amanda Welch, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Research has suggested that depressive symptoms are passed down in families and children of depressed mothers are at an increased risk of depression later in life (Hammen et al., 2011). Few studies have examined possible explanations (i.e., examining mediators) as to why depressive symptoms are transmitted from mothers to adolescents. Forman and Davies (2003) suggest that adolescence may be a critical period for developing a sense of security in the family context and may put adolescents at risk for depressive symptoms. Thus, we hypothesized that the association between maternal depressive symptoms and adolescent depressive symptoms will be partially mediated by emotionally insecurity.

To test this hypothesis, fifty participants (females=64%/Caucasian=78%) between the ages of 13 to 17 (M=15.1) completed surveys in the home. Mom depression was measured through the Center for Epidemiologic Studies Depression Scale (Radloff, 1977) and adolescent depression was measured through mom and adolescent reports of the Child Behavior Checklist Scale (Achenbach, 1991). Emotional insecurity was measured through the Inventory of Parent and Peer Attachment (Armsden & Greenberg, 1987).

Results from a multiple regression analysis indicated that maternal depressive symptoms were associated with adolescent depressive symptoms. Emotional insecurity partially mediated the pathway between maternal depressive symptoms and adolescent depressive symptoms with higher emotional insecurity resulting in higher reports of adolescent depressive symptoms. Results from this study may assist programs for adolescents and their mother's with depression by giving more information on ways to avoid/help depression and the potential sources of depressive symptoms in youth.

CELL BIOLOGY

LOCATED IN ROOM 105 ON THE 1ST FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 10:30 -11:30 AM

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS OF LEISHMANIA DNA

Christine Ortiz, Steven Symington, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Leishmania are parasitic organisms that are known to cause visceral and cutaneous leishmaniasis in humans. The manifestation of the parasite-induced symptoms is dependent on the specific species. The goal of this project is to identify unique genetic markers and genes that are expressed in different species of Leishmania. To this end, we utilized Amplified Fragment Length Polymorphism (AFLP), which is a PCR-based technique that can identify polymorphisms across genomes and differences in gene expression between species without prior sequence knowledge. The goal of this project was to use AFLP to analyze 4 different species of Leishmania; L. major, L. mexicana, (cutaneous) and L. major (visceral) in comparison to L. tarentolae, (a not pathogenic species) to identify genetic markers and genes expressed that are unique to the pathogenesis of each species. To accomplish this, we used sixteen unique EcoRI and Mse1 primer combinations to amplify sma ll fragments from previously prepared gDNA samples from each Leishmania species. Gel electrophoresis of the amplified fragments indicates that unique polymorphisms were detected with gDNA samples. Thus AFLP successfully detected genetic variation among Leishmania species with different disease profiles. Future studies will utilize AFLP to identify and sequence species-specific genes that are expressed in cDNA prepared from each of the species.

DETERMINING WHETHER UBE4B ACTIVITY EFFECTS SKELETAL MUSCLE PERFORMANCE

Jeremy Boutin, Sarah Spinette, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Skeletal muscle tissue develops through a complex process called myogenesis that is controlled in part by the regulated degradation of specific proteins. These substrates are degraded by the proteasome after being marked by the cell's ubiquitin conjugation system that is composed of three levels of enzymes (E1, E2 and E3). Ube4b is an E3 ligase enzyme that mediates ubiquitin conjugation through its U-box domain. To determine whether or not the function of Ube4b plays a crucial role in skeletal muscle development and function, we developed transgenic mice whose Ube4b U-box domain was removed in skeletal muscle when iCre-recombinase expression is controlled by the MyoD promoter. Removal of the U-box domain, leads to expression of an inactive E3 ligase. Mice lacking active Ube4b in their skeletal muscle exhibit reduced postnatal growth, skeletal muscle size and loss of sarcomere structure within many skeletal muscle fibers. These observations have led us to hypothesize that the Ube4b mutant mice should have diminished performance when tested with strength assays. In order to test this hypothesis, mutant and control littermates were subjected to two strength assays: the hanging wire and grip strength tests. The two strength assays showed that mice lacking Ube4b performed worse at two and four weeks old, but better at three weeks old. The data collected so far has not underlined an overall trend. Thus far only small groups (n=3-5) of mutant and control mice have been tested and none of the results were significant. Given the high variability in these assays they will be continued in order to increase the sample size and allow statistical analysis.

ACTIVATION OF SURVIVAL SIGNALS IN MELANOMA CELLS IN RESPONSE TO GLUCOSE DEPRIVATION

Garrett Cammarata, David Calianese, Yinsheng Wan, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Many cancer cells make use of altered cellular pathways to control proliferation, survival and internal regulation of metabolism. The use of these augmented pathways is necessary in order to proliferate and expand so aggressively but may make it possible to be targeted using cancer therapies. We studied the effects that lowering glucose concentration has on cell proliferation, growth and changes to its extracellular environment in WM266-4 Melanoma cells. When glucose concentration in medium is lowered, a wide variety of changes occur within the cells. The most visible ones being changes to morphology and inhibition of growth while there are more subtle changes like the alteration of its signaling pathways inside the cell. The cytoskeleton can interact with insulin containing vesicles, alter GLUT transporter translocation and alter glucose metabolism related enzymes. With glucose reduced, the cytoskeleton was disrupted after 48 hours which can be seen by decreasing dendricity. Inhibition of growth was found to be linearly correlated with decreasing glucose concentrations in medium, as was depletion of nutrients and changes in pH of the extracellular environment. Reduction in pH could be a mechanism of the cancer cells to proliferate and survive, from heavy production of lactic acid caused by increased rate of glycolysis, or both. Levels of p-S6, p-ACC and p-AKT were shown to be reduced and AMPK increased by glucose deprivation at 6 hours. S6 ribosomal protein, as well as MEK and ERK pathways were increased however at 48 hours showing the cancer cells are fighting back in order to survive. Finally, GLUT1 transporter expression as well as survivin, an anti-apoptotic molecule, was shown to be increased at 24 hours under glucose deprivation. The effects of glucose deprivation on melanoma are important in understanding the way these cells uptake their nutrients and may provide for therapeutic treatments in the future.

CHARACTERIZATION OF CAV1.3 IN RAT BRAIN NEUROLEMMA MICTROTRANSPLATED OOCYTES

Heather Conboy, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Cav1.3 is a voltage-sensitive calcium channel that is expressed in a variety of excitable cells, including brain, skeletal, and cardiac tissue. Activation of these calcium channels allows calcium entry into the cell and regulates muscular contraction, excitation, gene expression, or release of hormones or neurotransmitters. Over stimulation on Cav1 channels can result in excitotoxicity, as elevated levels of intracellular calcium activates signaling cascades which, and can degrade essential cellular structures. The purpose of this research was to examine Cav1.3 in its native state by microtransplanting rat brain neurolemma into Xenopus oocytes. To do this, we used a combination of western blotting, immunohistochemistry and electrophysiology to characterize rat brain neurolemma injected oocytes. Preliminary data indicates that Cav1.3 was incorporated into the Xenopus oocytes. These results indicate that microtranplanted rat brain neurolemma is a viable method to examine L-type voltage-sensitive channels microtransplanted into Xenopus oocytes.

EXPRESSION OF AN LDLIP3 FUSION PROTEIN IN LEISHMANIA DONOVANI

Tia Crowther, Steven Symington, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Lipases are a family of esterases that have been shown to be virulence factors in many organisms due to their ability to cleave triglycerides. Therefore it is proposed that LdLip3 may play a role in Leishmania's pathogenesis. This research was conducted to determine the optimal expression of LdLip3, a secretory lipase, in Leishmania donovani. An LdLip3 fusion protein was previously constructed by adding a HA tag to the lipase LdLip3 and transfected into Leishmania donovani. The supernatant of transfected Leishmania donovani was collected and the HA-LdLip3 was purified by affinity chromatography. Western blot analysis confirmed that the transfected Leishmania donovani cells expressed the HA-LdLip3 fusion protein. The enzymatic activity of purified HA-tagged LdLip3 was tested under varying conditions that consisted of a range of pH's (4-8) over a thirty minute incubation period at 37°C using a variety of different 4-MU substrates , palmitate and stearate. The results obtained from the enzymatic assays showed that HA-LdLip3 had less activity than a commercial lipase secreted by the organism, Rhizomucor miehei. Lastly, the results from the western blot analysis indicate that the HA-LdLip3 is overexpressed in the transfected cells after purification. Further experimentation is in progress to further optimize the expression of HA-LdLip3.

SYNTHESIS, CHARACTERIZATION AND IN VITRO CYTOTOXICITY OF MAGNETIC HOLLOW CUS NANOPARTICLES

Michela Cupo, *Department of Biology*, Roger Williams University, Bristol, RI; Yajuan, Li, Liangran Guo, Wei Lu, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Photothermal therapy of cancer has become a hot area of research in biomedical and pharmaceutical sciences. The magnetic (Fe3O4) hollow copper sulfide (CuS) nanoparticle (MHCuSNPs) drug delivery system combines the magnetic targeting function of the Fe3O4 and photothermal ablation effect of the poly(ethylene glycol) (PEG) decorated CuS nanoparticles. This drug delivery system provides the potential for cancer treatment under magnetic field and laser exposure. This study synthesized and characterized MHCuSNPs. Nanoparticles were prepared and coated with PEG. Next, water stable citric acid-coated Fe3O4 (CC-Fe3O4) was produced. MHCuSNPs were achieved by mixing the previously made products with a molecular ratio of 10:1 (Cu: Fe molecular ratio calculated by inductively coupled plasma mass spectrometry (ICP-MS). The MHCuSNPs were characterized with transmission electronic microscope (TEM) and UV spectrum. Cytotoxicity of MHCuSNPs with or without la ser exposure was tested on human breast cancer cells. The magnetic particles were attracted to the tumor cells by magnetic field exposure upon the cells in vitro. The hollow PEG-CuS nanoparticles (PEG-CuSNPs) needed partial surface area coverage of the magnetic solution in order for the nanoparticles to be controlled and attracted by exposed magnetic field. These findings suggested that the magnetic exposure and laser approach did in fact eliminate tumor cells following MHCuSNP treatment.

MOTILE PIGMENT GRANULES IN THE SQUID PHOTORECEPTOR ASSOCIATE WITH MICROTUBULES AND WITH THE MINUS-END DIRECTED MOTOR CYTOPLASMIC DYNEIN

Jennifer Cyr, Joseph DeGiorgis, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Within the squid visual system, photoreceptors contain large pigmented granules that move within the cell in response to light and act to absorb photons passing thought the cell membrane. The granules are found in association with microtubules and move at fast axonal transport rates suggesting that they are powered by microtubule-based motors. In previous work, we undertook a so-called expressed sequence tag project (EST) by single-pass sequencing mRNA isolated from the squid nervous system and identifying these sequences by bioinformatic analysis. In doing so, we identified a wide variety of kinesin, dynein and myosin motor proteins and generated peptide antibodies against many of these force-generating molecules. Here, we obtained pigment granules by cutting the retina with a blade and touching an EM grid to the cut surfaces to obtain a thin layer of cellular components including granules and microtubules. Through immuno-label EM we find tha t the granules contain a cytoplasm dynein light chain on their surfaces. This data suggests that dynein is involved in the transport of the granules and is likely responsible for minus-end directed microtubule-based movements from the distal ends of the photoreceptors to the photoreceptor base.

CONSTRUCTING A S. CEREVISIASE STRAIN CONTAINING A TEMPERATURE SENSITIVE MUTATION OF BCP1

Sabrina Elgar, Sarah Bilida, Saman Nayyab, Deborah Britt, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Responding to DNA damage is one of the most important functions in the survival of all living organisms. DNA repair is a very specific and complex series of events that include many components. In human cells, one such DNA repair protein is BCCIP, which interacts with the tumor suppressor BRCA2 and the cell cycle regulator CDKN1A. In response to DNA damage, BCCIP assists in halting the cell cycle and participates in homologous recombination repair with BRCA2. In order to better understand the role of BCCIP in DNA repair, we have chosen to study the protein's fungal homolog, Bcp1, in S. cerevisiae. In previous studies the hypothesis that Bcp1 plays a role in DNA damage response was evaluated by testing two strains of yeast. A strain containing a temperature sensitive mutation of Bcp1 known as AAY and its parent strain SEY, were treated with DNA damaging drugs and their growth was compared. The growth of SEY and AAY differed depending on which drug they were exposed to. In order to confirm that these results were due to the Bcp1 mutation and to facilitate new studies, a plasmid containing Bcpts was created. This plasmid was then transformed into a Bcptet regulated strain and its' parent strain R1158.

LOCALIZATION OF BXI1P/YBH3P IN THE ENDOPLASMIC RETICULUM AND THE VACUOLE OF THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE

Alfredo Gonzalez, *Department of Biochemisry*, Providence College, Providence, RI; Michael Matassa, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

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Bax inhibitor-1 (BI-1) is an anti-apoptotic gene whose expression is upregulated in a wide range of human cancers. Our laboratory has published data suggesting that the yeast gene BXI1 is a homolog of BI-1, which links the unfolded protein response and programmed cell death. Further studies in our laboratory with the yeast Saccharomyces cerevisiae suggested that Bxi1p is localized to the cell's endoplasmic reticulum. However, our colleagues in the Madeo Lab at the University of Graz in Austria have also published data that suggests that Bxi1p/Ybh3p is localized to the cell's vacuole. To reconcile these conflicting results, we obtained the Bxi1p/Ybh3p expression construct from the Madeo Lab and repeated their experiments. We show that over-expressed Bxi1p/Ybh3p-GFP does co-localize with the Sec63p-RFP that is known to be an endoplasmic reticulum integral membrane protein. Bxi1p/Ybh3p-GFP also localizes to the vacuole membrane. Howev er, long-term growth of these yeast cells reveals that the GFP-tagged Bxi1p/Ybh3p does eventually accumulate in the vacuole. Finally, we have also discovered that this plasmid slows the growth of cells. Therefore, our data suggests that the previous discrepancy in localization data arose because of the non-physiological overexpression of the tagged protein.

PH-LOW INSERTION PEPTIDE (PHLIP) TARGETS BREAST CANCER TUMORS IN TRANSGENIC MICE AND MEDIATES DELIVERY OF GOLD NANOPARTICLES TO A549 CANCER CELLS

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A hallmark of cancer is an acidic extracellular environment resulting from the Pasteur and Warburg Effects. In such environments, peptides within the pH-Low Insertion Peptide (pHLIP) family pH-dependently insert into cancer cell membranes and form stable transmembrane alpha-helices. In past experiments, it has been demonstrated that pHLIP also delivers cargo to tumor tissue both in vitro and in vivo. With pHLIP's potential for delivering agents able to improve cancer treatments, the purpose of this summer's work was two-fold: 1) to establish tumor targeting and distribution of pHLIP variants and colocalization with 2-deoxyglucose (2DG) in breast tumor transgenic mice and 2) to pH-dependently deliver pHLIP-monomaleimide nanogold conjugates to human lung carcinoma (A549) cells. Transgenic mice were first given a single intravenous injection of a pHLIP variant and 2DG, both labeled with infrared dyes. After 24 hours, whole body, organ , multiple tumor, and single tumor images were scanned with a LI-COR Odyssey Infrared Imaging System. In the second set of experiments, pHLIP was conjugated to 1.4 nm diameter monomaleimide gold nanoparticles and purified using high-pressure liquid chromatography (HPLC). A549 cells grown in media at pH 6.2 and pH 7.4 were treated with different amounts of either non-functionalized gold or monomaleimide nanogold-pHLIP, washed, and mixed with silver enhancement. This solution deposits silver on the surface of gold nanoparticles and enlarges them to micron sizes. Gold uptake was then observed using an Olympus IX71 Inverted Epifluorescence Microscope. The obtained data clearly demonstrate pHLIP's ability to target breast tumors in the mice transgenic tumor model and selectively deliver gold to cancer cells, which could be developed for enhancement of radiation during breast cancer treatment.

DETERMINATION OF THE CROSS-SECTIONAL AREA OF MYOFIBERS FROM UBE4B MUTANT AND CONTROL MICE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Ube4b is an E3 ligase that ubiquitinylates specific substrate proteins in order for degradation by the protostome. Its activity is dependent upon a conserved domain called the U-box, coded by exons 26 and 27 in the mouse gene. Based on previous work, we developed the hypothesis that Ube4b ubiquitylation plays an important role in the development of striated muscle tissue. In order to identify the function of Ube4b in skeletal muscle myogenesis, we developed a line of mice carrying a muscle-specific conditional Ube4b mutation. Mutant mice are homozygous for an allele containing lox P sites flanking exon 26 (ΔU) and heterozygous for the MyoDiCre allele in which iCre-recombinase is inserted in exon 1 of the MyoD gene. Since MyoD is expressed only in skeletal muscle cells, iCre only recombines the Ube4b allele in skeletal muscle. By approximately 4 days after birth most mutant mice are significantly smaller in size compared to control mice and display a slower growth curve which ultimately ends in early death. We aimed to determine if the muscles of the mutant mice are smaller because the individual myofibers are smaller in size, as opposed to having fewer total myofibers. Mutant and control mice at ages ranging from 4 to 21 days old were dissected and serial cross sections of the quadriceps muscles were stained with wheat germ agglutinin (WGA) to mark sarcoplasmic membranes using fluorescence microscopy. The average cross-sectional area (CSA) and Ferets diameter were measured using ImageJ. Preliminary results show that the myofiber CSA of mutant mice is smaller starting after P4. This could indicate that individual fibers are not growing normally postnatally.

MATRILIN-3 DEPENDS ON EPIDERMAL GROWTH FACTOR RECEPTOR FOR THE INDUCTION OF IL-1RA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Matrilin 3(MATN3) is an ECM protein commonly found in articular cartilage which has been shown to upregulate IL-1RA. Due to MATN-3's possible therapeutic effects, there is currently particular interest in how MATN-3 results in the upregulation of IL-1RA.

Based on the evidence that mutations in hMATN3 are associated with a variety of skeletal diseases, like HOA and our preliminary data which illustrates the inability of MATN3 to up regulate IL-1RA, COL2A1 and ACAN, when EGFR is chemically silenced in mature chondrocytes, we have hypothesized that the MATN3 signaling pathway is initiated by the binding of MATN-3's EGF domains to an Epidermal Growth Factor Receptor (EGFR). Therefore the purpose of this study was to investigate whether MATN-3 is dependent on EGFR for its induction of IL-1RA in a chondroprogenitor cell line.

This was done by using a siRNA to knockdown the chondroprogentor cell's expression of EGFR. Three different cell types were used including, WT-ATDC5 cells, and ATDC5 cells that either overexpress WT-MATN-3 or the mutated MATN-3 found in HOA. RT-PCR was used to quantify the gene expression of both IL-1RA and ACAN, which is dependent on presence of IL-1RA.

We found that MATN3 is dependent on EGFR for the induction of both IL-1RA and ACAN. This is illustrated by a 8-fold decrease in relative mRNA expression of IL-1RA in the WT-MATN3 ATDC5 cells that were treated with the EGFR siRNA. Furthermore we also saw there was no difference in either IL1-RA or ACAN expression in the silenced HOA cells, which have a mutant form of MATN 3. As a result, we can conclude that the mutant MATN-3 loses its ability to signal EGFR. Therefore as a result of these findings we have concluded that MATN 3 is dependent on EGFR for the activation of IL-1RA.

THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE ASSOCIATES WITH MOTILE PIGMENT GRANULES IN THE SQUID PHOTORECEPTOR

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RI-INBRE Summer Undergraduate Research Fellowship Program

The amyloid precursor protein is a progenitor of a peptide fragment termed "Abeta" a 36-43 amino acid sequence that builds up in the brain lesions of Alzheimer's patients. APP contains a single transmembrane domain and has been found in association with membrane-bound organelles within the nervous system, however a role for APP in neurons has not been established. Some data has suggested that APP may be bound to the microtubule-based motor protein kinesin and therefore may act as a trailer hitch linking kinesin to it intercellular cargo. Here, we find that the amyloid precursor protein is attached to pigment granules in photoreceptors of the squid retina that act to regulate the amount of light that reaches the photosensitive machinery of the cell and in doing so facilitates visual signaling. These granules move up and down a central shaft at rates consistent with fast axonal transport and they are attached to multiple microtubules at discrete domains along the pigment granule surface. Internal to the pigment granule lipid membrane lies an electron dense band reminiscent of a postsynaptic density a tightly pack proteinaceous structure at the end of dendritic spines. These dense bands lie adjacent to the pigment granule microtubule-binding site where microtubule-based motor are likely to be found. Thus the electron dense bands may be APP laden structures that serve as receptors for force generating molecules.

THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE CLUSTERS ON THE SURFACES OF AXOPLASMIC ORGANELLES AND LOCALIZES TO THE ORGANELLE/MICROTUBULE INTERFACE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Alzheimers disease afflicts an estimated 26 million individuals worldwide and its incidence is predicted to reach 100 million people by 2050. This debilitating neurodegenerative disease is characterized by loss of long-term memory, language degeneration, and cognitive impairment, which often leads to death. Mutations in the amyloid precursor protein (APP) have been shown to lead to heritable forms of this disorder, however, little is known about the wildtype function of APP or how mutations in the gene lead to AD pathogenesis. Here, we set out to study the distribution of squid APP in the giant axon at the ultrastructural level. Immuno-gold electron microscopy using antibodies raised against the C-terminus of squid APP reveals that the protein clusters on the surface of 100 nm vesicles in both reconstituted organelle/microtubule complexes and extruded axoplasm. Many of these organelles are bound to microtubules and APP often localizes to the organelle/microtubule interface. In addition, we are in the process of determining the concentration of APP in the squid giant axon by quantitative immunoblot. To develop a standard curve, we have cloned full-length squid APP from mRNA isolated from the stellate ganglion, which contains the cell body of the giant axon. Squid APP contains a 1839 nucleotide open reading frame that encodes a 613 amino acid sequence. We are currently overexpressing this gene with a 6xHis tag for nickel-based purification.

CHEMISTRY

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EVALUATION AND APPLICATION OF A MICROWAVE ASSISTED ESTERIFICATION METHOD

Edward Crosier, Lauren Rossi, *Department of Chemistry*, Roger Williams University, Bristol, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Diets rich in polyunsaturated fatty acids (PUFAs) have been associated with greater cardiovascular health and a decrease in the risk of some cancers. The common AOCS method to determine the amount of fatty acids within a sample involves an esterification process and gas chromatographic analysis. Limitations and variable effectiveness of this AOCS method have been shown, in particular with marine-based samples that are high in PUFA content. In order to optimize the preparation of fatty acid methyl esters (FAMEs) from fatty acids (FAs), several methods were examined in terms of efficiency and effectiveness. A method involving hydrochloric acid and copper acetate under microwave irradiation was optimized to convert long chain saturated, monounsaturated, and polyunsaturated fatty acids to the corresponding methyl esters. This method can also be applied to fish oil and fish tissue samples.

SINGLE-MOLECULE STUDIES OF CELL MEMBRANE STABILITY: LAYING THE FOUNDATION

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The cell membrane is a unique biological structure that defines a cell's boundaries and is key to controlling the transport of various chemicals between cell interior and exterior. The central element of the cell membrane is the lipid bilayer, a structural element whose stability is highly dependent on environmental conditions such as temperature, pH, chemical concentrations, and applied pressure. A variety of molecules can incorporate into lipid bilayers, including a special class of pore-forming molecules known as porins. The toxin alpha-hemolysin is one of these porins and it self-assembles into the bilayer to form stable channels that permit the passage of chemical species across the bilayer. We developed and tested a reliable set of procedures to form stable, long-lived bilayers. Giant unilamellar vesicles (GUVs) were pressure-driven to a glass microaperture, a micropore. Their collapse to form a lipid layer spanning the aperture w as monitored by measuring the change in current under applied voltage passing through the aperture: formation of a lipid layer resulted in a gigaohm resistance. Insertion of alpha-hemolysin pores into the bilayer resulted in a characteristic ~100picoamp current at 100mV. This observation confirmed that a bilayer, rather than multilayer, had been formed, and allowed us to monitor the long-term stability of the bilayer into which a porin had inserted. In a similar set of experiments, we attempted to use applied external pressure to force a GUV to rupture and coat the surface of a silicon nitride nanopore with a fluid lipid bilayer. A variety of custom components were designed and machined, including a pressure sensor holder that was fabricated using a 3D printer. Improvement in the ability to form long-lived, free-standing lipid bilayers and to bilayer-coat single-molecule nanopore sensors, could result in a multitude of useful bioanalytical applications, including personalized medicine.

ANTIOXIDANT ACTIVITIES OF AQUIDNECK HONEY EXTRACTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Honey is widely used in traditional medicine throughout the world and is one of the most unique products found in nature due to its potential to be composed of plant nectar from a large variety of species. One of the characteristics of honey attributed to its medicinal properties is the presence of phenolic compounds, which originate in plants via the process of Apis genus of bees collecting nectar. Phenolic compounds are known to exhibit antioxidant, anti-carcinogenic, and anti-inflammatory properties, among others. In order to investigate the properties of the phenolics in honey, column chromatography with Amberlite XAD-2 was used to separate the sugars in honey from the phenolics. The resulting methanolic extract was further fractionated by partitioning between water and ether to yield an aqueous extract (AE) and an ether extract (EE). The antioxidant properties of the crude honey and the aqueous and ether extracts were evaluated by testing their scavenging effect on DPPH radicals compared to vitamin C. The ether extract had the most potent antioxidant capacity, followed by the aqueous extract and the crude honey. High performance liquid chromatography (HPLC) was used to isolate the major compounds of the ether extract and the process of identifying the phenolics is underway. In addition, the antimicrobial properties of the extracts are also being investigated.

BIOCHEMICAL STUDIES OF NAMPT

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RI-INBRE Summer Undergraduate Research Fellowship Program

Nicotinamide adenine dinucleotide (NAD+) is required for over 200 redox reactions and is consumed during DNA repair signaling through the enzyme PARP1. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme of the NAD+ salvage pathway that restores depleted NAD+ and thus is crucial for maintaining stable cellular levels of NAD+ levels. Active NAMPT is a homodimer that generates two active sites along the dimerization plane. Successful alteration of the dimerization plane should affect enzymatic activity. Autodock Vina was used to prioritize the NCI Diversity set III library. NAMPT activity was monitored through conversion of NAMPT product to a fluorescent derivative. Two compounds that lie on NAMPT's dimerization plane show a significant increase in the enzyme's activity.

SYNTHESIS OF COMPOUNDS STRUCTURALLY SIMILAR TO QUINOLONE AUTOINDUCERS FOR USE AS POTENTIAL QUORUM SENSING INHIBITORS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Bacterial infections are currently the leading cause of death in the United States. Antibacterial use has caused outbreaks of drug resistant bacterial strains causing infection to become increasingly more difficult to treat. Quorum sensing (QS) is a process that bacterial species use to communicate with each other in order to act together. Autoinducers released by bacteria accumulate and ultimately cause the expression of virulence factors when the population of bacteria reaches its quorum. The structure of autoinducers varies among different QS systems but can include quinolone derivatives as well as N-acyl homoserine lactones (AHL). The focus of this project is to synthesize compounds structurally similar to the quinolones used in the Pseudomonas aeruginosa QS system. It is believed that these compounds will have the potential to inhibit quorum sensing by acting as competitive inhibitors. A one-step synthesis to prepare the desired scaffold has proven to be successful and the synthesis of a small focused library of analogs is underway. Each analog will be tested for quorum sensing inhibition to determine the inhibitory effects of different alkyl chain lengths as well as the effect of the nature and position of substituents on a phenyl ring. The compounds will be tested by the inhibition of known quorum sensing activities including bioluminescence in Vibrio harveyi and purple pigment production in Chromobacterium violaceum. The compounds synthesized during this investigation are anticipated to serve as valuable tools in the study of quorum sensing and provide potential new leads in the development of anti-infective agents.

MOLECULAR DOCKING TO IDENTIFY NOVEL INHIBITORS OF NICOTINAMIDE PHOSPHORIBOSYL TRANSFERASE (NAMPT)

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RI-INBRE Summer Undergraduate Research Fellowship Program

Current chemotherapies activate the poly (ADP-ribose) polymerase (PARP) family of DNA repair proteins. PARP enzymes consume NAD+, causing a depletion of cellular energy that is replenished through the activity of NAMPT. Thus NAMPT is a potential new target for chemotherapy intervention. NAMPT is a dimer that creates two active site channels spanning both monomers. Currently known inhibitors of NAMPT, such as FK866, compete for the active site and block substrate access. Alternatively, compounds that affect the dimerization should also inhibit the enzymatic activity. The freeware molecular docking program Autodock Vina was used to screen a virtual drug library for NAMPT dimerization inhibitors. The 1,521 ligand library was provided by the National Cancer Institute's Developmental Therapeutics Program. Docking surfaces targeted NAMPT's dimerization plane. Ligands bound into published crystal structures were removed and redocked into the active site. The calculated RMSD value for FK866 when redocked was within acceptable limits, whereby validating the method. Library compounds were scored and prioritized based on their docked binding energy, resulting in a listing of the top binding compounds. The binding energy of FK866 was used as the arbitrary cutoff to designate potential inhibitors of NAMPT. These compounds are being screened for their affect on NAMPT activity.

DESIGN AND SYNTHESIS OF CYCLIC DIPEPTIDES AS POTENTIAL QUORUM-SENSING INHIBITORS

Lindsey Coates, Taylor Braun, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; David Rowley, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Infectious diseases are traditionally treated with compounds that either kill or inhibit bacterial growth. Antibiotic resistance is a growing concern among the development of new drugs. Quorum sensing is the process by which bacteria communicate with one another through chemical signals. This allows the bacteria to coordinate their behavior and function as a multicellular organism rather than individual cells. Autoinducers are small molecules that are released by bacteria. These then bind to and stabilize receptor proteins causing the ligand-protein complex to initiate transcription of quorum sensing genes. Quorum sensing plays a role in regulating virulence in bacteria, thus providing an opportunity to control infectious bacteria without interfering with growth. The long-term goal of this project is the design and synthesis of small molecules that have the capability to inhibit quorum sensing. This includes the compound phevalin, which is a known regulator of virulence factor expression. By optimizing a synthetic procedure it will be possible to produce a library of phevalin derivatives to further investigate the potential of these compounds as quorum sensing inhibitors.

POLYSACCHARIDES FROM NATURAL SOURCES INTERFERE WITH BIOFILM FORMATION IN PSEUDOMONAS AERUGINOSA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Biofilms are structures that protect bacteria and other microbes from antibiotics and other environmental stress. Composed of molecular secretions from microbes themselves, biofilms can contribute to chronic and nosocomial infections and to industrial biofouling. Many organisms, from plants to bacteria, produce secondary metabolites that act on and regulate microbial activity, which provides protection from the microbes or a competitive advantage. These compounds may include polysaccharides, which have been shown to alter biofilm formation without direct biocidal effects or toxicity. To purify and characterize the molecules active in biofilm inhibition, carbohydrate extract from Panax ginseng was first precipitated with ethanol, then fractionated into six neutral and acidic components using anion exchange by fast protein liquid chromatography. Gel purification was used to separate an acidic fraction of interest by molecular size. The three fractions produced were analyzed with high performance liquid chromatography for estimated size with dextran standards. When sufficient amounts of these molecules are isolated, the fractions will be further elucidated for structure with NMR, and their inhibitory action demonstrated in biological assays.

SOLID STATE POLYPEPTIDE SYNTHESIS W/ ARYLPHOSPHONIUM SALT POLYPEPTIDE CONJUGATES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Polypeptides and polypeptides conjugated with an arylphosphonium salt were synthesized through the process of microwave assisted solid state synthesis. The synthesis begins with a "Wang" resin bead containing the first amino acid of the chain being synthesized, followed by a series of deprotecting, coupling, and washing cycles, and finished with a cleaving step in which the beads are separated from the peptide chain. Arylphosphonium salts conjugated on the end of these peptide chains have shown in previous research literature to be possible anti-cancer agents. Aryl phosphonium salts (APS) exhibit both lipophilic and cationic character, making them ideal phase transition catalysts. As such, they have been found to accumulate preferentially in tumor cells due to the high membrane potential of tumor mitochondria. The APS is carried into cells through transmembrane receptors known as integrins, which is assisted by the polypeptide it i s attached to; in this case the sequence RGD. Other non APS conjugated polypeptide chains were also synthesized to investigate other means of delivering anti-cancer agents into the cell. Polypeptide sequences following the motif known as "CendR", meaning the carboxylic acid end of the polypeptide contains either a lysine or arginine, were synthesized to explore penetrating cancer cells through the neuropilin- 1 receptor, which is a protein receptor in cells that can induce vascular and tissue permeation. The polypeptides were analyzed using mass spectroscopy.

SYNTHESIS AND BIOLOGICAL EVALUATION OF EUDISTOMIN U AND DERIVATIVES

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A branch of heterocyclic, aromatic amines known as carbolines have attracted interest within the field of organic chemistry due to their diverse biological activity. Carbolines are best described as indole alkaloids that consist of a three-ring fused system. Our research investigates substitution at the 1-position of the ring. We will describe a 5-step synthesis for the naturally occurring β–carboline Eudistomin U, which includes a key Suzuki cross coupling reaction. Finally, we will also report Eudistomin U's biological activity against various bacteria and human pathogens, as well as its interaction with DNA.

THE THERMODYNAMICS OF CINNAMYLTRIPHENYL PHOSPHONIUM CHLORIDE BINDING TO DNA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Arylphosphonium salts (APS) that bind to DNA have been reported. In a recent study the APS compound, cinnamyltriphenyl phosphonium chloride (CTP-Cl), appeared to inhibit amplification of qPCR products in mM concentrations. Although this result suggests CTP-Cl binds to DNA directly, currently no crystallographic data exists for DNA:APS complexes in public databases. To date, efforts by this lab to crystallize DNA:CTP-Cl have been unsuccessful. Without atomic coordinates of the complex available, the exact manner in which CTP-Cl binds with DNA is unknown and the characterization of binding dynamics remains challenging. This study attempts to parse CTP-Cl binding events through calorimetric and spectroscopic methods. The possibility of cooperative binding, binding sites spanning several base pairs, and non-classical binding modes will be considered. A tentative binding model will be described in anticipation of further studies.

INTERACTIONS BETWEEN ARYLPHOSPHONIUM SALTS (APS) AND DNA

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Arylphosphonium Salts (APS) are organic compounds that are cationic and lipophilic. APS facilitate transport across lipid bilayers to accumulate in cellular compartments of carcinoma cells selectively. Previous studies demonstrated an interaction between APS and DNA. Cinnamyl triphenylphosphonium chloride (CTP-Cl), showed the greatest interaction as determined by DNA gel shift assays and qPCR. This study evaluated the intermolecular forces driving the DNA interaction by modifying the electronics of the triphenyl rings on the phosphonium atom. Addition of methoxy groups in the ortho or para positions increased the ability of APS to bind DNA. Binding could be partially reversed by pre-incubating DNA with metal cations, although complete reversal was never achieved. Intercalation of the cinnamyl arm into the DNA helix likely affects the strength of binding.

FLUORESCENCE BASED PROTEIN-PROTEIN INTERACTION ASSAY FOR NAMPT

Endurance Okoeka, Karen Almeida, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the NAD+ salvage pathway and has been implicated in numerous human disorders including diabetes, heart disease and cancer, making NAMPT an attractive drug target. NAMPT forms a homodimer whose active site is located along the dimerization plane. We have developed a yellow fluorescence protein (YFP) based protein interaction assay to assess the dimerization capabilities of NAMPT. Plasmids were generated to include the coding sequences for the protein fusion of YFP1-155 (YN)-NAMPT and YFP155-238 (YC)-NAMPT. Protein was expressed in BL21 Codon Plus cells and purified on Ni-NTA resin. Validation of the dimerization assay will be presented.

SYNTHESIS OF CRYPTOPHANE-1.1.1 AND ITS APPLICATIONS TO MAGNETIC RESONANCE IMAGING

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RI-INBRE Summer Undergraduate Research Fellowship Program

Cryptophanes are hollow, cage-shaped molecules of interest with unique molecular encapsulation properties. These properties enable them to trap molecules or atoms, such as xenon, within their structure. Xenon-129 Magnetic Resonance Imaging (Xe-MRI) is a powerful tool capable of non-invasive scanning of biological tissue. Xenon is an inert, non-toxic, noble gas that displays no MRI background signal in biological tissue. When inhaled, xenon rapidly enters the bloodstream, distributing the gas to all the body's organs, including the brain. A new technique using hyperpolarized xenon-129 with MRI (HP-Xe MRI) provides an increase in magnetic resonance, allowing for imaging with 100,000 times higher resolution than traditional MRI. Further signal amplification required to image at picomolar concentrations can be achieved through hyperpolarized chemical saturation transfer (HyperCEST), which capitalizes on the diffusion of xenon in and out of t he cryptophane cages.

Cryptophane-1.1.1 displays the highest xenon binding constant in organic solution known to date. Originally, it was believed that xenon's high affinity for cryptophane-1.1.1 made it optimal for sensing applications such as HP-Xe MRI. However, we hypothesize that xenon's high affinity for cryptophane-1.1.1 may no longer be optimal for HyperCEST Hp Xe-MRI. We predict that cryptophane-2.2.2, a slightly larger cage molecule, would be better suited for HyperCEST enhancement because this cage allows for more rapid xenon diffusion. Optimal cage structure still remains undetermined until a systematic study of novel cryptophane cages is completed.

The goal of this work is synthesis of functionalized cryptophane-1.1.1 molecules, which could target biological areas of interest such as tumors and areas of inflammation. Water-soluble functionalized cryptophanes could provide the capability of non-invasive imaging of biological processes at the molecular and cellular level. Such technology could be applied Hp-Xe MRI both in-vivo and in-vitro, providing opportunity for major biological and medical advancements.

MICROFLUIDIC BASED IMMUNOSENSOR FOR ELECTROCHEMICAL DETECTION OF PROTEIN CANCER BIOMARKERS P53 AND VEGF IN SERUM

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RI-INBRE Summer Undergraduate Research Fellowship Program

Cancer is the 2nd leading cause of death in the US. Early detection is the best means to minimize the damage cancer takes on a patient. Tumor suppressor p53 and vascular endothelial growth factor (VEGF) are found in high levels in patients with head and neck squamous cell carcinoma (HNSSC) and can be measured as a means to detect cancer. Accurate, quick and cost effective protein cancer biomarker detection is a promising means of early detection and disease monitoring, for better patient outcomes. Herein we report on an ultrasensitive microfluidic immunosensor based on novel multi-labeled magnetic beads, (HRP/MB/Ab_2)-PEG with specially designed polyethylene glycol polymer brushes to minimize non-specific binding (NSB) and particle aggregation. GSH-AuNPs were bioconjugated to the primary antibodies (Ab1). They were then attached to the HNSSC biomarkers, p53 and VEGF, which were then bound to the multi-labeled magnetic beads. The (HRP/MB/Ab2)-P EG based immuonsensors show great promise for the fabrication of ultrasensitive biosensor microarrays for point-of-care cancer diagnosis. This microfluidic immunoarray based on a panel of 2 biomarkers is promising for point of care diagnostic, as it provides a rapid, low cost system for the detection of multiple cancer biomarkers.

CHARACTERIZATION AND OPTIMIZATION OF A MULTIPLEX NANOSTRUCTURED MICROFLUIDIC IMMUNOSENSOR ARRAY BASED ON PEG-COATED MULTI-LABELED CONJUGATE FOR ULTRASENSITIVE ELECTROCHEMICAL DETECTION OF PROTEIN CANCER BIOMARKERS IN SERUM

Brian Somba, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Multiplexed biosensor arrays for clinical measurements of biomarker proteins are critically important for early detection and monitoring of cancer, which will lead to accurate, inexpensive devices for reliable POC cancer diagnosis, improved therapeutic outcomes, new personalized therapies and decreased patient stress. Herein, we report an ultrasensitive, 8-unit, electrochemical microfluidic array, designed for the simultaneous detection of a panel of two protein biomarkers for oral cancer in serum. The prototype 8- sensor immuno-array based on carbon paste was designed to measure Interleukin-8 (IL-8) and interleukin-6 (IL-6) in a single serum sample. All of these proteins are elevated in serum of patients with oral cancer with relatively similar levels of serum concentration. To minimize non-specific binding (NSB), the nanostructured array was decorated with gold nanoparticles coated with carboxyl polyethylene glycol (AuNPs-PEG-COOH) upon which the capture antibody is attached. The sandwich type Immunosensor was coupled to PEG-coated HRP multi-labeled magnetic beads conjugate to further minimize NSB and particle aggregation. This provided the necessary higher sensitivity required for IL-8 and IL-6 detection at physiological levels. The immuno-array magnetic bioconjugate was optimized with 10kDa PEGs in PBS buffer which gave minimum particle aggregation using DLS experiments. The number of Ab2 and HRP molecules per magnetic bead was characterized to be ~115,000 and ~55,000 respectively which promise to give limits of detection within the attomolar region and a wide dynamic linear range in a ~1hr assay for the two protein biomarkers. These results show great promise for real time, rapid, extremely sensitive and accurate multiplexed cancer biomarker detection for point-of-care diagnostics.

SMALL POLYPEPTIDE CONJUGATES AND ESCORTS FOR INTRODUCING CATIONIC LIPOPHILES INTO CELLS

Adrian Soto, John Williams, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Arylphosphonium salts (APS) are cationic cytotoxic lipophiles that can cross cell membranes. APS are selectively taken up by malignant cells and mitochondria in healthy cells and have been used as transport molecules to deliver DNA-alkylating molecules into mitochondria. This makes APS and their conjugates potential anti-cancer agents. Some possible targets are cell cycle arrest, tubulin polymerization, mitochondrial hexokinase disassociation, and calcium channel inhibition. Conjugates to the APS can make small or major changes in structure that may alter the mechanism and potency of their cytotoxic action. Capping a polypeptide synthesized on a polystyrene resin with APS can make a conjugate molecule designed to target specific cell types and regions of cells. Polypeptides, usually pentamers or less, can be efficiently made by microwave assisted organic synthesis to any specified amino acid sequence. Wang-resin based solid-state synthesis has been used to synthesize a variety of amino acid trimers that were then conjugated to arylphosphonium salts. By combining these amino acid sequences and APS the accumulation of APS in non-malignant cells may be prevented. All steps in SSPS were done using a microwave in about 1/40 of the overall reaction time with high yields.

CYCLIC PEPTIDE-CAPPED SELENIUM NANOPARTICLES AS A NANO DRUG DELIVERY SYSTEM

Brian Sullivan, Amir Shirazi, Keykavous Parang, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Selenium nanoparticles (SeNPs) have become subjects of major interest for biomedical applications. Recently, the surface-decorated SeNPs have been employed for the intracellular delivery of drugs. Peptides containing diverse amino acids can be used for the surface functionalization or capping of SeNPs. Herein, we report the synthesis and evaluation of cyclic peptide [W5R4C] containing arginine, tryptophan, and cysteine residues, for generating and capping SeNPs. The selection of amino acids was based on the requirement of the presence of an optimal balance between positively charged and hydrophobic residues in the structure of the carrier to enhance the interactions with the corresponding negatively charged and hydrophobic groups in cell membrane and to improve the cellular permeability through the lipid bilayer. The size of c[W5R4C]-SeNPs were found in the range of 300-350 nm as shown by Transmission Electron Microscopy (TEM). Flow cytometry results showed that c[W5R4C]-SeNP s improved the intracellular uptake of a fluorescently-labeled dasatinib (F′-Das), a model anticancer drug, by 2-fold compared to F′-Das alone in human leukemia adenocarcinoma (CCRF-CEM) cells after 2 h incubation. The antiproliferative activity of a number of anticancer drugs was examined in the presence of c[W5R4C]-SeNPs. The results revealed that the anticancer activity of all anticancer agents (5 μM) including gemcitabine, clofarabine, etopside, camptothecin, irinotecin, epirubicin, fludarabine, dastatinib, and paclitaxel was enhanced in the presence of c[W5R4C]-SeNPs (50 μM) after 48 h incubation in human ovarian adenocarcinoma (SK-OV-3) cells by 49%, 36%, 36%, 31%, 30%, 30%, 28%, 24%, and 17%, respectively, presumably by improving the cellular uptake of the drugs. This data demonstrates the potential application of c[W5R4C]-SeNPs for enhancing the antiproliferative activity of the anticancer drugs.

SYNTHESIS OF ANNULATED β-CARBOLINES VIA METAL MEDIATED [2+2+2] CYCLIZATIONS

Jonathan Varelas, Seann Mulcahy, *Department of Chemistry and Biochemistry*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Carbolines are a specific class of heterocyclic amines that contain two nitrogen atoms within a fused, three-ring system. These molecules have been shown to have exquisite biological properties, but their preparation poses a significant challenge to the field of synthetic chemistry. We will describe various cyclization strategies to synthesize a library of chemically unique β-carbolines, as well as our initial findings concerning a Palladium catalyzed [2+2+2] cyclization.

ENVIRONMENTAL SCIENCES

LOCATED IN THE MAIN HALLWAY AT THE END CLOSEST TO PHARMACY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 10:30 -11:30 AM

CARCINOGENIC AMINE AND HEAVY METAL CONTENT OF RIVER SEDIMENT FROM THE BLACKSTONE RIVER

Eimear Black, Julia Crowley-Parmentier, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Blackstone River has a long history of industrial use. In 1990 the United States Environmental Protection Agency characterized the Blackstone river as the most polluted river in the country with respect to toxic sediments. Due to the historic presence of textile plants, tanneries and mills along the river there has been discharge of textile dyes and heavy metals. Most of this toxic material has become trapped behind dams as it accumulates along the river. Studies have shown industrial pollution may be a driving force in emerging antibiotic resistance. Therefore our research goal is to characterize the sediment behind dams along the Blackstone river for heavy metals and decomposition products of textile dyes prior to screening for antimicrobial resistance in the river microbial community.

Using high pressure liquid chromatography (HPLC) we have developed a method to analyze the presence of decomposition products of several azo-dyes used in the textile industry. These azo-dyes decompose under reducing conditions to yield carcinogenic and mutagenic amines such as aniline, phenylenediamine and benzidine. Additionally inductively coupled plasma mass spectrometry (ICP-MS) was used to quantify heavy metals such as Pb, Cr, and Hg present in the river sediment in order to establish a baseline for future studies on the river.

SHOULDER GIRDLE MOVEMENT DURING ALLIGATOR STRIDES (ALLIGATOR MISSISSIPPIENSIS)

Brigid Garrity, Christopher Pellichero, David Baier, *Department of Biology*, Providence College, Providence, RI; Sabine Mortiz, Ryan Carney, *Department of Ecology & Evolutionary Biology*, Brown University, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Alligators have played a significant role in evolutionary studies of archosaurs. Given that several major shifts in forelimb function (including 2 of the 3 origins of vertebrate flight) occur within this group, the relatively basal position of crocodilians is of particular importance in assessing evolutionary studies of archosaur locomotion. Skeletal movement is critical for linking form/function relationships of living animals to the varied morphology seen in fossils, but only a few studies have explored skeletal kinematics relevant to archosaurs. These studies were limited to either single X-ray views or standard light cameras and revealed conflicting data from lizards on the role of the coracosternal joint during terrestrial locomotion. A fixed coracosternal joint would provide stability, but a mobile coracosternal joint would increase stride length by increasing the fore/aft excursion of the shoulder joint. Previous investigations of alli gator forelimb bones suggested a substantial amount of coracosternal movement; however, motion of the scapulocoracoid could only be quantified relative to the vertebral column because the cartilaginous sternum was not visible on the X-ray video. Hence, movement of the scapulocoracoid could result from 1) coracosternal joint movement 2) rotation of the sternum, or 3) some combination of both. In this study, we employ marker-based x-ray reconstruction of moving morphology (XROMM) to measure movement of the scapulocoracoid, vertebral column, and sternum of alligators walking on a treadmill or trackway. Surgically implanted radio-opaque markers permit clear measurements of each of the elements in the kinematic chain. Simultaneous dual X-ray videos, combined with CT scans were used to reconstruct the movements of the shoulder girdle. Initial analysis suggest that both sternal and coracosternal rotations contribute to scapulocoracoid movement. This is particularly interesting beca use rotations of the sternum, which indicate rib movement, have yet to be demonstrated in any vertebrate.

DISTRIBUTION OF MAJOR AND TRACE METALS IN PORE WATERS AND SEDIMENTS FROM THE INTERTIDAL ZONE AT INDUSTRIAL SITES ON THE PROVIDENCE RIVER, RI

Eric Hopf, Julia Crowley-Parmentier, Dan McNally, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The purpose of this study is to analyze the relationship between industrial sites and the distribution of trace metals in the shoreline sediments and pore water along the Providence River, at the northern reaches of the Narragansett Bay estuary. Elevated levels of certain trace metals can have harmful effects on the marine ecosystem. We collected our samples from three public access points on the North and West sides of the Providence River: India Point Park (IPP), Public Way (PW) and Oxford Street (OX). Using Sanborn maps, we identified past industrial activities including a former railroad yard (IPP), petroleum storage sites (OX) and coal and petroleum storage site (PW). PW is also adjacent to a metal recycling facility.

To extract pore water, we centrifuged wet sediment samples. Sediment samples were digested in HCL and HNO3 acids. Sediment and pore water samples were analyzed for major and trace metals using an Agilent 7700 ICP-MS. Organic content in the sediment samples, determined using loss on ignition, ranged from 0.2-26%, with most samples having less than 1.5%. We identified the area around Oxford Street to be the most contaminated of our sampling sites. Copper, lead, and nickel were elevated in pore water at all locations in relation to the EPA guidelines for chronic and acute levels of contaminants. Sediment samples have elevated levels in both copper and lead in relation to ERM levels set by NOAA. The elevated levels of contaminants in the pore water suggest that they may be impacting the water quality of the Providence River.

DETERMINATION OF THE NAPHTHALENE BIODEGRADATION POTENTIAL OF A DIESEL CONTAMINATED SITE ON PRUDENCE ISLAND, NARRAGANSETT BAY, RI

Allison Hubbard, Sarah Pierce, Mary Penniman, Dan McNally, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The T-Dock on Prudence Island is considered to be the source of contamination even after the removal of the fuel lines from the Naval Refueling Depot. There is a layer of diesel several inches thick that is migrating into Narragansett Bay at the intertidal zone. Previous studies have shown when bacteria are exposed to diesel contamination they develop a unique ability to use compounds, such as aromatics, as a carbon and energy source. In many cases, the aromatic compounds were mineralized into CO2 and H2O. The goal of this study was to determine the presence of PAH degrading bacteria in the diesel contaminated sediment. Conditions at the site, which were in constant change, included a high water table, brackish water, tidal action, and hypoxic sediment. Specifically, the site conditions were as follows: □T 27.8□C, 7.72, conductivity 13.86 ms/cm, and NO3- 53.2 mg/L; TP2; pH 8.11, conductivity 5.46 ms/cm, D.O. 1.6 mg/L, and NO3- 127.2 mg/L. Nitrate levels this high have been shown to be toxic to typical marine organisms. The preliminary results of the contaminated layer for growth conditions: Total Petroleum Hydrocarbon (TPH) of 5400 ppm, (comparatively) Prudence Island diesel has weathered ~84%, and only high-molecular weight compounds remain, which can be highly toxic. The media (LB, Nutrient Broth, Pore Water, PTYG, BSM, and Marine Broth) were compared in growing and culturing bacteria to cultivate a naphthalene degrader. The bacteria were identified and enumerated using spread plate technique and streak plating techniques in order to obtain a pure sample. Each species of bacteria were put through naphthalene screening tests: naphthalene ether, naphthalene vapor, and verification/enrichment cultures. Additional work is needed on whether there is a specific naphthalene degrader, or if a consortium of bacteria is required to degrade naphthalene to determine the bioremediation potential of the site.

USING ANAEROBIC GROWTH CHAMBERS AS SUITABLE ENVIRONMENTS TO SUPPLEMENT IRON REDUCING MICROORGANISMS SUCH AS GEOBACTER METALLIREDUCENS, AND OTHER MICROBIAL GROWTH

Peter Killian, Oluwatobi Raji, , *College of Environmental and Life Sciences*, University of Rhode Island, Kingston, RI; T. Julie Scott, *Department of Geosciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Serpentinization is a water-rock alteration process that transforms Fe- and Mg-rich rocks of Earth's deep crust and mantle into rocks dominated by serpentine minerals. This process creates hydrogen, methane, building blocks of organic compounds, and diverse metals abiotcally. Microorganisms found in these environments are able to survive extreme conditions: chemical compositions lacking dissolved inorganic carbon, very basic pH, and elevated temperatures. There is a direct connection between these microbes and the first organisms that inhabited the Earth, as they share a common environmental niche.

In order to enrich for iron-reducing microorganisms from a serpentinizing system, we obtained groundwater from a well array in northern California through the Coast Range Ophiolite Microbiological Observatory. We sought to enrich under anaerobic conditions for Geobacter metallireducens in petri dish experiments. Parallel experiments were arranged to compare microbial growth by testing: temperature (room temperature vs. incubated at 40^0 Celsius), pH (varied from 6 to 10.4 to span acidic, basic, and near neutral pH values), light (some samples in constant light, others in constant darkness), and oxygen availability (sealing petri dishes with parafilm). An Fe-rich groundwater sample from RI was collected and used for comparison.

Petri dishes filled with agar were streaked with water samples after a nutrient mixture was made to enhance growth; buffer solutions were added to each plate to ensure desired pH values. Powdered magnetite (Fe3O4), was added to samples to promote the growth of Geobacter metallireducens. The most favorable conditions were seen at pH 7 and pH 10.4. Geobacter metallireducens appeared to be found at pH 7 in the dark from site QV 1,1. A different organism was instead cultivated, as this microbe was found to be gram negative while G. metallireducens should be Gram positive. In the future, we will re-structure experiments isolate G. metallireducens.

IDENTIFYING GUT MICROBIOTA IN RIBBED MUSSELS WITH NEXT-GENERATION SEQUENCING

Sara Moore, Brea Govenar, *Department of Biology*, Rhode Island College, Providence, RI;
Serena Moseman-Valtierra, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Salt marshes have been considered as “sinks” for greenhouse gas emissions, but under conditions of climate change, may instead act as “sources,” of carbon dioxide, methane, and nitrous oxide. As part of a larger project to understand the shifts in trophic dynamics and greenhouse gases fluxes as a result of elevated temperatures and nutrient levels in estuaries, we are investigating the composition of bacteria and archaea in the gut content of the dominant benthic invertebrate, the ribbed mussel Geukensia demissa, in coastal marshes, along the historic nitrogen-loading gradient in Narragansett Bay. In 2012, we collected mussels from two salt marshes where greenhouse gas fluxes were measured. Then, in the laboratory, we dissected the digestive tract to extract DNA from different regions of the gut to test the hypotheses that 1) the diet of the mussels would vary along the nitrogen-loading gradient, and 2) the foregut woul d include a greater proportion of ingested bacteria capable of denitrification and the hindgut would include a greater proportion of resident methane releasing microbes. From DNA extractions, we used PCR to amplify the V4 hypervariable region of the 16S ribosomal RNA gene for next generation sequencing for taxonomic classification and comparison of microbial diversity and we will use qPCR to quantify the abundance of denitrifiers and methanogens. This work will help us to identify the role of the gut microbiota in ribbed mussels in contributing to the shifts in greenhouse gas fluxes resulting from impacts of climate change in Narragansett Bay.

INVESTIGATING BIOREMEDIATION OF POTENTIAL DIESEL CONTAMINATED SEDIMENT ON PRUDENCE ISLAND, RI BY IDENTIFYING, ENUMERATING NAPHTHALENE DEGRADING OF BACTERIA

Sarah Pierce, *Biotechnology*, Community College of Rhode Island, Warwick, RI; Allison Hubbard, Mary Penniman, *Department of Environmental Science*, Bryant University, Smithfield, RI; Dan McNally, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

During World War II the T-Dock on Prudence Island located in Narragansett Bay, RI was used as a Navy refueling Depot. There were underground piping and storage tanks for oil and fuel. After the area was given back to Rhode Island the piping and tanks were removed. Significant contamination of the sediment near the area of the piping was evident. An analysis of the sediment using GC-MS gave a Total Petroleum Hydrocarbon (TPH) of 5400ppm, which is highly contaminated. The contamination has weathered about 84% compared to a fresh diesel spill. The compounds that remain have high molecular weights and can be very toxic. Bioremediation is a possible solution for decontamination of the sediment on Prudence Island. Therefore the bacterial population was characterized and enumerated to determine the total numbers, total viable numbers and number of biotypes. The different biotypes were tested for their ability to utilize Naphthalene as a sole carbon and energy source or as a co-substrate. Samples were taken from different depths, 6” and 12” for both test pits, and 8” from the second test pit, at the intertidal zone. Samples from a stream and offshore were taken as well. The total bacterial count ranged from 18,000,000 to 47,500,000. The highest bacterial count came from Test Pit 2 at a depth of 8”. The viable cell count ranged from 3,000 to 64,000. The highest viable cell count came from Test Pit 2 at a depth of 6” on LB broth media. The number of Biotypes ranged from 2 to 6 which varied among the different samples and media. The results of the Naphthalene degradation tests were inconclusive, however the methods of enumerating and classifying the bacterial population can help further the process for finding a PAH degrading bacteria for bioremediation of diesel contaminated sediment of Prudence Island.

HEAT SHOCK PROTEINS IN GEUKENSIA DEMISSA COLLECTED FROM SITES IN AND AROUND NARRAGANSETT BAY AS INDICATORS OF CLIMATE CHANGE

Victoria Thermuda, John Williams, *Department of Physical Sciences*, Rhode Island College, Providence, RI; Breea Governor, *Department of Biology*, Rhode Island College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

When extreme fluctuations in temperature occur cells of Geukensia demissa express heat-shock proteins to aide in the folding and unfolding of thermally denatured proteins. Heat shock proteins (Hsp) are also known as molecular chaperones and help protect the denaturation of proteins and refolding of damaged proteins. In some cases heat shock proteins chaperone irreversibly damaged proteins out of the cell. We have established a protocol for extracting and identifying Hsp-70 from mussels kept in thermostated water to compare high and low temperature expression of these proteins.

Collection sites were; Passeonquis, Warwick, RI, Watchemocket, East Providence, RI, and Fox Hill, Jamestown, RI. Samples were stored at 8C, then 18C or subjected to to a 40C bath for one hour before sacrifice.

In this experiment I focused my attention on proper protocol in order to achieve the ultimate goal of analyzing Hsp 70 expression in Geukensia demissa and their response to climate change. Heat shock proteins are expressed the most in the cells of the gill tissue in these organisms. Dissection of the gills and homogenization using a tris-HCl pH 8.0 homogenization buffer was used to lyse the cells. The samples were centrifuged for 30 minutes and the supernatant was removed from the solid pellet, stored at 8C overnight and vertex spun again then stored at -80C. Protein gel electrophoresis was run at 200V for 45 minutes followed by a ponceau stain at 100V for 1 hour (for transfer to nitrocellulose membrane) for total protein expression. Four to six of the collected mussels (G.demissa) were exposed to 40oC water bath for 1 hour to amplify expression, then were put back into 18C water for 24 hours. Samples were dissected and homogenized and will be incubated with Mouse anti- Hsp70 antibody with Goat anti-mouse(IgG) for proper tagging and expression.

PFOS AND FATTY LIVER DISEASE

Kimberly Ezeama, Danna Salter, Angela Slitt, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

PFOS, or perfluorooctanesulfonic acid, is a synthetic fluorosurfactant and an environmental pollutant that is abundant in society and has been shown to cause hepatotoxic effects. This study looks at the mechanism of PFOS on the lipogenesis pathway in C57BL/6 male mice and its possible induction of fatty liver disease. The mice were dosed 0.1mg/kg of PFOS or vehicle daily and either fed ad libitum (freely) or undergoing caloric restriction (a diet) for five weeks. In methods such as Western Blotting, there is a slight increase in P-AMPK and SCD-1 in mice that were treated with PFOS, especially the mice that underwent caloric restriction. It has been shown that PFOS induces Nrf2 activation and hepatic lipogenesis through the downregulation of lipogenic genes. PFOS may inhibit the expression of transcription factors in the liver that promote triglyceride accumulation and decrease lipid export from liver, and may somehow work in other lipogenic pathways.

DELTAMETHRIN MODULATES RAT BRAIN ION CHANNEL CURRENTS MICRO-TRANSPLANTED IN XENOPUS LAEVIS OOCYTES

Craig Irving, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The use of Xenopus laevis oocytes as a model system for the analysis of specific ligand- and voltage-sensitive ion channels is a widely used method to elucidate the mechanisms of action of various neurotoxicants. Micro-transplantation of foreign tissue into X. laevis oocytes provides for a means of overcoming the limitations of traditional cRNA expression of ligand- and voltage-sensitive ion channels. This is because it allows for a viable strategy of incorporating multiple native state receptors in the presence of their regulatory subunits. The purpose of our study was to determine the affects of deltamethrin (a pyrethroid insecticide) on the functional attributes of ion channel currents from rat brain neurolemma micro-transplanted into X. laevis oocytes. Deltamethrin is a neuroexcitant that targets multiple receptors in the central nervous system, including voltage-sensitive sodium and calcium channels. Rat brain neurolemma micro-transplanted oocytes were exposed to increasing concentrations of deltamethrin. Preliminary data indicate that rat brain micro-transplanted channels exhibit a biphasic current response to increasing concentrations of deltamethrin. In the presence of niflumic acid, a specific calcium gated chloride channel inhibitor, a more traditional concentration response curve was observed. This data supports the hypothesis that the action of deltamethrin on micro-transplanted rat brain tissue is complex and involves a variety of ion channels.

GENETICS

LOCATED NEAR THE CENTRAL STAIRWAY ON THE 1ST FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 9:30 -10:30 AM

EXPLORING THE INVASION OF RED ALGAL PARASITES: A STUDY OF THE POPULATION GENETICS BETWEEN VERTEBRATA LANOSA, CHOREOCOLAX POLYSIPHONIAE, AND ASCOPHYLLUM NODOSUM

Taylor Clement, Eric Salomaki, Christopher Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Parasitism among red algal species is a common and complex evolutionary strategy within the phylum. Red algal parasites are generally classified as either adelphoparasites (which are close in genetic relation to their host, having diverged recently) or alloparasites (which can often infect numerous and more distantly related hosts). Red algae are prone to parasitism due to their characteristic pit plugs, by which parasites deposit their cellular components into the host. There is little known about what makes some hosts more susceptible than others, and why the parasites occur where they do, on both a local and oceanic geographic scale. The focus of this study is the host, Vertebrata lanosa and its obligate parasite, Choreocolax polysiphoniae. Choreocolax forms characteristic cysts within the host's cells. This system is made more intricate by the fact that V. lanosa is an obligate epiphyte of Ascophyllum nodosum, a brown alga. While pre vious studies have focused on the genetic variability of A. nodosum, within the mtDNA-IGS and mtTrnW loci, no research has been published on relationships between V. lanosa and Choreocolax in regards to their geographic diversity. Samples continue to be collected from different areas around the North Atlantic, with a current focus on Rhode Island, looking at data within the cox 1 gene to gain a genetic barcode, and cox 2-3 spacer in order to look for haplotype distinction. This study of the population genetics of these three species can hopefully begin to offer insight as to the dynamics within this system of algae, and ultimately see whether there is any pattern to the parasitism.

DETECTING DIFFERENCES IN GENE EXPRESSION OF LEISHMANIA BY AFLP

Stephanie Marvel, Steven Symington, Alison Shakarian, *Department of Biology and Biomedical Sciences,* Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The protozoan parasite Leishmania can cause a cutaneous and visceral form of the disease leishmaniasis, which affects about 12 million people in Africa, India and South America. This project aims to identify unique genetic markers and different genes expressed in Leishmania that may give insight into the pathogenesis of leishmaniasis in humans. To accomplish this, we used amplified fragment length polymorphism (AFLP), a technique based on selective PCR amplification of restriction fragments from the digestion of gDNA or cDNA. We screened digested gDNA and cDNA samples with 16 unique primer combinations and observed differences in the fragment banding patterns of four different species of Leishmania examined. Thus these results indicate that we have identified unique genetic markers and genes expressed in different species of Leishmania. Future studies are underway to sequence and identify the genes that were expressed.

DNA FINGERPRINTING OF PORTULACARIA AFRA

Katherine Gladsky, *Department of Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Portulacaria afra grows across the veld habitat of South Africa's Eastern Cape. P. afra is drought tolerant and switches from the C3 pathway to the CAM pathway when water stressed. It has an increased ability to store carbon and is being considered for use in carbon sequestration. Throughout the varied habitats of the Eastern Cape, P. afra plants have differing phenotypes. Perhaps this is an indication of differing ecotypes of P. afra. This study used DNA fingerprinting to search for genetic differences in these possible ecotypes. Leaf samples were collected from various sites across the Eastern Cape. DNA was isolated from the samples and the presence of DNA was confirmed in all samples using Rubisco primers. With the use of RAPD primers genetic differences were seen between leaf samples. More research needs to be done, but there is evidence indicating that there may be some genetic differences between the ecotypes of P. afra. Microsatel lite research will be continued with the help of Dr. Larry Wimmmers of Towson State University for further analysis of the P. afra DNA.

SITE-DIRECTED MUTAGENESIS OF A UNIQUE B-SHEET REGION OF THE FANCONI ANEMIA D2 PROTEIN

Stephanie Komjian, Elizabeth Vuono, Niall Howlett, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi anemia (FA) is a very rare genetic disease. It is characterized by bone marrow failure, congenital defects, and cancer susceptibility (a large portion of patients developing acute myeloid leukemia). Biallelic mutations in any of sixteen known genes will cause FA. The sixteen FA proteins make up the FA-BRCA pathway and function to repair damaged DNA. FA patient-derived cells are characteristically hypersensitive to one class of DNA damaging agents that generate DNA interstrand cross-links (ICLs). The monoubiquitination of the FANCD2 and FANCI proteins is an essential step in the activation of the FA-BRCA repair pathway. The FANCD2 protein is a large 1451 amino acid protein with few known or functionally characterized domains. The crystal structure of FANCD2 has recently been solved and has revealed an exposed region comprised of two anti-parallel β-sheets, unique as the protein is extensively α-helical. This β-sheet region could represent an important protein-protein or protein-DNA interaction surface. The goal of this research project was to use a site-directed mutagenesis approach to disrupt the β-sheet region and to uncover its importance for FANCD2 function in ICL repair.

INVESTIGATING THE CONSERVATION AMONG GENES INVOLVED IN THE CELL CYCLE AND CONSTITUTIVELY EXPRESSED PATHWAYS IN PLANTS AND ANIMALS

Stephanie Liptak, Kelsey Stafstrom, Alexandria Bierce, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gallic acid, a secondary metabolite found in the head of developing Rubus prickles has the potential to contribute to the field of medicine as a natural treatment for certain cancers. When investigating the effects gallic acid has on the cell cycle it is critical to look into the genetics underlying this pathway. To this end, we are testing the effects of gallic acid exposure on patterns of cell cycle genes in both plant and animal cells. To investigate gene expression we employ a qPCR which requires constitutive genes to act as controls. As we look at the cell cycle in both plants and animals simultaneously, it is imperative that we ensure that they are comparable. Therefore, we hypothesize that control genes and cell cycle genes are from similarly conserved pathways consequently we should see cross amplification of genes. To test the hypothesis, specific primers were designed using a combination of the BLAST algorithm and Primer3. These primers were tested with plant or animal template DNA specific to the primer from which they were designed after which they were then tested for cross amplification in both plant and animal DNA. Ten primers were shown to work with their specific species and eight primers were shown to cross amplify between species. Cross amplification shows that the genes are biologically conserved between the two species, and also ensure comparative data.

THE CIMRF N-TERMINUS: A FUNCTIONALLY IMPORTANT EVOLUTIONARY NOVELTY?

C.J. Pickett, Thomas Meedel, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Myogenic regulatory factors (MRFs) are basic helix-loop-helix (b-hlh) transcription factors that play crucial roles in metazoan muscle development. Typically these proteins display a high degree of functional conservation; for example avian and fly MRFs are able to rescue a loss of function mutation of the nematode MRF. It was, therefore, surprising that non-ascidian MRFs were unable to elicit muscle gene activity when we expressed them in the developing notochord of our model organism, Ciona intestinalis, even though ascidian MRFs could direct such activity. Sequence comparisons revealed that ascidian MRFs contained an extensive N-terminal domain (≈ 300 amino acids in length), which is absent from the MRFs of other organisms. We propose that this large N-terminal domain may be an evolutionary novelty essential for MRF function in the context of the ascidian embryo. Here we present our initial analysis of the N-terminus of the Cion a intestinalis MRF (CiMRF). Consistent with an essential role for the N-terminus in myogenesis, we found that chimeric constructs in which the b-hlh domain of non-ascidian MRFs replaced the b-hlh domain of CiMRF were myogenic. We further show that a chimeric protein consisting of just the CiMRF N-terminus and the b-hlh domain and C-terminus of the Drosophila melanogaster MRF, Nautilus, is myogenic, whereas the full-length Nautilus protein is not. The possibility that the N-terminus participates in interactions with other factors, presumably proteins, which are essential for myogenesis in Ciona is currently being investigated. Finally, we examined the CiMRF N-terminus for known protein motifs and although we found none we identified a region within the b-hlh domain that was predicted to act as a nuclear localization sequence. Here we report the experimental validation of that prediction.

EXAMINING GENETIC PROFILES OF HUMAN STOMACH CANCER AND RUBUS CALLUS AFTER GALLIC ACID EXPOSURE

Kelsey Stafstrom, Stephanie Liptak, Alexandria Bierce, Alyssa Guarracino, Rhiannon Morrissey, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Songhua Zhang, Steven Moss, *Liver Research Center*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gallic acid is a phenolic found in certain Rubus varieties that has the potential to be a highly effective nutraceutical based upon its putative ability to initiate developmentally regulated signal transduction pathways. Previous studies focused on HeLa cells and Rubus callus response show that low concentrations (10-25 uM) of gallic acid exposure initiate rapid cell proliferation, but high concentrations (>25 uM) cause apoptosis at the G2/M phase of the cell cycle. Therefore that are exposed to certain concentrations of gallic acid will have elevated levels of G2/M checkpoint regulating genes. It is necessary to characterize gene expression profiles for mammalian and plant cells in order to provide understanding of the relevance of each system in regards to its respective response to gallic acid. Quantitative PCR (qPCR) is being employed to examine the real time expression of optimized genes in both AGS cancer and Rubus tissue extracts a fter exposure to gallic acid. The levels of expression can be compared between the two species to validate the extent of the effect of gallic acid. Preliminary results show samples from both species which were exposed to 25 uM gallic acid have an elevated expression of selected cell cycle genes when compared to expression profiles of control (0 uM) sets. Control genes were validated by both the 0 uM and 25 uM exposed cell sets by displaying consistent degrees of expression. Up regulation of the cell cycle genes for the G2/M phase show that the cell cycle is being affected by gallic acid. From those results we can ascertain the overall effect gallic acid could have as a naturopathic treatment for stomach cancer.

MARINE SCIENCES

LOCATED IN THE MAIN HALLWAY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 9:30 -10:30 AM

THE EFFECTS OF SUBSTRATE TYPE ON THE DIVERSITY AND ABUNDANCE OF MARINE INVERTEBRATES ALONG NEWPORT NECK

Kirston Barrett, Sarah Matarese, Jameson Chace, *Department of Biology*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Sea levels are rising due to increasing global temperatures. While it is clear that sea level rise will affect intertidal zonation, it is unclear how this will affect the marine invertebrates living along the coastline of Newport Neck, RI. The current supertidal zone in this area is primarily composed of large boulders, bed rock and man-made seawall, while the current intertidal zone is primarily composed of sand, cobble, and small boulder. As the sea levels rise, this supertidal zone will eventually become the intertidal zone. The summer of 2013 marks the third year in a five-year study examining the effects of climate change on the marine invertebrates along Newport Neck. This study specifically examines how the rocky substrate affects the diversity and abundance of the marine intertidal community. Several types of data were collected at nine sites: quantification of substrate type, abundance of intertidal organisms from modified lobst er traps, quadrats and systematic searches. It was hypothesized that species diversity and abundance would decrease in sandy sites because of decreased sheltering locations. The results show a weak negative effect of sand cover on intertidal diversity, periwinkle (Littorina littorea) abundance, barnacle (Cirripedia sp.) abundance, Asian Shore Crab (Hemigrapsus sanguineus) abundance, and mussel (Mytilus edulis) abundance. Sand comprises 14.3% of the intertidal zone today and 12.2% of the current supertidal zone. In comparison, large boulder comprises only 7.4% of the current intertidal zone while making up 19.2% of the current supertidal zone. With a sea level rise of one to two meters by 2100, we expect the composition of sandy substrate to decrease and large substrate to increase leading to more habitat available for these key foundational members of the intertidal community.

COMPARING DECOMPOSITION RATES IN RHODE ISLAND SALT MARSHES ALONG A NITROGEN GRADIENT

Emily Bishop, Sierra Moseman-Valtierra, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Elizabeth Watson, *Atlantic Ecology Division*, Environmental Protection Agency, Narragansett, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Spartina alterniflora is a cordgrass commonly found in the lower elevations of New England salt marshes, close to the water. This species has the ability to create a buffer zone in which nutrient runoff from anthropogenic sources can be utilized by the plant instead of running directly into the ocean. High inputs of nitrogen can significantly affect the productivity, community composition, and biogeochemistry of salt marshes. To assess the effects of nitrogen on the decomposition rate of cordgrass plant material, we deployed litter bags containing dried S. alterniflora aboveground biomass at three marshes along an anthropogenic nitrogen gradient in Narragansett Bay, RI. By collecting litter bags at multiple times during the summer, we found a significantly faster pace of decomposition at the sites with more N loading ($F_{3,4} = 30.5$, $p = 0.0032$). We also found that the conditioning phase, which is characterized by enzymatic activity and takes place approximately between 3-15 days after senescence, had a faster rate of decomposition than the leaching phase (0-3 days). We hypothesize that this could be due to enzymatic differences at these sites, or increasing ambient temperatures over the course of this summer study. These data are contributing to a more comprehensive assessment of the functioning of salt marsh ecosystems which are subject to anthropogenic pressures of nitrogen enrichment. Increased decomposition rates of plants with a higher anthropogenic nitrogen load may change the elevation of marshes, or result in more decomposing plant biomass than biomass contributing to net primary productivity, factors which would influence the reaction of marshes to continuing sea level rise and climate change.

THE COMPARATIVE ANALYSIS OF VOLUNTEER AND PROFESSIONAL REEF MONITORING SYSTEMS IN THE BRITISH VIRGIN ISLAND

Dennis Conetta, Graham Forrester, *Department of Marine Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Volunteer data collection has the potential to be a valuable resource to many fields of science however, even though these volunteer collections are highly encouraged at times their viability is always at question. In this study the volunteer reef monitoring program, Reef Check was compared to professionally acquired data to see if volunteer programs are a reliable option for monitoring coral reefs over time. The volunteer program and the professional data both analyzed the benthic composition and reef fish populations of various reefs in the British Virgin Islands. The two data sets counted the percentage of hard coral cover (HC), soft coral cover (SC), nutrient indicator algae cover (NIA), rubble (RB), sand (SD), and the number of sponges (SP) for benthic reef compilation. The two also counted butterfly fish, snappers, grunts, parrotfish, grouper, Nassau grouper, and the urchin species Diadema for fish and invertebrate population assessments . Each variable was run through a Kendall-Mann test with 95% confidence interval which tested if there was an increase, decrease, or no change in the tested variable over time. The variables that showed significant trends and were most consistent were hard coral, soft coral, gorgonians, rubble, and sand. Hard coral cover in all sites except for Spyglass reef decreased, while soft coral, gorgonians, sand, and rubble showed increased cover for the majority of the sites. The fish count data was too sporadic and inconsistent to say any trends were present. Overall, the professional data produced trends that had higher incidence of significance than that of the volunteer data. However, the trends for hard coral, soft coral, gorgonians, rubble and sand did correlate between the two data sets proving that although they are not as precise; the volunteer data did match the overall trends from the professionally acquired data.

A SURVEY AND ANALYSIS OF LITERATURE CONCERNING THE FLEXIBILITY PROPULSORS

Khushbu Desai, Jack Costello, *Department of Biology*, Providence College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Animals utilize a variety of methods to move through fluids. Propulsion is examined because various animals propel themselves in different ways. Patterns of biological propulsion are important for emulation by bio-inspired vehicles. These vehicles would be able to efficiently propel themselves through fluids. In this survey, analysis focused on literature concerning the effect of flexibility on propulsion. Literature on flexible propulsors was collected through various online sources. A growing database, so far consisting of 255 related references, has been created containing information including: standard bibliographic information, the discipline of investigation, the approach used to collect data, the mode of propulsion considered, the type of movement considered, the location of the flexible component of the propulsor, whether span-wise or chord-wise flexibility was investigated, the quantities evaluated, the conclusion on the effect of flexibility, and whether the propulsors were homogenous or anisotropic. The database was used to determine patterns of growth in this field of study. This database will be useful as a reference within the growing field of bio-inspired propulsion.

IDENTIFYING ABUNDANCE AND RICHNESS OF ULVA ALGAL SPECIES IN NARRAGANSETT BAY, RI

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Increases in temperature and nutrient loading over the past several decades have led to many predictions that harmful macroalgal blooms will increase in duration and frequency worldwide. Macroalgal blooms are an annual summer occurrence in Narragansett Bay, RI, and are known to cause ecological and economical disturbances to the community. In Narragansett Bay, these blooms are primarily dominated by two morphologically similar Ulva species, U. rigida and U. compressa. Until recent, all Ulva species in the Bay were identified as U. lactuca, but due to current molecular sequencing, our understanding of bloom taxonomy has improved. However, there is little known about the dynamics of these two bloom-forming species in terms of their population cycles, distribution, and abundance. To quantify abundance of Ulva species present in the bay, we conducted repeated monthly surveys at two Narragansett Bay sites, Chepiwanoxet and Brushneck Cove in Wa rwick, RI, for the 2012 and 2013 summer seasons. Each Ulva individual was identified to species level by microscopic cellular examination. By observing percent cover, total biomass and seasonal trends of each species we were able to determine a continued lack of U. lactuca, and an overwhelming abundance of co-competing U. compressa and U. rigida. The abundance of both Ulva species varied significantly among sites and months for both U. compressa and U. rigida, and it was uncommon that both would have large biomass at the same time at the same site. Research from this study will help identify ecological trends occurring in macroalgal bloom formation and dynamics, as well as support associated flow cytometry, microsatellite, and genomic research on Ulva.

THE EFFECTS OF THE NARRAGANSETT BAY NITROGEN GRADIENT VS. THE EFFECTS OF COPPER ON GEUKENSIA DEMISSA N2O PRODUCTION

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Geukensia demissa, or the ribbed mussel, is one of the most abundant invertebrates in the salt marshes of Narragansett Bay. As filter feeders, these mussels may accumulate chemical toxins in the water column and thus may be good model organisms for evaluating salt marsh water quality. Bacteria in the guts of these mussels also have the potential to release green house gases (GHGs) like N2O through processes like denitrification (the reduction of nitrate to N2). Copper (Cu) is a heavy metal found in contaminated coastal water that is required in small amounts by the enzyme nitrous oxide reductase (N2OR), which catalyzes the last step of the complete denitrification pathway. This experiment tests the effects of copper on N2O production by Geukensia demissa. In this experiment four CuSO4 treatments were applied (0 μg/L, 0.4μg /L, 1.4 μg/L, 14 μg/L) to mesocosms containing live G. demissa collected from 3 salt marshes in Rhode Island: FoxHill, Passeonkquis and Watchemoket. These sites fall upon a nitrogen gradient with FoxHill being fairly pristine and Watchemoket being the most polluted. Three mussels were placed in each of 12 mesocosms (3 mesocosms per CuSO4 treatment). A series of 35mL water samples were taken every 2 hours for 4 hours. These water samples were then analyzed on a gas chromatograph (GC) to evaluate the N2O concentrations. The data from this experiment suggest that N2O production varied based upon the different sites rather than the different copper treatments ($f=8.12$, $p<0.01$: TF-T1, $f=6.89$, $p<0.5$: TF-T0). This suggests that the nitrogen gradient within Narragansett Bay is more important for salt marsh invertebrate GHG emissions rather than Cu exposure. Water sample analysis using inductively-coupled plasma (ICP) showed that each site contained the same amount of Cu (~7.7μg/L). This could explain the lack of response observed in each Cu treatment (no statistical significance).

UNDERSTANDING ULVA SPP. BLOOM FORMATION IN NARRAGANSETT BAY, RHODE ISLAND USING FLOW CYTOMETRY

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The genus Ulva consists of green macroalgae that show characteristics of rapid bloom formation. These bloom formations of Ulva are becoming an increasing problem in Narragansett Bay, RI. Ulva compressa and Ulva rigida are the two most abundant species in Narragansett Bay that contribute and form these harmful algal blooms. Ulva has a biphasic, isomorphic life cycle in which individuals alternate between distinct yet morphologically indistinguishable haploid and diploid phases. We used flow cytometry to distinguish the ploidy level of Ulva individuals. Ulva was collected in June 2013 from three sites of different levels of bloom intensity in Narragansett Bay, RI and processed for flow cytometry. Out of 50 samples run, 34 samples showed florescence. Of these, we found 8 diploid individuals and 26 haploid individuals. We assessed haploid:diploid ratio for all individuals combined as well as U. compressa and U. rigida separately using chi-squared analyses. Although we found no significance difference between the ratio of haploid:diploids from null model predictions, there is a trend toward more haploid individuals in both U.rigida and U. compressa. Future research will involve surveying Ulva populations in July and October 2013 and setting up mesocosm experiments to compare growth rates of haploid and diploid individuals.

ALGAE AS PALEOTHERMOMETERS IN NARRAGANSETT BAY: CALIBRATION OF AN ALKENONE-BASED TEMPERATURE PROXY

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Over the past century, Narragansett Bay has been subjected to excess eutrophication, hypoxia, and thermal pollution due to anthropogenic activities. In order to better predict the future of the Bay under projected warming conditions, it is necessary to look deeper into the past. An open-ocean proxy for paleotemperature has recently been successfully applied to the estuarine environment of Narragansett Bay. This temperature proxy is based on the production of lipid biomarkers, called alkenones, by members of the haptophyte algae. However, the species of algae commonly associated with the production of these compounds in the open ocean have not been observed in the 50+ year time series of phytoplankton abundance in the Bay. Further, preliminary work shows that temperatures calculated from this alkenone proxy often track measured temperature in the Bay only during certain periods of the year. To test how different species of algae produce alkenones in relation to ambient temperatures, we grew haptophyte algae using Narragansett Bay water amended with nutrients under 12:12 light/dark cycle at 12, 14, and 18 degrees Celsius. We determined the growth rates for the cultured haptophyte algae in order to better understand their ecology, and calculated experimentally derived calibrations for the determination of temperature based on alkenone production. We compared these experimentally derived calibrations to the well-established open ocean calibration in order to explain the variance observed between instrumental T and calculated T at different times of the year. Separately, we measured alkenone concentrations in the water column at the University of Rhode Island Graduate School of Oceanography, and compared the proxy-calculated temperatures to the instrumentally measured temperature value.

INVESTIGATION OF THE RELATIONSHIP BETWEEN RIBBED MUSSELS AND CORDGRASS ALONG THE NITROGEN-LOADING GRADIENT OF NARRAGANSETT BAY

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Anthropogenic activities have increased the level of nutrients in Narragansett Bay and have consequently created a nitrogen-loading gradient from south to north. Geukensia demissa is one of the dominant organisms in salt marshes fringing Narragansett Bay, and can serve as a bioindicator of the condition of salt marshes. The primary obejective of this experiment is to track the changes in biomass, shell length, and overall physiological condition of ribbed mussels (Geukensia demissa) along this nitrogen-loading gradient in order to examine the scale of spatial and temporal variability in the mussel populations and determine the role of ribbed mussels in the impacted salt marsh ecosystem in Narragansett Bay. Another obejctive of this experiment is to investigate the relationship between ribbed mussels and the cordgrass, Spartina alterniflora. For this experiment, ribbed mussels and cordgrass were collected from 6-25 x 25 cm sample plots randomly selected at 3 different site along the nitrogen-loading gradient. Mussel samples were dried at 60 degrees Celsius and combusted at 500 degrees Celsius to obtain ash free dry weights and cordgrass samples were dried at 75 degrees Celsius for 3 days. Mussel density, biomass, shell length, and condition index were compared to the density, biomass, and height of the cordgrass. The density of mussel populations was shown to increase with an increase of nitrogen load. The more densely populated sites were also shown to have a much higher proportion of small mussels, showing that recruitment was higher at sites with higher levels of nitrogen-loading.

DEVELOPMENT OF MICROSATELLITE DNA MARKERS FOR POPULATION GENETIC STUDIES OF MARINE DIATOM, THALASSIOSIRA GRAVIDA

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Marine diatom populations are known to harbor high genetic diversity, suggesting that high adaptive potentials may play a critical role in their long history of success in a marine environment. Tracking their genetic diversity and structures can help us gain insights of how they survive, thrive, and evolve in response to changing environments. Microsatellites are among the most powerful genetic markers commonly used for such population genetic studies. However, a microsatellite marker is often species-specific due to its high variability nature. Development of specific microsatellite markers for a species is thus an important first step to its population genetic studies. In this study, we attempted to develop microsatellite DNA markers for a representative marine diatom species, Thalassiosira gravida. Thousands of microsatellites were first identified from the 454 genomic sequencing data of a T. gravida lab strain. Twenty of them were carefully selected for validity tests as genetic markers. For each marker, optimal conditions for polymerase chain reaction (PCR) amplification were first tested on the source T. gravida strain, including various reagent concentrations and thermal cycling programs. Variability of a genetic marker was then tested using field samples of T. gravida from the North Atlantic ocean. PCR amplified microsatellite alleles were visualized and verified in polyacrylamide gels and through fragment analysis. Of the 20 tested loci, 9 have yielded promising results with reliable PCR amplifications and variations across individuals. A preliminary population genetic analysis based on 3 microsatellite loci revealed a considerably high level of genetic diversity in North Atlantic T. gravida populations, indicative of strong adaptive potentials of this cosmopolitan species to changing environments.

ZOOPLANKTON OF RHODE ISLAND WATERS: THE SEARCH FOR SIPHONOPHORES

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

There are many evolutionary pathways to multicellularity. Humans are vertebrates with cells that develop to become unique organs in a singular body. Siphonophores are cnidarians with a different type of multicellularity. Siphonophores have cells that separate into individual bodies with specific functions. These bodies, called zooids, bud from a single stem and work together as a colonial organism. Siphonophores have been found all over the world, from tropical islands to sub-polar seas. Our goal for the summer has been to discover whether siphonophores exist in the waters around Rhode Island and to delineate the physical and biological factors that contribute to distribution of these organisms.

Along with the search for siphonophores our goal has been to create a fuller picture of other zooplankton populations that can be found in and around Rhode Island. Taking samples in Narragansett Bay and Block Island Sound we found an abundance of zooplankton including ctenophores, copepods and medusas. Sampling continues and we are working to track and understand local plankton populations.

Our studies suggested that Narragansett Bay, with harsher conditions influenced by anthropogenic runoff, would not provide a tolerable environment for siphonophores. Looking at Gulf Stream patterns, temperature and salinity levels, phytoplankton, and zooplankton blooms, we predicted that if siphonophores were to be found in Rhode Island waters they would most likely be seen miles offshore, where the western moving Gulf Stream could funnel these animals toward the coast.

Led by our studies we collected samples off the Southwest coast of Block Island, and we successfully found siphonophores. The search continues bolstered by evidence from this initial find. According to our research, warming ocean temperatures and a rise in salinity during August and September will create an ideal environment for these delicate animals, and we hope to find a greater abundance as the summer continues.

TROPHIC CASCADES IN THE INTERTIDAL ZONE: ECOLOGICAL EFFECT OF THE INVASIVE SPECIES, HEMIGRAPSUS SANGUINEUS ALONG NEWPORT NECK

Conor Marino, Sarah Matarese, Jameson Chace, *Department of Biology*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Asian shore crabs (Hemigrapsus sanguineus) and European green crabs (Carcinus maenas) are both invasive species. Asian shore crabs have come to populate New England, specifically New Jersey in 1988 and have moved northward since. They have out-competed the previously well-established green crab causing their decline. Littorina littorea, a snail commonly referred to as a periwinkle, are also found in vast numbers along Newport neck. Green crabs often prey upon periwinkles, but with the influx of Asian shore crabs, green crabs have declined and periwinkles have increased a possible Trophic Cascade. If Asian shore crabs continue to dominate the Newport neck region, then periwinkle populations will directly correlate with a rise in their abundance. Two methods were used to test this hypothesis. Systematic timed searches for crabs and quadrats sampling, both at – nine sites summers 2011-2013. In each of the ten quadrats at all sites, substrate was quantified. The combination of the two methods has revealed a large number of both Asian shore crabs and Periwinkles. There was a weak positive correlation between Asian shore crab abundance and periwinkle abundance ($R2 = 0.1685$, $P = 0.033$) and negative correlation between green crab abundance and periwinkle abundance ($R2 = 0.00016$, $P = 0.9494$). Sea ducks winter along the coast of Rhode Island, and their abundance and distribution was determined by shore line censusing Nov – Apr 2010-2012. Common Loon (Gavia immer), Common Eiders (Somateria mollissima) and Buffleheads (Bucephala albeola) increase in abundance in locations with a greater density of Asian shore crabs ($R2 = 0.12$, 0.12 and 0.23 respectively). Increases in crabs appear to have increased periwinkle abundance and prey items for wintering for several sea birds, and we predict increases in grazing periwinkles affects sessile algae. Changes in substrate with sea level change will affect distribution and abundance of crabs.

OCEAN ACIDIFICATION EFFECTS MORPHOLOGY AND MINERALOGY IN OTOLITHS OF LARVAL REEF FISH

Jacqueline Mitchell, Drew Canfield, *Department of Biology and Marine Biology*, Roger Williams University, Bristol, RI; Eric Wilcox-Freeburg, *School for the Environment*, University of Massachusetts Boston, Boston, MA; Bradford Borque, *CEED*, Roger Williams University, Bristol, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The pH of the ocean is declining in response to increased atmospheric carbon dioxide concentrations from anthropogenic production. This process of Ocean Acidification (OA) has become subject of great importance. Enhanced carbon dioxide absorption into the ocean results in changed carbonate system chemistry in the ocean. Otoliths, the inner ear bone of fishes, are calciumcarbonate structures that are essential for navigation and orientation in the water column. Not much is known about otolith development with respect to these changing conditions. If OA changes the development of otoliths, impaired navigational ability may be a consequence.

CHARISMATIC MICROFAUNA: GIGAPIXEL IMAGING OF MARINE PLANKTON FOR PUBLIC ENGAGEMENT

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The field of science communication seeks to transmit knowledge generated through specialized research. Predominant methodologies, however, often fall short in engaging, informing, and empowering audiences outside the science community because they rely on passive didactics. Alternatively, a reciprocal approach to science communication combines scientific knowledge and diverse perspectives to cultivate public interest and maximize active learning. This approach allows an interchange of ideas between researchers and the public, which helps democratize – and even generate – a communal scientific awareness, leading to greater potential for positive social and environmental impact.

Phytoplankton and zooplankton are essential to planetary photosynthesis, carbon cycling, and oceanic ecosystems. Unfortunately, the general public lacks basic literacy in microscopic marine science; most planktonic species are invisible to the unaided eye. Whereas charismatic megafauna (e.g., polar bears, lions, pandas) are seen as emblematic of their respective ecosystems and easily rally public interest, microscopic marine life can be challenging to represent in a manner that elicits comparable support. Nonetheless, it is not for lack of inherent charisma that plankton are underappreciated. Instead, it is an issue of making their charisma accessible to the general public via context, imaging strategy, and manipulation of scale.

The diversity of planktonic life can sustain public interest and education if it is thoughtfully presented in accessible and inspiring ways. The authors developed compositing techniques for imaging microscopic plankton at high resolution for large format prints; produced dramatically scalable image files approaching gigapixel thresholds for interactive display on multitouch screens; and, created an interdisciplinary exhibit informed by art and design perspectives. Our data indicate that multitouch interactivity, custom imaging, and careful orchestration of presentation space can improve science communication and catalyze public engagement with a specialized body of knowledge.

IS THERE A SEX-BIAS AMONG NEAR-SHORE HOMARUS AMERICANUS POPULATION ALONG THE NEWPORT NECK?

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Recently there has been a decline in the lobster abundance of near-shore American lobster Homarus americanus populations and according to NOAA the Southern New England stock of lobster is below a sustainable population size. Male biased sex ratios reduce population growth potential and a known sex ratio is an important parameter for fisheries management. The goal of this study was to determine the habitat-specific population density and sex ratio of mature and immature lobster along Newport Neck, RI. There are known sex biased density-dependent mortality factors that might affect the sex ratio such as shell disease. Lobster traps modified to capture undersized lobsters were set at 54 locations along Newport Neck between May and October 2011-2013, primarily at near-shore (< 25 m) and up to 50 m depths. Overall we found that there is a strong male biased sex ratio of 3.45:1. The strong male sex bias is consistent among the shallowest trap s and among the deepest traps. Both males and females were caught significantly more often in July but only in the deepest of our traps, suggesting they are migrating from the deeper areas towards the shoreline in mid-summer. There is a strong month-location interaction in capture rates because the near-shore, shallow captures do not decrease while deeper trap captures increase in July. The influx of lobsters towards shallower water tends to be female biased as sex ratios become more balanced, but still male-biased, as summer progresses towards fall. The strong male-bias sex ratio among breeding and future breeding lobster in the near-shore environment of Newport Neck will affect potential population growth. Not only does female-biased mortality pose a threat to local lobster population, it also affects the potential harvest of lobster in Newport, and potential population response as the climate changes.

PREDICTING THE EFFECTS OF SEA LEVEL RISE ON THE DIVERSITY OF MARINE INVERTEBRATES IN THE SUBTIDAL ZONE ALONG NEWPORT NECK

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Climate change causes a warming effect producing oceanic thermal expansion, glacial melting and sea level rise. Sea level is expected to rise 5 meters in Rhode Island. Our goal was to determine habitat selection of near shore marine subtidal organisms in order to predict future abundance and distribution of these organisms with changing substrate following sea level rise. In 2011-2013, as part of a five year study, we used modified lobster traps in shallow (<25m) subtidal regions at each site (n = 41) to determine the measures of species abundance and diversity. We then dove to the sea floor to quantify the substrate by specific sizing criteria used in previous years. Total relative abundance decreased with subtidal bedrock and total species diversity declined with percentage of small boulder and bedrock in subtidal substrate. Complete observations of the subtidal sea floor continue to be obtained for full analysis. The preliminary results suggest that a rising sea level will increase the smaller substrate composition (sand, cobble, small boulder) which may decrease relative abundance and diversity of subtidal marine invertebrates.

MODELING THE MERCURY EXPOSURE OF RHODE ISLAND RECREATIONAL ANGLERS AND THEIR FAMILIES

Patrick Williamson, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In this study we used an exposure assessment model to estimate mercury (Hg) intake by RI anglers and their families owing to the local fish consumption. We sought to: (1) measure muscle Hg concentrations of a variety of recreationally-targeted fishes using atomic absorption spectrometry; (2) disseminate food frequency questionnaires (FFQ) to RI anglers and their families to ascertain each person's fish eating habits, and; (3) model Hg exposure rates in this sensitive subpopulation by coupling their dietary habits with fish Hg data. Muscle Hg content was positively related to fish length across all species, indicating Hg bioaccumulation. Moreover, for striped bass, bluefish, and tautog, Hg concentrations were near the US EPA action level (0.3 ppm) at their legal catch size, while remaining fishes had contaminant levels mostly below this threshold. The FFQ was completed by 284 individuals, of which 78.2% were male and the mean age was 52. 4 (range = 15-81). Respondents' mean fish consumption rate was 7.8 meals per 30 days (range = 0-30), which is significantly higher than estimates for national and coastal populations, and equivalent to the high-end fish eating habits of NY/NJ harbor anglers. The mean estimated Hg exposure rate for RI anglers and their families was significantly higher than rates reported for other US coastal regions. Moreover, 38% of the respondents were estimated to have Hg exposure rates above the US EPA reference dose through their fish consumption. These results reveal that many RI anglers and families are highly exposed to Hg due to elevated rates of fish consumption. Hence, continuing research on fish consumption rates and Hg exposure in this sensitive subpopulation will support public health risk assessments and risk management decisions related to the issuance of fish consumption advisories.

MERCURY CONCENTRATION IN SCUP (STENOTOMUS CHRYSOPS)

Sean Maiorano, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

Rhode Island Saltwater Anglers Association

Mercury (Hg) is a toxic element that commonly bioaccumulates in the tissues of marine fish. Scup (Stenotomus chrysops) is a recreationally important coastal fish that is found throughout the Northwest Atlantic, yet there is no reported information on Hg contamination in this species. In this study, scup were collected from numerous sites throughout both Narragansett Bay (NB) and Rhode Island/Block Island Sound (RIBIS). The Hg content (ppm wet wt) of scup muscle tissue was analyzed using automated combustion absorption spectrometry, and results were examined relative to fish total length and geographic location (NB vs. RIBIS). A significant positive relationship was observed between muscle Hg concentration and total length, indicating the bioaccumulation of Hg. Furthermore, scup collected from NB had significantly higher Hg concentrations than conspecifics from RIBIS. With respect to other recreational fish species, scup had significantly lower Hg levels then striped bass (Morone saxatilis), bluefish (Pomatomus saltatrix), and tautog (Tautoga onitis), and equivalent levels to black sea bass (Centropristis striata) and summer flounder (Paralichthys dentatus). Furthermore, only 8.4% of legal size scup (n = 9 out of 106 fish) had Hg concentrations exceeding the US EPA action level of 0.3 ppm wet weight, indicating that this species is relatively safe for human consumption. Future research aims to gather additional samples from both locations for dietary analysis in order to understand how trophic level plays a role in mercury bioaccumulation.

ABUNDANCE, GROWTH AND DIET OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) AND SUMMER FLOUNDER (PARALICHTHYS DENTATUS) IN RHODE ISLAND TIDAL RIVER

Christopher Mills, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

Rhode Island Science and Technology Advisory Council

Summer flounder, Paralichthys dentatus, and winter flounder, Pseudopleuronectes americanus, utilize estuaries as a nursery habitat during early life history stages. From 2009-2013, we sought to identify the spatiotemporal overlap and potential biotic interactions between flounder species in two Rhode Island tidal rivers (Seekonk River, RI; Taunton River, MA). This study specifically focused on the abundance, growth, and dietary habits of the juvenile flounder life stage. From May to August, sampling occurred fortnightly in the tidal rivers, and collected flounder were enumerated, measured for total length (mm), and a sub-sample was preserved for subsequent stomach content analysis. Irrespective of location and year, winter flounder were more abundant than summer flounder.. Further, the cumulative abundance of flatfish was higher in the Seekonk River (1.8 fish $\cdot$ m^{-2}) than the Taunton River (0.13 fish $\cdot$ m^{-2}) across all years. The growth rate of summer flounder (0.92 mm $\cdot$ d^{-1}) was also significantly faster than winter flounder (0.37 mm $\cdot$ d^{-1}), which may be explained by dietary differences across species.. Specifically, amphipods and crangonid shrimp were the principle prey of summer flounder, while amphipods, nematodes, and copepods comprised the most abundant food item in winter flounder stomachs. Fish remains, including winter flounder, were observed in summer flounder stomachs, albeit to a limited extent. The results indicate considerable spatiotemporal overlap between summer and winter flounder, but predator-prey and competitive interactions are minimal.

SPATIO-TEMPORAL DISTRIBUTION OF THE BLUE CRAB (CALLINECTES SAPIDUS) IN THE NARRAGANSETT BAY, COASTAL PONDS AND TIDAL RIVERS (RI/MA, USA)

Michael Pallotta, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

Rhode Island Science and Technology Advisory Council

The blue crab, Callinectes sapidus, is a temperate species that occupies Mid-Atlantic estuaries during several life history stages. Recent empirical data further indicate that the abundance of blue crabs has significantly increased in southern New England estuaries, suggesting a northward shift in their distribution. Spatial and temporal dynamics in blue crab abundance and size structure in Rhode Island (RI) estuarine and coastal waters were synthesized from current and historical data provided by the RI Department of Environmental Management and Roger Williams University seine surveys. Blue crab abundance was maximal in July in the upper reaches of Narragansett Bay, including several cove locations, e.g., Greenwich Bay. In contrast, peak crab abundances occurred in late May/early June and September in tidal rivers and coastal ponds, respectively. Blue crab abundance also demonstrated pronounced annual variations, with extremely high catc hes observed in 2010. The size structure of blue crabs in tidal rivers differed considerably across years, with smaller age classes observed in 2013. Further, in both tidal rivers and coastal ponds, distinct cohorts were detected from monthly length-frequency distributions. Future research will examine the foraging ecology of blue crabs via stomach content and stable isotope analyses to better understand the crabs' potential impact on the benthic community.

MICROBIOLOGY

LOCATED IN THE MAIN HALLWAY NEAR THE CENTRAL STAIRCASE ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 10:30 -11:30 AM

ACTIVITY OF AMPICILLIN OR VANCOMYCIN ALONE AND IN COMBINATION WITH RIFAMPIN AGAINST BIOFILM FORMING STAPHYLOCOCCUS AUREUS IN A TIME KILL ON ENDOTRACHEAL TUBES MODEL

Alana Stempien, *Department of Chemical Engineering*, Rochester Institute of Technology, Rochester, NY; Megan Luther, Kelsey Confreda, Kayla Babcock, Kerry LaPlante, *Department of Pharmacy Practice*, University of Rhode Island, Kingston, RI and Veterans Affairs, Medical Center, Providence, RI

Bacterial growth on indwelling medical devices is a major source of nosocomial infections, and the formation of biofilm on these devices makes the infections more difficult to treat. Biofilm growth can occur on many different materials such as prosthetic joints, pacemakers, catheters, and endotracheal tubes, and can cause such illnesses as ventilator-associated pneumonia, bacteremia, osteomyelitis, and urinary tract infections.

Staphylococcus aureus (S. aureus), a biofilm forming bacteria, was grown on polyvinyl chloride endotracheal tubes (ETT) to simulate ventilator-associated pneumonia. Vancomycin and ampicillin in monotherapy, as well as in combination with rifampin, were tested against two strains of S. aureus- methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible Staphylococcus aureus (MSSA). A 24h time kill model was used to quantify the activity of vancomycin, ampicillin, and rifampin at 0.5x, 1x, 2x, and 4x the minimal inhibitory concentration (MIC). It is hypothesized that the addition of rifampin to ampicillin or vancomycin will improve the bactericidal activity in biofilm-forming S. aureus.

Ampicillin demonstrated bactericidal activity against both MRSA and MSSA strains at 24h, with average decrease 3.18±0.32 log10CFU/mL. Vancomycin demonstrated bactericidal activity against both S.aureus strains at 24 hours, with average decrease 3.88±0.37 log10CFU/mL. Rifampin alone was not active against either strain. In combination, ampicillin and rifampin demonstrated bactericidal activity against both S.aureus strains at 24 hours, with average decrease 4.87±0.83 log10CFU/mL. Vancomycin and rifampin in combination demonstrated bactericidal activity against both S.aureus strains at 24 hours, with average decrease 3.78±0.76 log10CFU/mL.

In a study of S. aureus on medical grade PVC ETT, bactericidal effects of ampicillin, vancomycin, or rifampin was demonstrated with greater than two times the MIC at 24 hours with monotherapy or greater than one times the MIC at 24 hours with combination therapy. This indicates that in a clinical setting, combination therapy may lead to increased activity as compared to monotherapy.

KIN DISCRIMINATION, CRYPTICITY AND PATHOGENESIS IN THE ENTAMOEBA LINEAGE

Kevin Schindelwig Franca, Joshua Leitao, Harsha Kumar, Layla Ferland, Hanna Sobon, Steve McDonough, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI; Guillermo Paz-y-Mino-C, *Department of Biology*, University of Massachusetts Dartmouth, Dartmouth, MA

RI-INBRE & RI NSF EPSCoR Summer Undergraduate Research Fellowship Programs

Entamoeba histolytica causes 100,000 deaths annually. More than 50 million people get infected with Entamoeba histolytica. Amoebic adherence and killing of target cells including phagocytosis are crucial. Signaling and aggregative behavior could impact pathogenicity and virulence. Chemical cues and chemokinesis have been reported for E. histolytica but not E. dispar, suggesting cell-communication between con-specifics and behavioral differences with relevance for disease. Using Entamoeba to explore effects of environmental stresses (climate change) in marine and fresh water protists. Little is known about the basic biology of marine and fresh water amoebozoans, their complex behaviors and interactions, or the effect of climate change on these unexplored groups. The Entamoeba lineage is an ideal model to analyze comparative cell signaling between/among ameba with morphological, multigene, and ecological studies in a laboratory setting. We demonstrate that by color tagging and pair-mix-culturing six Entamoeba varieties, the difficulty of discerning among apparently similar taxa can be resolved. When grown together with different amoeba strains, free-living/opportunistic (E. moshkovskii Laredo), commensal (E. moshkovskii snake) or parasitic (E. invadens IP-1, E. invadens VK-1:NS, E. terrapinae, E. histolytica) trophozoites aggregate only with members of their own lineage. Clusters of trophozoites from each amoeba show distinctive rate of aggregation, density of cells per cluster, and distance between clusters. By using these behavioral cues, and identifying the genes involved in cell-signaling for cluster formation, distinctive amoeba taxa can be characterized quantitatively and discern aggregative signals relevant for pathogenesis.

CHARACTERIZATION OF YEAST BAX INHIBITOR, BXI1, FUNCTION IN THE UNFOLDED PROTEIN RESPONSE AND CALCIUM SIGNALING IN SACCHAROMYCES CEREVISIAE

William Cavedon, B. Michael Berry, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bax inhibitor-1 (BXI1) is an anti apoptotic gene whose human homolog's expression is upregulated in a wide range of human cancers. Studies have shown that Bxi1p is localized in the endoplasmic reticulum and is involved in the unfolded protein response (UPR) that is triggered by endoplasmic reticulum (ER) stress. BXI1 is thought to act via a mechanism involving altered calcium dynamics. In this paper, we provide evidence that suggests that the Saccharomyces cerevisiae protein facilitates Ire1p clustering in response to ER stress and alters calcium signaling. First, we confirm that BXI1 is involved in the UPR with DTT induction experiments using Δbxi1 mutants and different UPR GFP reporters. Significantly, our data suggests that Bxi1p facilitates clustering of Ire1p during ER stress. Finally, our experiments suggest that Bxi1p is not involved in the calcium pathway but Ire1p is involved in connecting the UPR and calcium signaling. In total, our data suggests that the Bxi1p, like its eukaryotic homologs, is an ER-localized protein that is involved in the clustering of IRE1 during the unfolded protein response and IRE1 affects calcium signaling and connects the UPR and calcineurin complex.

ANALYSIS OF THE ROLES OF SHEWANELLA ONEIDENSIS HFQ IN OXIDATIVE STRESS RESPONSE

Meghan Keane, Taylor Hunt, Brett Pellock, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Shewanella oneidensis is a dissimilatory metal reducing bacterium that can utilize a wide variety of terminal electron acceptors when grown under anaerobic conditions. Our goal is to identify and characterize genes that encode small regulatory non-coding RNAs (sRNAs) in S. oneidensis. sRNA genes are regulated by changes in environmental conditions and can mediate both positive and negative regulatory outcomes by inexact base pairing to their mRNA targets. Hfq is an RNA chaperone protein broadly implicated in sRNA function in bacteria. Earlier work in our lab showed that loss of hfq in S. oneidensis results in a variety of mutant phenotypes, including an exquisite sensitivity to oxidative stress induced by either hydrogen peroxide or superoxide.

We are currently focused on understanding the mechanisms underlying the sensitivity of the hfq mutant to oxidative stress. Because the active sites of catalase, the enzyme that breaks down hydrogen peroxide, contain heme, we hypothesized that defects in heme biosynthesis underlie oxidative stress sensitivity in the hfq mutant. We have found that addition of heme boosts oxidative stress resistance in both wild type and hfq mutant cells. In addition, we have shown that pre-treatment with a low dose of hydrogen peroxide increases survivorship of both wild type and hfq mutant cells when compared to cultures that are naïve to hydrogen peroxide. Compared to wild type cells, pretreatment of the hfq mutant results in a much higher degree of protection relative to naïve cells, suggesting that the hfq mutant is capable of adapting to oxidative stress conditions, but is less efficient at handling large doses of hydrogen peroxide. This suggests that the hfq mutant may be defective in DNA repair. We are currently using next generation RNA sequencing technology to identify sRNAs and sRNA targets involved in the oxidative stress response in S. oneidensis.

ANALYSIS OF THE ROLES OF SHEWANELLA ONEIDENSIS HFQ IN GROWTH AND IRON HOMEOSTASIS

Nicholas Mazzucca, Jessica Leonard, Christopher Brennan, Brett Pellock, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Shewanella oneidensis is a dissimilatory metal reducing bacterium that can utilize a wide variety of terminal electron acceptors when grown under anaerobic conditions. Our goal is to identify and characterize genes that encode small regulatory non-coding RNAs (sRNAs) in S. oneidensis. sRNA genes are regulated by changes in environmental conditions and can mediate both positive and negative regulatory outcomes by inexact base pairing to their mRNA targets. Hfq is an RNA chaperone protein broadly implicated in sRNA function in bacteria. Earlier work in our lab showed that loss of hfq in S. oneidensis results in a variety of mutant phenotypes, including growth and survival defects and exquisite sensitivity to oxidative stress.

Our current work is focused on understanding the mechanisms underlying hfq mutant phenotypes and the role of Hfq and sRNAs in S. oneidensis iron homeostasis. We have found that the exponential phase growth defect of the hfq mutant is rescued by the addition of heme. Our analyses indicate that the hfq mutant growth defect is the result of lowered heme levels caused by misregulation of the first committed step of the heme biosynthesis pathway. We have also begun characterizing the S. oneidensis ryhB sRNA, which is regulated by iron availability. We have shown that ryhB levels are lower in the absence of Hfq protein, suggesting that Hfq promotes ryhB stability. Our results also suggest that ryhB regulation of the sodB gene, which encodes an iron-dependent superoxide dismutase depends on Hfq. We are currently using next generation RNA sequencing technology to identify additional ryhB targets in S. oneidensis for further analysis.

CASPOFUNGIN INDUCES AIF1-DEPENDANT PROGRAMMED CELL DEATH IN SACCHAROMYCES CEREVISIAE AND CANDIDA ALBICANS

Morgan McCarthy, Faith Donaghey, Christopher Chin, Katherine Helming, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Caspofungin was the first member of a new class of antifungals called echinocandins to be approved by a drug regulatory authority. In recent years, several laboratories have shown that a wide range of antifungal drugs leads to programmed cell death (PCD) in yeast that is reminiscent of apoptosis in mammalian cells. We now provide evidence that Saccharomyces cerevisiae cells cultured in media containing caspofungin manifest the classical hallmarks of PCD in yeast, including the generation of reactive oxygen species (ROS), the generation of caspases, change in the mitochondrial membrane potenetial and fragmentation of mitochondria. In addition to causing PCD in yeast, caspofungin also causes PCD in Candida albicans, a fungus that causes many infections in humans. Our data suggests that PCD triggered by caspofungin requires AIF1 but not YCA1 in both Saccharomyces cereisiae and Candida albicans.

CHARACTERIZATION OF AN ENDO-ACTING N-ACETYLGLUCOSAMINIDASE FROM CLOSTRIDIUM DIFFICILE

Ryan Miller, Danielle Gutelius, Garrett Holmes, Zachary Suter, Joshua Jones, Kristen Hokeness, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Peptidoglycan (PG) is composed of alternating β-1,4N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), cross-linked by the peptide tetramer extending off of MurNAc, creating a meshed sheath-like structure that comprises the major structural component of the bacterial cell wall. This project characterizes the protein encoded by cd1034 from Clostridium difficile 630. The gene has been tentatively annotated as a mannosyl-glycoprotein endo-β-N-acetylglucosaminidase (GlcNAcase), and as an FlgJ homolog (Sphingomonas sp. A1). The protein CD1034 is predicted to cleave β-1,4 glycosidic linkages between GlcNAc and MurNAc, producing GlcNAc at the reducing end. Based on sequence alignment, CD1034 is classified in the Carbohydrate Acting Enzyme database (CAZy) as a member of glycosyl hydrolase family 73 (GH73). This family is poorly characterized, despite essential involvement in bacterial life. In characterizing this GlcNAcase, there is potential for elucidating the functions of similar enzymes in this family. Bacterial GlcNAcases function in a variety of roles such as septum degradation, toxin export, PG recycling, and flagellar assembly. By exploring GlcNAcases and elucidating mechanism of action, we can better understand this family of enzymes. Optimal expression conditions in Escherichia coli (BL21(DE3)pLysS) were found for CD1034d44, a CD1034 clone with an N-terminal His-tag lacking the N-terminal transmembrane domain. Purification from inclusion bodies was successful, yielding a near-homogenous sample of CD1034d44. In vitro reaction conditions were established by evaluating the effect of temperature, pH, and divalent cation requirements in turbidometric assays with purified PG.The role the PG stem peptide plays in substrate binding was explored using purified peptidoglycans of varying chemotype as well as synthetic substrates (GlcNAc-pNp).

EVALUATING THE ACTIVITY OF COMBINATIONS OF AMPICILLIN AND CEPHALOSPORIN'S ON ENTEROCOCCUS IN AN IN VITRO PHARMACODYNAMIC MODEL

Thomas Rylah, *Department of Microbiology*, University of Rhode Island, Kingston, RI; Megan Luther, Kerry LaPlante, Kayla Babcock, *Department of Pharmacy Practice*, University of Rhode Island, Kingston, RI and Veterans Affairs, Medical Center, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

BACKGROUND: Enterococci are the causative pathogen in ~10% of infective endocarditis cases, and the most frequently isolated species are Enterococcus faecalis and E.faecium. Synergistic, bactericidal activity is necessary against E.faecalis, with either ampicillin+gentamicin (risk of nephrotoxicity) or ampicillin+ceftriaxone (risk of multi-drug-resistant bacterial colonization).

HYPOTHESIS: The combination of ampicillin+cefepime will be as active as ampicillin+ceftriaxone against E.faecalis and E.faecium. Enhancement of activity will be demonstrated with dual beta-lactam therapy.

METHODS: A high-inoculum (8log10CFU/mL) in vitro pharmacodynamic (IVPD) infection model was used to simulate human pharmacokinetics of ampicillin(2g-q4h), cefepime(2g-q12h), ceftriaxone(2g-q12h), ampicillin+cefepime, ampicillin+ceftriaxone, and no antibiotic(growth-control). Pharmacokinetic parameters of ampicillin (peak150mcg/mL, protein-binding-20%,half-life-1-hour), ceftriaxone (peak257mcg/mL,protein-binding-90%,half-life-6-hours), and cefepime (peak163.9mcg/mL,protein-binding-20%,half-life-2-hours) were used to simulate free drug concentrations over 24hours. One strain of E.faecalis[HH22-Ampicillin(Susceptible (S))-Gentamicin(Resistant (R)] and three strains of E.faecium[D344S-Ampicillin(S)-Gentamicin(S),D366-Ampicillin(S)-Gentamicin(S),and C68-Ampicillin(R)-Gentamicin(R)]were evaluated. Enhancement of activity was defined as >2 log decrease in CFU/mL (kill) from the most active component, with ≥2log kill from initial inoculum.

RESULTS: Against E.faecalis HH22, ampicillin killed 0.26logCFU/mL, ampicillin+ceftriaxone killed 2.80logCFU/mL, ampicillin+cefepime killed 3.41logCFU/mL. Combinations of ampicillin+cephalosporins demonstratedsynergy versus ampicillin alone. Against E. faecium D344S, ampicillin killed 5.70logCFU/mL, ampicillin+ceftriaxone killed 5.62logCFU/mL, ampicillin+cefepime killed 5.62logCFU/mL. Against D366, ampicillin killed 5.94logCFU/mL, ampicillin+ceftriaxone killed 5.36logCFU/mL, ampicillin+cefepime killed 5.64logCFU/mL. Against C68, ampicillin killed 2.43logCFU/mL, ampicillin+ceftriaxone grew 0.55logCFU/mL, ampicillin+cefepime grew0.01logCFU/mL. The activity of ampicillin+cefepime was equivalent to ampicillin+ceftriaxone. Cefepime and ceftriaxone demonstrated no activity when tested alone.

CONCLUSIONS: We conclude that in a high-inoculum PD model, combinations of ampicillin+cefepime are as active as ampicillin+ceftriaxone against enterococci. Enhanced activity was demonstrated in E.faecalis. Ampicillin susceptibility determines activity and synergy.

CHARACTERIZATION OF COMBINATORIAL EPITOPE ASSEMBLIES FOR OPTIMAL EXPRESSION, SOLUBILITY AND IMMUNOGENICITY

Andrew Shumate, *Department of Biology*, Brown University, Providence, RI; Rui Liu, Annie De Groot, Leonard Moise, *Institute for Immunology and Informatics*, University of Rhode Island, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

A major hurdle facing reverse vaccinologists has been how to harness the many candidate antigens identified by T cell epitope predictors in order to construct an effective, immunogenic vaccine. A promising solution to this problem is to deliver multiple epitopes as a concatameric protein. However, little attention has been given to the impact of epitope order on expression and immunogenicity of concatameric proteins. We hypothesize that different epitope orders will result in variable expression, solubility, and immunogenicity of concatameric epitope proteins. To address these critical vaccine properties, a complete combinatorial set of concatameric proteins is recombinantly constructed. Expression and solubility are evaluated using green fluorescent protein reporter assays. Immunoreactivity is measured using MHC tetramer reagents for each epitope and enumerating epitope-specific T cells expressing cytokines following peripheral blood leukocy te stimulation with purified concatameric proteins. Recombinant expression constructs were designed and synthesized for all 24 possible epitope concatamers of four HLA-A2 restricted T cell epitopes derived from CMV, EBV, and influenza. Variable protein expression in E. coli was observed in fluorescence imaging analyses, suggesting that epitope order impacts concatameric protein expression (quantitative expression analyses ongoing). 10 of the 24 (42%) concatameric proteins advanced to solubility screenings, which will be followed by immunoreactivity analyses. The results of this study will provide insight for the future development of computational and experimental methods for handling significantly larger numbers of epitopes.

MOLECULAR BIOLOGY

LOCATED IN ROOM 240 ON THE 2ND FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 9:30 -10:30 AM

DEMONSTRATING THE ANCIENT ORIGIN OF THE MYOGENIC CODE

Thomas Meedel, CJ Pickett, Emmanuel Asiedu, Megan Warburton, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE & RI NSF EPSCoR Summer Undergraduate Research Fellowship Programs

Our laboratory studies the myogenic regulatory factor (MRF) family of basic helix-loop-helix (b-hlh) containing transcription factors that are key regulators of metazoan muscle development. All MRFs examined to date exhibit a high degree of functional conservation and all possess an alanine-threonine (A/T) dipeptide at the same relative position of the basic domain. In vertebrate MRFs, and in the MRF of our model organism, the invertebrate chordate Ciona intestinalis (CiMRF) this dipeptide is known to be critical for myogenesis. Based on its presence in all MRFs and its importance for chordate MRF activity the A/T dipeptide has been hypothesized to constitute a "myogenic code" that is an ancient and conserved feature essential for the myogenic activity of MRFs. In order to further examine the properties of MRFs, and particularly to evaluate the requirement for the A/T dipeptide in myogenesis, we have developed misexpression as says that exploit the ability of MRFs to convert non-muscle cells to a muscle phenotype. Previously, we showed that chimeric proteins in which the b-hlh domain of CiMRF was replaced by the b-hlh domain of vertebrate, arthropod, echinoderm, or nematode MRFs were able to elicit muscle gene activity in the notochord of Ciona embryos, and that mutating the A/T dipeptide severely impaired this activity. Here we extend this analysis to the MRFs of two lophotrochozoans – a major invertebrate taxon whose MRFs have not previously been characterized. We show that chimeric proteins containing the b-hlh domain of a mollusk and an annelid MRF are able to direct muscle gene expression in our notochord assay, and that mutating the A/T dipeptide of these proteins reduces or eliminates that ability. Our results demonstrate a deep functional conservation of MRF activity and indicate that the myogenic code predates the divergence of lophotrochozoans, ecdysozoans, and deuterostomes, consistent with it proposed great antiquity.

DENV REPORTER SYSTEMS FOR LIVE CELL ANALYSIS

Casey Vendettoli, Carey Medin, Alan Rothman, *Institute for Immunology and Informatics*, University of Rhode Island, Providence, RI

Coastal Fellows Program

The overall objective of this project is to develop and evaluate modified constructs for labeling of dengue (DENV)-infected cells for live-cell analysis. DENV is a mosquito-borne flavivirus of global importance, which predominantly infects myeloid-derived cells. DENV infection is known to induce significant changes in cellular function, but most in vitro studies have either used unsorted cultures, reflecting both direct and indirect effects of viral infection, or have destroyed cell integrity by permeabilization and fixation to identify DENV-infected cells by immunostaining. This project will initiate development of novel technology to identify live DENV-infected cells. For this project, we are developing two reporter systems for detection of DENV infection. Both systems will utilize the proteolytic processing of viral proteins NS4B and NS5 by the DENV protease upon viral infection of the cell. The first system will be engineered to turn on GFP expression only in the presence of DENV infection. The second system will induce a color change from green to red when cells are infected with DENV. These reporter systems will be useful tools in live-cell analysis of virus-infected cells by fluorescence microscopy or flow cytometry and represents a promising approach to investigate virus-cell interactions. For several viruses, live cell imaging has revealed new mechanisms critical to viral replication or cell-to-cell spread and has been used for antiviral drug screening.

NEXT GENERATION SEQUENCING ANALYSIS OF MICROSATELLITES IN ULVA RIGIDA AND ULVA COMPRESSA

Matthew Breseman, Noe Mercado, Kristen Dostie, Shennel Gelin, Amy Battocletti, Tania Aires, JD Swanson, *Department of Biology*, Salve Regina University, Newport, RI; Carol Thornber, *Department of Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The macroalgal genus Ulva is present in shallow coastal systems worldwide. During the summer months, Ulva forms large macroalgal blooms which can have negative ecological and economic impacts on the coastal ecosystem. Of two common Ulva species, U. rigida and U. compressa, only 396 nucleotide sequences have been submitted to GenBank as of July 2013. The aim of our research is to utilize di- and tri- nucleotide repeats found in the genome, known as microsatellites, to create unique fingerprint profiles of U. rigida and U. compressa. Nine microsatellite primer pairs designed for U. intestinalis have been previously found to amplify microsatellite sequences in U. rigida and U. compressa. We have successfully optimized the annealing temperature for seven of the primers to be between 47.9 oC and 52.3 oC. To further elucidate additional Ulva microsatellite markers, 100mg of extracted U. rigida DNA was sent to Washington State University to be seque nced using PacBio third-gen sequencing. We have obtained 5x coverage of the 134 Mbp genome and have completed a primary genome assembly. An additional run of HiSeq2000 will soon be completed. The HiSeq2000 data will result in smaller reads and will provide far greater, more accurate genome coverage than the PacBio data which provided scaffold information due to their long, less accurate read length. Combining the two data sets will give us more accurate assemblies from which we can design future primers for U. Rigida and U. Compressa. Currently, we have scanned the 25 largest contiguous sequences from the PacBio assembled genome for potential microsatellite regions using Msatcommander. Using Msatcommander we have designed 15 primer pairs unique for U. Rigida and U. Compressa. We are in the process verifying the primer pairs by amplifying them across test DNA samples collected during the summer of 2012.

STRUCTURE-FUNCTION ANALYSIS OF NICOTINIC ACETYLCHOLINE RECEPTORS

Helen Lord, *Department of Molecular Pharmacology, Physiology, and Biotechnology*, Brown University, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

I investigated neuronal nicotinic acetylcholine receptors, and the effect that reactive oxygen species (ROS) have on the receptor. Neuronal nAChRs are ligand-gated, inwardly rectifying receptors that are made up of 2α and 3β subunits. These neuronal subunits contain a cysteine in the M1-M2 linker region of exon V at the pore of the receptor. It is hypothesized that this cysteine is the target for ROS in α3β4-containing and α4β2-containing neuronal nAChRs. The ROS create a conformational change in the nAChRs, causing the receptors to enter a prolonged inactivated state. This study is especially important in relation to neurodegenerative diseases and diabetes, which are associated with an increase in ROS. In order to eliminate this prolonged inactivation, I made a target vector of exon V of alpha 3 with a cysteine to alanine mutation. I then transformed the mutated vector into XL-10 gold ultracompetent cells and amplified the colonies of interest. Finally, the region with the intended mutation was sequenced in order to find one particular sample with the cysteine to alanine mutation. In the next month, the mutated target vector should be transfected into a mouse.

MACROALGAL BLOOM FORMATION PATTERNS IN RESPONSE TO TEMPERATURE THRESHOLDS ASSESSED BY RT-qPCR

Noe Mercado, Tania Aires, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Carol Thornber, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Macroalgal blooms can cause serious ecological and economic impacts to marine communities and are thus a growing interest in several recent studies. As the climate changes, the magnitude and duration of the blooms are predicted to increase, which may have negative impacts on the overall stability of ecosystems. In order to determine the harmful effects of the macroalgal blooms and link their growth dynamics with global environmental changes, more information about the life history and genetic makeup of these macroalgae (seaweed) must be explored. At present, the genus Ulva includes more than 100 species, which are widely distributed in the intertidal zones of several estuarine environments. Their abundance is particularly due to their high tolerance of salinity levels and water temperatures, along with high growth rate. The primary focus of this study is the green macroalgae Ulva rigida, which is found in large deposits around marine communiti es and known to develop into harmful blooms in shallow coastal systems worldwide. Real Time qPCR was performed to observe the expression pattern of a specific gene (LhcSR) known to play an important role in the photo-protective mechanism only found in algae. In previous studies, the LhcSR gene was found more highly expressed at higher temperature ranges. Two housekeeping genes (Histone 2 and 18S) were used as controls for the normalized expression of the target gene. Expression patterns were compared between three different bloom periods (May, July, and September) in three different U. rigida populations in the Narragansett Bay (Chepiwanoxet, Sandy Point and Oakland Beach) and in both tidal levels (intertidal and subtidal). Normalized LhcSR gene expressions in U. rigida demonstrated that the target gene (LhcSR) was being upregulated during the warmer time periods (July and September) for all the populations studied.

INVESTIGATING REAL TIME QPCR PRODUCTS WITH CYCLINS AFTER GALLIC ACID EXPOSURE TO RUBUS CALLUS

Alexandria Bierce, Kelsey Stafstrom, Stephanie Liptak, Tia Crowther, JD Swanson, *Department of Biology and Biomedical Sciences,* Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gallic acid is a secondary metabolite found in many species including the genus Rubus. Obtaining a better understanding of this phenolic can lead to development of naturopathic medicines. Previous research has indicated that when plant callus is exposed to high concentrations of gallic acid, the plant cell cycle is halted, but in lower concentrations it speeds up the cell cycle. To investigate the effect of gallic acid on plant cells, raspberry leaves have placed onto media containing thidiazuron to form callus. The plates of raspberry leaves are kept sterile in an incubator on a cycle of 18 hours in the light and 6 hours a day in the dark. It has been found that the callus survives the best when being placed in the dark at room temperature for four days and then they are transferred into the incubator at 23˚C. Callus will then be exposed to 0 uM, 25 uM and 40 uM gallic acid for 0, 12, 24, and 48 hours. RNA will be extracted from Rubus callus leaves using a ‘RNeasy Plant Mini Quiagen kit’. Prior research has speculated that gallic acid affects the plant cell cycle in the G2/M phase, thus primers for genes that work in the G2/M phase such as Cyclins A1, B1, G1, Y, and CDK 7 were designed. Extracted RNA will be tested with primers using real time qPCR. This will provide insight into gene expression of several cyclins in response to gallic acid. To further validate our hypothesis about the effect of gallic acid on Rubus leaves, protein has been extracted from the leaves exposed to 25 uM and 40 uM of gallic acid for 48 hours. A western blot will be run to measure the protein level after exposure to gallic acid compared to the qPCR products.

DETERMINING HOW BCP1 INACTIVATION INFLUENCES NONHOMOLOGOUS END-JOINING IN S. CEREVISIAE

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RI-INBRE Summer Undergraduate Research Fellowship Program

BCCIP is a mammalian protein that interacts with BRCA2 in homologous recombination repair of double strand DNA breaks (DSBs). The nuclear export protein, Bcp1, in S. cerevisiae is a homologue of BCCIP, but its role in the DNA damage response is undetermined. Previous work in this lab has shown that when Bcp1 is inactivated, yeast exhibit an increased sensitivity to the DNA damaging drugs bleomycin and phleomycin, suggesting that Bcp1 is involved in DNA damage response. Through a series and transformations and plasmid isolations using a wildtype (SEY) strain and a temperature sensitive Bcp1 mutant (AAY) strain of yeast, this study was designed to determine the effects of Bcp1 inactivation on repair of double strand breaks generated by restriction enzymes. It was discovered that the yeast repaired the blunt-end break through homologous recombination repair even when Bcp1 was inactivated.

CHARACTERIZATION OF ENDOPLASMIC RETICULUM (ER) INVOLVEMENT IN THE CHRONOLOGICAL LIFESPAN OF THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE

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RI-INBRE Summer Undergraduate Research Fellowship Program

All organisms age. In humans, who have a life expectancy of approximately eighty years, aging leads to a general physiological deterioration of numerous organ systems. Recently, several hallmarks of aging have been described suggesting that aging is a pleiotrophic phenotype. Because of their relatively long lifespans, the aging process of humans and other mammals is not easily studied. Therefore, model organisms have been investigated to uncover the fundamental mechanisms behind aging. With an average chronological life span of just three weeks, Saccharomyces cerevisiae is a good model organism for aging experiments. In this study, we look at the effect of ER stress on yeast cells throughout their chronological lifecycle. ER stress leads to an unfolded protein response (UPR) that helps the ER deal with the buildup of unfolded proteins. Our preliminary data suggests that the UPR decreases as wildtype yeast cells age and die, indicating that ER function is important for long-term viability. To test this hypothesis, we are measuring the chronological lifespan of wildtype yeast cells that are continuously exposed to low levels of ER stress to determine if activating the UPR extends longevity.

HUMAN MALIGNANT MELANOMA CELLS RESIST OXIDATIVE STRESS DUE TO OVERACTIVE PI3K/AKT/MTOR PATHWAY AND OVER-PRODUCTION OF ROS REGULATORS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Melanoma is considered one of the most aggressive and less treatable cancers. While various models and mechanisms have been proposed, the transformation from tranquil melanocytes to invasive melanoma cells remains an enigma. Alteration of cellular redox status has been suggested to be associated with cellular transformation. We compared the responses to oxidative stress in primary human melanocytes and melanoma cells. Both cells lines were treated with H2O2, to simulate oxidative stress, at doses between 50 and 250μM. Data obtained showed that melanoma cells are more resistant to the abrasive H2O2 treatment. Furthermore, melanoma cells have constituent activation of AKT and mTOR, which are key signaling proteins associated with cell survival and protein synthesis pathways. To further elucidate this phenomenon, experiments were conducted to investigate the enzymes that regulate reactive oxygen species within the cells, specifically, Super Oxide Dismutase 1 (SOD 1), Super Oxide Dismutase 2 (SOD 2), Super Oxide Dismutase 3 (SOD 3), and Catalase. Western blot and confocal analysis showed that these proteins are over-produced in melanoma cells relative to the normal levels found in melanocytes. Interestingly, when melanoma cells were challenged with AKT and mTOR inhibitors (LY294002 and Rapamycin respectively), the production of SOD 3 decreased significantly. Collectively, our data suggest that active AKT/mTOR and up-regulation of ROS regulators contribute to the resistance of melanoma cells to oxidative stress and attribute to their aggressiveness. This data provide insights into the understanding of the molecular mechanisms of the transformation from melanocytes to melanoma and support the notion of cells reducing apoptotic signals in stressful situations for survival. Focusing on how these cells neutralize ROS through these important cell-signaling pathways can identify important targets for better clinical management of melanoma.

MOUSE LIVER SULFOTRANSFERASE ISOFORMS AND THEIR METABOLIC ROLE IN THE SULFONATION OF BPA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Bisphenol A (BPA) is known to be a common endocrine disrupting drug that can be metabolized and excreted from the body with the aid of sulfotransferases, which are enzymes that catalyze the transfer of a sulfonate group from a donor molecule to a particular substrate. BPA toxicity within the body of an organism occurs as a result of direct BPA ingestion; however, the physiological effects of this toxicity can be minimized by the action of sulfotransferase enzymes present within the liver that prepare BPA for excretion. Previous studies conducted in Dr. King's lab have shown that there is more than one type of sulfotransferase active within the liver cytosol of mice that contributes to the sulfonation of BPA. This study investigates the rate of sulfonation of BPA, at various concentrations, catalyzed by specific sulfotransferases present in the mouse liver. Competent E.coli cells were transformed with cDNA for mouse sulfotransferases (mSu lt1a1, mSult2a1, mSult1c1, mSult1c2) and cultured until sufficient growth was reached. The cells were pelleted, lysed and centrifuged at 100,000xg to obtain the cytosol, which contained the protein of interest. The expressed sulfotransferases were characterized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and by the Western Blot method for selective antibody reactivity.

ANTI-MELANOGENIC POTENTIAL OF THYMOQUINONE ISOLATED FROM BLACK CUMIN (NIGELLA SATIVA L.) SEED OIL

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RI-INBRE Summer Undergraduate Research Fellowship Program

Thymoquinone (TQ) is the predominant bioactive constituent present in black cumin (Nigella sativa) seed oil, a culinary spice widely consumed worldwide and used as a traditional medicine in Eastern cultures. TQ has been extensively studied for its anti-cancer, diabetic and inflammatory properties but is yet to be investigated for its anti-melanogenic potential. Given the growing research interest in the cosmeceutical applications of natural compounds, herein we investigated the anti-melanogenic potential of TQ (from previous isolation by our laboratory) in B16F10 melanoma cells. TQ significantly decreased melanin production in B16F10 cells (at 2.5-20 μM), but did not inhibit the activity of tyrosinase enzyme which is involved in melanin synthesis as suggested by both the cellular and enzymatic assays. However, TQ was effective in lowering the expression of the melanogenesis-related genes, MITF (19-16% reduction), TYRP-1 (70-98% reduction) and TYRP-2 (10% reduction), which was assessed by qPCR at 2.5 and 10 μM. Western blotting analysis also revealed that TQ down-regulates MITF, TYRP-1 and TYRP-2 protein expression in a dosage-dependent manner. These findings suggest that TQ should be further investigated for cosmeceutical applications pertaining to skin hyperpigmentation disorders.

HIGH-YIELD EXPRESSION AND PURIFICATION OF ENTAMOEBA HISTOLYTICA ALCOHOL DEHYDROGENASE 2 (EHADH2)

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RI-INBRE Summer Undergraduate Research Fellowship Program

The bifunctional alcohol/aldehyde dehydrogenase enzyme in E. histolytica (EhADH2) belongs to the ADHE iron dependent family of enzymes and is essential for trophozoite survival. Using bioinformatics and sequence comparisons with distant structural homologs, we have identified the optimal domain boundaries for the isolated ADH and ALDH domains of EhADH2. We are fully confident that we will obtain protein yields suitable for structure determination using x-ray crystallography. Five constructs, EhADH2 full length, EhADH2 truncated, ALDH, ADH, ADH truncated, have been successfully cloned into pRP1B-NheI vector and sequence verified. The N-terminal ALDH domain expresses to high levels, has been purified to homogeneity and is a monomer, as determined using size exclusion chromatography. The protein is also stable, can be concentrated to more than 39 mg/ml (40x higher than the 1.5 mg/ml of the previous protocol). This domain has produced small crysta ls that are being optimized. The pRP1B-Nhe_EhADH2 five constructs have been transformed in an E. coli adhe deficient strain to test for activity. We have obtained enzymatic activities for EhADH2 full length and ALDH domain that are twice more active than the proteins expressed with the original expression vector. Once diffraction quality crystals of these constructs are obtained, data will be collected and phased by molecular replacement using structures 3K9D and 1RRM as search models.

LIPIDS RAFTS: FACT OR FICTION? AN ANALYSIS OF THE CRITERIA USED TO DEFINE THE MEMBRANE COMPARTMENTS KNOWN AS “LIPID RAFTS”

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RI-INBRE Summer Undergraduate Research Fellowship Program

Membrane compartmentalization is an important way for the cell to organize cellular processes and enable complex actions in cells. Originally, plasma membrane was considered to be a two-dimensional fluid in which the proteins and lipids freely diffused. Later it was suggested that the plasma membrane is organized into compartments such as lipid rafts. Lipid rafts are defined by portions of the membrane that are enriched in cholesterol and sphingolipids and are insoluble in non-ionic detergents such as Triton X-100. Under this theory, when the membrane is treated with a cholesterol chelating agent such as methyl-β-cyclodextrin (MβCD) the lipid rafts are disrupted and the proteins contained in the lipid raft become soluble in Triton X-100. Although Triton X-100 and MβCD are used to identify lipid rafts proteins, the processes of treating cells with Triton X-100 or MβCD disrupts cell functions and does not necessarily confirm that these proteins are contained within lipid rafts. This persuaded us to develop an experiment that would determine if lipid rafts represent functional plasma membrane compartments. We are using a proximity biotinylation assay to determine if lipid raft proteins, Lyn Kinase and Flotillin, are really compartmentalized and separated from a protein known to not reside in lipid rafts, KRAS. By measuring the ability of a non-lipid raft protein (KRAS) to interact with a supposedly lipid raft protein (Flotillin or Lyn Kinase) we can determine the accessibility of non-lipid raft proteins have to proteins residing in lipid rafts. Interestingly, we discovered that only a small minority of the supposedly confirmed lipid raft protein Lyn Kinase is insoluble in Triton X-100 but most of the Flotillin is insoluble. Additionally, we found that cholesterol chelation has no effect on the amount of insoluble Flotillin. These findings further identify inconsistencies in the theory of lipid rafts as membrane compartments.

SELECTIVE TARGETING OF CANCER CELLS BY ARYLPHOSPHONIUM SALT-PEPTIDE COMPLEXES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Polypeptides and arylphosphonium salt (APS) were synthesized utilizing microwave assisted solid state synthesis. Arylphosphonium salts have shown in previous research literature to be possible anti-cancer agents. Arylphosphonium salts are lipophilic cations and as such they have been found to accumulate preferentially in tumor cells due to the high membrane potential of tumor mitochondria. These characteristics also make them ideal phase transfer catalysts. The peptide sequence RGD (Arg-Gly-Asp) has been previously shown to be the recognition sequence for α,β integrins. These intergrins are only present in cells undergoing angiogenesis. APS bound via an ester link to RGD has a greater affinity to cancer cells than do APS alone. APS accompanied by co-administered RGD "escort" molecules is expected to have an even greater affinity for α,β integrin receptors. The hypothesis is that APS is carried 1) as covalentl y bound cargo to integrin proteins embedded in the cells' plasma membrane, and once there the APS is endocytized along with the polypeptide into the cell by neurophilin-1 proteins which are also located on the cell surface and have a greater affinity for RGD than do integrins, and 2) RGD acts as an escort molecule for the APS possibility by a phase transition catalytic mechanism. This greater affinity is due to the "CendR" rule, polypeptide sequences following the motif known as "CendR", meaning the carboxylic acid end of the polypeptide contains either a lysine or arginine. Other non-APS conjugated polypeptide chains were also synthesized to investigate other means of delivering anti-cancer agents into the cell.

BINDING OF THE GLOBAL POLLUTANT PERFLUOROOCTANE SULFONATE (PFOS) TO DNA REVISITED

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RI-INBRE Summer Undergraduate Research Fellowship Program

Perfluorooctane sulfonate (PFOS) is an organic chemical prevalent in many industrial and commercial products that accumulate in the aquatic environment and mammalian tissues, particularly, liver and kidney. Despite its environmental prevalence, the present knowledge about its long-term effect on humans and the environment is very limited. The 2009 study by Zhang and coworkers [BMC Molecular Biology (2009), 10(16), doi: 10.1186/1471-2199-10-16] has shown that PFOS non-covalently binds to the DNA and alters its secondary structure. This finding is significant because of possible genotoxic implications. In the present study, we revisited their work to verify the binding of PFOS to the DNA at various conditions. To that end, we have employed three different biophysical techniques (Circular Dichroism, CD; Isothermal Titration Calorimetry, ITC and 19F Nuclear Magnetic Resonance spectroscopy, 19F NMR) on three different types of DNA (16-mer, 44-mer and Calf Thymus CT). We analyzed the DNA samples in presence of increasing amounts of PFOS and the data was compared with the non-fluorinated octane sulfonate (OS) control. Our CD data suggest that PFOS does not bind to the shorter DNA (16-mer and 44-mer), but exhibited 1:1 binding with the CT DNA. In contrast, no binding was observed for OS in all the three cases. While the CD results were in accord with the findings of Zhang et al, the ITC and 19F NMR do not show any evidence of binding. Thus, further investigation is needed to confirm its DNA binding as well as its potential to induce mutagenesis and carcinogenesis and disruption in transcription. We also used the well-known DNA minor groove binder DAPI to probe the conformational heterogeneity of arylamine modified DNA adducts. The results of this study will be discussed.

DETERMINING THE EFFICIENCY AND SPECIFICITY OF CRE MEDIATED UBE4B RECOMBINATION AND ITS EFFECTS ON MYOBLAST DIFFERENTIATION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Protein turnover in cells is controlled by the ubiquitin conjugation system which marks proteins for degradation by the proteasome and is composed of the three classes of enzymes E1, E2, and E3. Ube4b is an E3 ligase which has a conserved U-box domain that is essential for mediating ubiquitin conjugation. Protein degradation is one process that controls myogenesis, the growth and differentiation of muscle. Studies have shown that skeletal muscle cells express alternative splice forms of Ube4b at different time periods during myogenesis. To determine how the lack of Ube4b activity could affect skeletal muscle in vitro and in vivo, a line of transgenic mice was developed in which the U-box domain of Ube4b was removed only in skeletal muscle through expression of iCre-recombinase controlled by the MyoD promoter. The studies discussed here demonstrate that iCre-dependent recombination of Ube4b occurs only in skeletal muscle and not in any other t issues analyzed from these mice, indicating that the Ube4b is inactivated specifically in skeletal muscle. Other experiments performed to determine the efficiency of the recombination in skeletal muscle have not yet shown 100% recombination of the Ube4b allele so further studies will be required to address the reasons for this observation. A second aim was to determine if expression of the inactivated form of Ube4b could affect skeletal muscle differentiation in vitro. Primary cell cultures of muscle myoblasts are challenging to grow, and experiments thus far have been focused on determining the critical parameters such as the age of the mice, cell morphology, and cell plating density which are optimal for induction of differentiation. Results have shown that control and mutant myoblasts cultures are able to differentiate in vitro, but further experiments will need to carried out to perform a complete comparison of the differentiation capacity of the two groups.

RESHUFFLING KINASE STRUCTURES TO UNDERSTAND THEM

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RI-INBRE Summer Undergraduate Research Fellowship Program

Protein tyrosine kinases are a large family of enzymes that phosphorylate and regulate the functions of other proteins in a cell. Even though all PTKs share a similar core catalytic structure, each PTK possesses a unique set of biochemical properties, enabling it to play a unique role in the cellular regulatory network. The structural bases for this functional specificity among PTKs are still poorly understood. We are developing a directed evolutionary strategy to elucidate this structure-function relationship. This strategy involves a DNA shuffling approach to generate a large number of random off-spring chimeras of a set of parental PTKs, and a screening approach to identify chimeras displaying a given property. A comparison of structures of a set of chimeras displaying a common property reveals the structural basis for a given property. This strategy should apply to investigation of just about any PTK.

NANOPARTICLE-BACTERIAL MEMBRANE INTERACTIONS

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RI-INBRE Summer Undergraduate Research Fellowship Program

This study aims to determine the effects that nanoparticles have on the outer membrane of bacterial cells. Studies have shown that certain nanoparticles exhibit cytotoxic effects on cells through adverse nanoparticle-membrane interactions, but little has been done to determine the physical mechanisms. Model membranes were initially used to simulate the cell membranes of bacteria. The lipidsdipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) provided a net anionic membrane, which is characteristic of bacteria. Using dynamic light scattering, nanoparticle binding was investigated using silver (Ag) nanoparticles with anionic, neutral, and cationic surface coatings. While the results did correlate generally within known toxicity behavior, a deeper understanding was sought of how these results would correlate to actual bacterial membranes. For this reason, membranes extracted from prokaryotic Escherichia Coli (E. coli) bacteria were used in place of the model membranes. A procedure was developed where the membranes of bacteria were exposed to nanoparticles in a controlled aqueous trough within a Langmuir-Blodgett system. This allowed us to detect changes in lipid interactions (via surface pressure) due to nanoparticle binding. Direct imaging was achieved by placing a hydrophobic slide onto the interface of the water containing E. coli membranes, thereby transferring a membrane monolayer to the slide. Nanoparticle binding was confirmed by field emission scanning electron microscopy (FE-SEM). Our preliminary work suggests that this approach provides a new pathway for determining how nanoparticles bind to and disrupt membranes.

PREVENTION OF GASTRIC CANCER IN A MOUSE MODEL OF H. PYLORI INFECTION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Helicobacter pylori bacteria live in the human stomach, causing gastritis, peptic ulcers and gastric cancer. Eradicating H. pylori with antibiotics prevents ulcers but whether it also prevents gastric cancer is controversial. Normal (wild-type) mice do not develop cancer after experimental H. pylori infection, but mice lacking the tumor suppressor p27 (p27 knockout mice) do so, and are a good experimental model of H. pylori-induced cancer.

Aim: To examine whether H. pylori eradication prevents gastric cancer in p27 knockout mice.

Methods: p27 knockout mice were infected with H. pylori. Mice in group 1 received “early” combination antibiotic eradication (15 weeks post infection, WPI). Group 2 received the same therapy “late” (45 WPI). H. pylori non-eradicated mice served as controls. All mice were euthanized 70 weeks after infection. H. pylori status was evaluated by serology, culture and PCR. Stomach tissue was harvested for pathology scores and expression of multiple cytokine/chemokines.

Results: H. pylori-infected mice developed advanced gastric pathology, including high-grade dysplasia, whereas those given antibiotics did not. Pathology scores in group 1 vs. 2 were similar. Both were significantly decreased compared with controls for overall pathology ($p<0.05$ for 15 WPI, $p<0.01$ for 45 WPI vs. controls), inflammation ($p<0.01$), hyperplasia ($p<0.05$ for 15 WPI, $p<0.01$ for 45 WPI vs. controls) and dysplasia ($P<0.05$). IP-10 and MIG levels were significantly decreased in the stomachs of mice who received antibiotics ($P<0.001$ for 15WPI, $P<0.01$ for 45WPI vs. control). There were no significant differences in expression of IFN-gamma, TNF-alpha, IL-1 Beta, RANTES, MCP-1, MIP-1 alpha or MIP-1 beta among the three groups.

Conclusion: H. pylori eradication given either early or late after H. pylori infection significantly lowered gastric inflammation, hyperplasia, and dysplasia in the p27-deficient model of H. pylori-induced gastric cancer. The mechanisms of this protection may involve reduced expression of IP-10 and MIG.

THE INTEGRITY OF THE ENDOPLASMIC RETICULUM AND THE UNFOLDED PROTEIN RESPONSE ARE COMPROMISED WHEN THE ERMES COMPLEX IS MUTATED IN SACCHAROMYCES CEREVISIAE

Seann Murphy, Douglas Biancur, Nicole Byrnes, Faith Donaghey, Ryan Frazier, Katelyn Higgins, Michael Kondik, Benjamin Lichtenfels, Nicholas Mazzucca, James O'Brien, Jenna Perry, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Endoplasmic Reticulum – Mitochondria Encounter Structure (ERMES) is a protein complex in Saccharomyces cerevisiae that tethers the endoplasmic reticulum (ER) to the mitochondria. The ERMES complex is thought to facilitate communication and cooperation between the ER and mitochondria via the intra- organelle transport of biologically important molecules such as phospholipids and calcium. The ERMES complex is made up of four proteins: Mdm10p, Mdm12p, Mdm34p and Mmm1p. To determine the role of the ERMES complex on ER structure and function, we imaged mutant strains with deletions in each ERMES gene to examine the structure of the ER and the integrity of the unfolded protein response (UPR). The UPR is a stress-response signaling pathway that is activated when the protein-folding capacity of the ER is exceeded. Our data suggests that ER structure and UPR function are compromised when the ERMES genes are deleted.

ONCOGENIC POTENTIAL OF LRH1 IN HUMAN PANCREATIC CELLS

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RI-INBRE Bridges to Graduate School Program

Pancreatic cancer (PC), the 4th leading cause of cancer-related deaths in the United States, has the lowest 5-year survival rate in all malignancies. Genome wide association study (GWAS) has identified a region containing the liver receptor homolog 1 (LRH1) gene as being highly associated with individual susceptibility to PC. LRH1 is expressed in endoderm derived tissues where it regulates cholesterol, fatty acid, and bile acid homeostasis. In adult mammals, it is primarily expressed in the liver, pancreas, and intestine. Accumulating evidence has implicated the critical involvement of LRH1 in a variety of tumors. Noticeably, we have observed that LRH1 is overexpressed in patients with pancreatic cancer. To explore the mechanism of LRH1 in pancreatic oncogenesis, Capan-1, a human pancreatic ductal adenocarcinoma cell line, was employed for all assays. A wound healing assay was performed to examine cell migration and interaction. In this assay, a pipette tip is used to scratch (simulating a wound) cells seeded in a multi-well plate. Migration is then observed after 18 hours. Colonosphere formation assay, a three dimensional growth model in matrigel, was performed to analyze cell invasion, which mimics how the cells may potentially grow in vivo. Lastly, proliferation was examined through a growth curve based on crystal violet staining. The results demonstrate that LRH1 enhances cell proliferation, migration, and invasion. All of these characteristics indicate oncogenic properties of LRH1, illustrating that overexpression of LRH1 can lead to pancreatic cancer progression. Future studies examining LRH1 expression in a variety of human pancreatic cell lines, as well as in vivo studies of the tumorigenicity of LRH1 on nude mice, would be able to better understand the essential role of LRH1 in development and progression of pancreatic cancer.

DEFINING THE ROLE OF BCP1 IN HOMOLOGOUS RECOMBINATION AND NON-HOMOLOGOUS END JOINING

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RI-INBRE Summer Undergraduate Research Fellowship Program

DNA damage could lead to cell death and mutations; thus it is important for cells to repair properly to maintain genome and cell viability. The goal of this study was to assess the role of BCP1 protein in homologous recombination and non-homologous end joining in the organism Saccharomyces cerevisiae. Plasmid DNA was cut with either SmaI or HindIII restriction enzyme then transformed into S.cerevisiae with either the wild type Bcp1(SEY strain) or a mutated Bcp1(AAY strain). Only yeast able to repair the plasmid could grow on selective agar plates. The plasmid was then rescued and sent for sequencing. We discovered that the cut plasmid was repaired by homologous recombination in the absence of the Bcp1 protein, suggesting Bcp1 is not required for homologous recombination repair in yeast.

MICTROTRANSPLANTATION OF CAV2.2 INTO XENOPUS OOCYTES

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RI-INBRE Summer Undergraduate Research Fellowship Program

N-type voltage sensitive calcium channels are transmembrane multiprotein complexes that conjoin membrane depolarization to calcium entry into the cell. These calcium channels (Cav2.2) are located primarily in the nervous system, and are important for neurotransmitter release at the presynaptic nerve terminals. The purpose of this study was to confirm the presence of the Cav2.2 channel in rat-brain neurolemma microtransplanted into Xenopus oocytes. To accomplish this goal, we utilized western blot, immunohistochemistry and electrophysiological approaches. Our results indicated that microtransplated neurolemma possess a current that is sensitive to ω-conotoxin MVIIC, a potent Cav2.2 channel blocker. Thus, microtransplanted rat brain neurolemma is a viable method to examine the properties of Cav2.2 in the X. laevis oocytes.

ISOLATION AND PURIFICATION OF WATER SOLUBLE PROTEINS FROM GINGER ROOT (ZINGIBER OFFICINALE)

Ana Ortez Sandoval, *Biotechnology*, Community College of Rhode Island, Warwick, RI; Sameer Surti, Aftab Ahmed, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI; Farwa Hashmi, Atia Tulwahab, Iqbal Choudhary, *International Center for Chemical & Biological Sciences*, University of Karachi

RI-INBRE Summer Undergraduate Research Fellowship Program

Ginger is the rhizome of the plant, Zingiber officinale. It is an important ingredient in the traditional South Asian cuisines. In Indian, Pakistani and Chinese folk medicine, ginger is used for gastro-intestinal disorders, nausea, vomiting, inflammatory diseases, muscle and joint pain. Limited studies have been reported on the isolation of proteins from ginger extract. In the current study, water soluble proteins were extracted from ginger root and successfully purified by using two-dimensional liquid chromatography (2D-LC) approach. The ginger root was washed with distilled water; skin removed and then grounded using an electric blender. Sample was stirred for four days at 4oC and filtered using cheese cloth followed by high speed centrifugation at 26,000 x g. The extracted proteins were precipitated using both 90% cold Ethanol. Combination of 2D-LC techniques including gel filtration, ion-exchange and reversed phase HPLC were successfully achieved. Purity of the isolated proteins were confirmed by SDS-PAGE gel electrophoresis. Future work will be conducted on the protein characterization using mass spectrometry and Edman N-terminal protein sequencing.

A SEARCH FOR FANCONI ANEMIA GENES IN AN INVERTEBRATE CHORDATE

Beatrice Pratt, Steve Irvine, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; David Vierra, Niall Howlett, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

Fanconi Anemia (FA) genes have attracted intense research due to its important role in the recognition and repair of damaged DNA. To date, 15 FA genes in a complex biochemical pathway have been discovered, including the breast cancer susceptibility gene BRCA2. Although rare, genetic defects in these genes lead to early developing childhood cancers and leukemia. Many interesting findings can be determined by studying rare genetic diseases including the DNA repair pathways in a normal functioning system. To better understand the human disease we will use a simpler organism, Ciona Intestinalis, a "sea squirt" to study both homologous and similar genes. C. intestinalis is a good test subject because it appears to have a simpler set of FA genes than humans, and sea squirts are the closest invertebrate group to the vertebrates.

To test when FA genes are expressed, we gathered embryos at different stages and isolated the mRNA to make cDNA. We are testing these cDNA pools with degenerate primers designed to amplify FancL, FancD2, ATR and FancM. We are also constructing phylogenetic trees with amino acid sequences of possible FA genes from various species to determine which genes in C. intestinalis are homologous to human FA genes.

A GENETIC SCREEN FOR HIGH COPY SUPPRESSORS OF BXI1 IN THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Bax inhibitor-1 (BI-1) is an anti-apoptotic gene whose expression is upregulated in a wide range of human cancers. Our laboratory has published data suggesting that the yeast gene, BXI1, is a homolog of BI-1, which links the unfolded protein response and programmed cell death. We have initiated a high copy suppressor screen using a yeast genomic library to find other genes that will help us better understand the function of BXI1. These include genes that function in a manner similar to that of BXI1 or genes that operate within the same pathway as BXI1. We are both selecting for and screening for high copy suppressors that allow Δbxi1 cells to survive exposure to a high temperature of 37°C when grown on minimal media, a condition which is known to induce programmed cell death in yeast. Fluorescent reporters of the unfolded protein response will be used to verify that potential candidates are legitimate suppressors of the Δbxi1 phenotype. Once the screen itself is complete, potential suppressors will have their genomes sequenced to identify the mutations responsible for their phenotypes.

DETECTION OF DENGUE VIRUS INFECTION IN LIVE CELLS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Dengue virus (DENV) is a mosquito-borne human pathogen of global medical importance. DENV causes an acute febrile illness that is sometimes associated with a life-threatening plasma leakage syndrome, dengue hemorrhagic fever (DHF). Given the limitations of clinical studies and existing animal models, cell culture models remain an important approach to studying DENV infection and host responses. Live-cell analysis of virus-infected cells by fluorescence microscopy represents a promising approach to investigate virus-cell interactions. However, current methods to detect DENV-infected cells use antibody staining, which generally requires permeabilization and fixation thus killing the cells. These methods are destructive and are not suitable to intact cell imaging or sorting. We have developed a plasmid-based reporter to allow robust non-destructive identification of DENV-infected cells. The reporter utilizes the ability of the DENV NS2B3 protea se to proteolytically process the site linking NS4B and NS5, allowing the GFP portion of the fusion protein to move from the cytoplasm to the nucleus. Cells transfected with the reporter plasmid and infected with DENV showed nuclear GFP as early as 8 hours post infection. Immunostaining for DENV antigen confirmed that nuclear GFP correlated with the presence of DENV antigen. Additionally, we found that infection of cells transfected with this reporter plasmid with each serotype of DENV induced nuclear localization of GFP after 24 hours. We have this technology to analyze mitochondrial changes in DENV infected cells and observed an increase in mitochondrial lengths in DENV infected cells when compared to uninfected cells. The DENV reporter described is a promising strategy for identifying live DENV-infected cells by fluorescence microscopy, with potential applications for detection of virus and for studies of virus-cell interactions.

DELATMETHRIN STIMULATED NEUROTRANSMITTER RELEASE IN NEUROLEMMA-INJECTED XENOPUS LAEVIS OOCYTES

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Current approaches to toxicity testing are time consuming, expensive and typically rely on investigations that evaluate observable changes to whole animals. New approaches capable of assessing environmental contaminants in a cost and time efficient manner are required to provide information necessary for sound evaluation of the health effects of adverse environmental agents. To this end, we evaluated neurolemma-injected oocytes as a system to evaluate the toxic action of pyrethroids. Our results obtained using pyrethroids on neurolemma-injected Xenopus oocytes validate initial biochemical findings obtained using synaptosomes. Rat brain neurolemma-injected oocytes are capable of reconstituting native ion currents into their plasma membrane and undergo neurotransmitter release. Microtransplanted ion currents were sensitive to TTX, omega-conotoxin MVIIC, and chlorotoxin, indicating the presence multiple ion channels (VSSC, VSCC, VSClC) germane to the neuroexcitatory action of pyrethroids. Neurolemma-injected oocytes were also capable of current depolarized, Ca2+-dependent, neurotransmitter release, a hallmark of neurotransmission that operates in intact nerve. Furthermore, deltamethrin (a CS-syndrome pyrethroid) enhanced neurotransmitter release under depolarizing conditions in a stereospecific manner and was substantially blocked by omega-conotoxin MVIIC. Thus, the neurolemma-injected oocyte expression system is a good approach that has the potential to serve as an in vitro model system to examine the direct and comparative actions of pyrethroids and possibly other neurotoxicological agents.

CHARACTERIZATION OF LEISHMANIA USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM

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Salve Regina University Work Study Program

Leishmania are parasitic organisms that can result in visceral and cutaneous leishmaniasis in humans. Visceral leishmaniasis, an enlargement of internal organs is transmitted by L. donovani and cutaneous leishmaniasis, which causes skin sores and lesions, is transmitted by L. major and L. mexicana. In this study, we used AFLP to characterize polymorphisms and differences in gene expression for four different species of Leishmania; L. mexicana, L. donovani, L. tarentolae, L. major. Purified gDNA and cDNA was obtained from each of the four species and subjected to restriction digest and adapter ligation using Mse1 and EcoR1 specific sites. Individual fragments were amplified with multiple successive rounds of PCR using 16 unique EcoR1 and Mse1 primer combinations and analyzed of gel electrophoresis. We identified several primer combinations that resulted in unique amplified fragments for both gDNA and cDNA. These results indicate that that AFLP is a viable technique to assess polymorphic differences in the Leishmania genome and differences in gene expression.

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