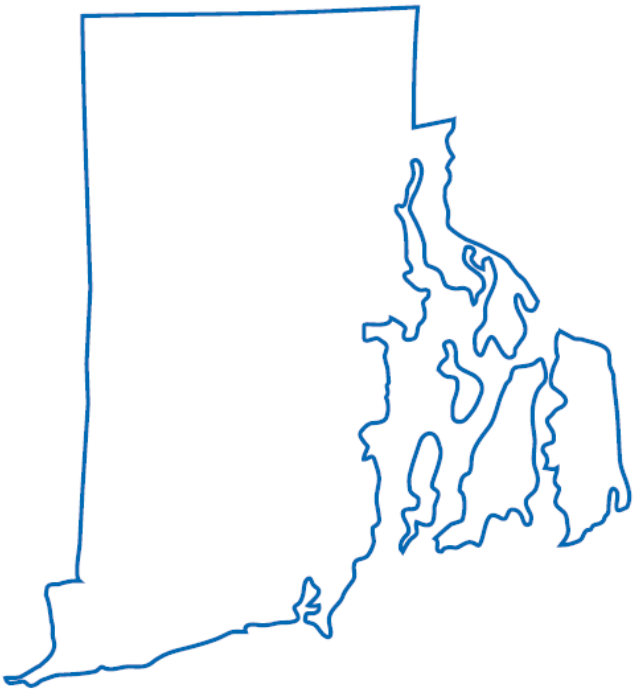
2011 Rhode Island Summer Undergraduate Research Fellowship Conference



Friday, July 29, 2011
8:00 AM

The Ryan Center, University of Rhode Island

Supported by

Rhode Island NSF EPSCoR
Experimental Program to Stimulate Competitive Research

RI-INBRE & RI EPSCoR
SUMMER UNDERGRADUATE RESEARCH FELLOWS (SURF) CONFERENCE

FRIDAY, JULY 29, 2011
THE RYAN CENTER CONCOURSE
UNIVERSITY OF RHODE ISLAND
KINGSTON, RI

8:00 – 9:00 AM	***CONTINENTAL BREAKFAST & SURF GROUP A POSTER SET-UP***
9:00 – 9:15 AM	***WELCOMING REMARKS***
	DR. DAVID DOOLEY, PRESIDENT, UNIVERSITY OF RHODE ISLAND
	DR. ZAHIR SHAIKH, RI-INBRE PRINCIPAL INVESTIGATOR & PROGRAM DIRECTOR, UNIVERSITY OF RHODE ISLAND
	DR. PETER ALFONSO, RI EPSCOR PRINCIPAL INVESTIGATOR & PROJECT DIRECTOR, AND VICE PRESIDENT FOR RESEARCH AND ECONOMIC DEVELOPMENT, UNIVERSITY OF RHODE ISLAND
	MS. CHRISTINE SMITH, INNOVATION PROGRAM MANAGER, RHODE ISLAND SCIENCE & TECHNOLOGY ADVISORY COUNCIL, RHODE ISLAND ECONOMIC DEVELOPMENT CORPORATION
9:15 – 10:45 AM	***SURF POSTER SESSION* - GROUP A**
10:45 – 11:00 AM	***INTERMISSION & SURF GROUP B POSTER SET-UP***
11:00 – 12:30 PM	***SURF POSTER SESSION* - GROUP B**
12:30 PM	***LUNCH***

LIST OF SUMMER RESEARCH FELLOW POSTERS

***Please note that the poster numbers listed in the following tables also correspond with the page numbers in the abstract book. In instances where the Summer Fellow is from an institution other than where the summer research was performed, the Summer Fellow's home institution has also been listed.*

University of Rhode Island

Poster #	Summer Fellow	Mentor
1	Cesar Alejo	Walter Besio
8	Thomas Carbone, Providence College	Angela Slitt
27	Hilary Friedman	Aftab Ahmed
31	Mallory Goding, Salve Regina University	Navindra Seeram
36	Veronica Heard	Clinton Chichester
20	Elisabeth Elmstrom, University of Vermont	Art Gold
24	Megan Ferguson	Graham Forrester
44	Annaliese Jones	Bethany Jenkins
49	Kristina Klara, Brown University	Bongsup Cho
51	Genna Kyriakides, Salve Regina Univeristy	Paul Cohen
54	Victoria Lomas	Keykavous Parang
59	Louis Marchetti	Brenton DeBoef
63	Evan Mello	Geoffrey Bothun
66	Karissa Neira	Roberta King
71	Beatrice Pratt	Geoff Bothun
73	Samy Ramadan, Roger Williams University	Wei Lu
71	Megan Reidy, Providence College	Niall Howlett
78	Justin Schumacher	Daniel Udwary
82	Sarah Showalter, Salve Regina University	David Rowley
85	Madeleine Suits	Mindy Levine

University of Rhode Island (Continued)

Poster #	Summer Fellow	Mentor
74	Shelby Rinehart	Carol Thornber
20, 97	Molly Welsh	Art Gold

Brown University

Poster #	Summer Fellow	Mentor
17, 29, 69	Lauren Pirrman, Salve Regina University	Stephen Sheinkopf
7	Caitlin Brisson	Heather Leslie
35	Ryan Handoko	Jay Tang
58	Anthony Marcello, University of Rhode Island	Wayne Bowen
64	Eshan Mitra	Sarah Delaney
100	Kevin Zheng	Rebecca Page
9	Kristina Carrero, Community College of Rhode Island	Edward Hawrot
33	Mathew Griffin	John Williams

Providence College

Poster #	Summer Fellow	Mentor
26	Moira Farrell	Elisabeth Arévalo
75	Emily Roblee	Nicanor Austriaco
80	Christian Selinksi	
80, 87	Brendan Swan	
4, 21	Brittany Blumenthal	Christopher Bloom
4, 21	Nicole Eslinger	
4, 21	Liz Sokolowski	
70	Paul Poidomani	Joseph DeGiorgis
79	Brianne Scollins	
92	Kristen Tucker	
79	Rylie Walsh	
61	Thomas McDonough	Christopher Laperle
6	Chris Brennan	Brett Pellock
6, 14	Christina D'Agostino	
6, 14	Zachary Sexton	
23	Anne Fast	Jennifer Van Reet
23	Colleen McInnis	

Rhode Island College

Poster #	**Summer Fellow**	**Mentor**
30	Angela Gargano	Karen Almeida
67	Steven Ortiz	
30	Valarie Zabala	
25, 55, 86	Xenia Fernandez	Deborah Britt
25, 55, 86	Alise Lombardo	
25, 55, 86	Catherine Svetcharnik	
32	Melissa Marcotte	Beverly Goldfield
32	Tabitha Newman	
32	Lauren Whittle	
48	Sathiarith Chau	Thomas Malloy
	Lauren Chaunt	
	Jessica Hunter	
	Emilee Ray	
13	Nicole Cote	Rebeka Merson
13	Jessica Fernandes	
40	Katherine Holfelder	
99	Rilwan Yusuff	
16, 89	Jennifer Desjarlais	Robin Montvilo
16, 65, 89	Jacquelyn Morgan	
65, 89	Owen Tidwell	
84	Titilayo Adedeji Campbell	Sarah Spinette
72	Irina Maglysh	
72	Megan Radka	

Rhode Island College (Continued)

Poster #	**Summer Fellow**	**Mentor**
53, 88	Micaela Dunn	Steven Threlkeld
53, 88	Cynthia Gaudet	
53, 88	Jason Lennox	
5	Ursula Brandl	John Williams
28	Christopher Funk	
42	Angela Jacavone	

Roger Williams University

Poster #	**Summer Fellow**	**Mentor**
60	Caroline Martin	Loren Byrne
37	Katelyn Higgins	Avelina Espinosa
56	Tianmeng Luo	
76	Lauren Salerno	
37	Haylee Zubrycki	
15	Diana Denio	Lonnie Guralnick
22	Jesse Farruggella	Dale Leavitt
34	Allison Hall	David Taylor
50	Nicholas Kutil	
52	Garrett LeBlanc	
68	Danial Palance	

Salve Regina University

Poster #	Summer Fellow	Mentor
94	Gina Varuzzo	Sarah Matarese
2	Alexander Antonopoulos	Bernard Munge
47	Gregory Keras	
47	Kara Lombardo	
83	Brian Somba	
17, 29	Rachel Basset	Sheila Quinn
17, 29	Samantha DeMartin	
17,29	Marissa Dickinson	
39	Lana Hoertz	Alison Shakarian
77	Heather Nicholson	
77	Carlos Santos	
3	Wayne Bainter	Steven Symington
3	Justin Gay	
41	Craig Irving	
95	Priscilla Villa	

Bryant University

Poster #	**Summer Fellow**	**Mentor**
57	Hilary Lux	Kirsten Hokeness & Christopher Reid
62	Mike McGovern	
62	Samantha Whitham	
93	Alejandro Vando	Christopher Reid

FLEXIBLE PIN ELECTRODE IMPEDANCE VARIANCE

Cesar Alejo, Walter Besio, *Department of Engineering*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Planar tripolar concentric ring electrodes have been shown to have significantly better signal-to-noise ratio, spatial resolution, and mutual information than conventional disc electrodes. However, planar electrodes suffer from poor skin-to-electrode contact due to scalp contours and hair. Normally impedance matching paste is used to improve the contact. In this experiment we are trying to improve the connection of electrodes to the human scalp. Now we are trying to make a different approach to record the EEG signals by using spring-loaded pin electrodes, these springs allow the pin electrodes to move up and down and adjust to the shape of the human scalp. They also go through the hair like a comb. We are analyzing four different types of pins in the bipolar and tripolar concentric ring electrode configuration for skin-to-electrode impedance and quality of signal. We also test three different skin preparations: (1) no preparation, (2) Nuprep a mild skin abrasive, and (3) Ten20 paste, an impedance matching paste. The skin-to-electrode impedance and brain activity with eyes closed are recorded every 15 minutes for two hours. When the eyes are closed alpha waves are generated. We performed spectral analysis to determine if the alpha waves were present. The research is not completed yet but our preliminary data show that the impedances are beyond the range of the impedance meter without any preparation, within 10 KOhms with Nuprep and Ten20 paste. We also found that the impedance varies with time. From the spectral analysis we were able to determine that the pin electrodes were recording the alpha waves. In conclusion the pin electrodes worked with Nuprep and Ten20 paste. For future work more experiments need to be conducted on multiple people.

LABEL FREE SURFACE PLASMON RESONANCE (SPR)-BASED IMMUNOSENSOR FOR THE DETECTION OF INTERLEUKIN-8 CANCER BIOMARKER IN SERUM

Alexander Antonopoulos, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Methods for measuring protein biomarkers with high sensitivity and ultralow detection limit (DL) promise to provide valuable tools for early diagnosis of diseases such as cancer, and for monitoring therapy and post-surgical recurrence. Surface plasmon resonance (SPR) coupled to nanoparticle-antibody labels for signal amplification in immunoassays is an emerging approach for detecting proteins in biomedical samples. Herein, we report on a rapid label free sensitive SPR-based immunosensor for the detection of Interleukin-8, a cancer biomarker protein in serum. SPR immunoassays involve attaching capture antibodies (Ab1) to an SPR chip and measuring signals after capture of the protein analyte from the sample. The Ab1 concentration was optimized to lower NSB which often controls the sensitivity and DL. This approach provided a DL of 10 pg/mL-1 in 10 uL calf serum samples which compares favorably to the standard hospital ELISA method. Work is in progress to enhance the sensitivity and lower the DL using nanoparticle-antibody labels signal amplification strategy.

PYRETHROID MODULATION OF T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL ISOFORMS

Wayne Bainter, Justin Gay, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroids are synthetic derivatives of the naturally occurring pyrethrins that are widely used in agriculture and residential pest control programs. Human exposure is virtually assured because of their widespread use, but little is known about how these compounds affect human receptors. Previous studies have shown that T-type calcium channels from a variety of organisms are modified by pyrethroids, however, effects vary depending on the pyrethroid used and the channel examined. To better understand this phenomena, we investigated the structural activity relationship of three different but related pyrethroids (deltamethrin, permethrin, and fenpropathrin) on T-type voltage-sensitive calcium channel isoforms expressed in Xenopus oocytes. To do this, cRNA was transcribed from linearized cDNA encoding Cav3.2 and Cav3.3 using the mMessage mMachine T7 ultra in vitro transcription kit. Newly transcribed cRNA was injected into oocytes (0.1 μg/μl and 0.3 μg/μl) and channel expression was verified using two electrode voltage clamping with known inhibitors for Cav3.2 (mibefradil) and for Cav3.3 (nickel). Results indicate that only deltamethrin inhibited Cav3.2 in a concentration-dependent manner while permethrin and fenpropathrin had no effect. In contrast, all pyrethroids examined inhibited Cav3.3 in a concentration-dependent manner. These results suggest a stereospecific interaction of pyrethroids on T-type voltage-sensitive calcium channels that is unique to specific calcium channel isoforms.

A NOVEL ANIMAL MODEL OF NSSI: PAIN TOLERANCE & ENVIRONMENTAL STRESSORS

Brittany Blumenthal, Liz Sokolowski, Nicole Eslinger, Christopher Bloom, *Department of Psychology*, Providence College, Providence, RI; Ryan Bastings, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Non-suicidal self-injury (NSSI) refers to a form of intentional physical self-damage or self-harm that is not accompanied by suicidal intent or ideation.

Recent work reported decreased levels of CSF endogenous opioids in self-injurers. Opioids have long been implicated in the regulation of pain and modify sensitivity in response to stress, fear and physical damage. Changes in pain sensitivity have been reported in some self-injurers making this a potential area of exploration in understanding the role of opioids in NSSI.

The current experiment explores a novel animal model of NSSI for the investigation of the role of endogenous opioids in this phenomenon by quantifying changes in pain sensitivity in rodents exposed to multiple environmental stressors that represent the proposed human experience in NSSI. It has been proposed that self-injury results as a coping mechanism in response to non-painful, environmental stressors. This stress is then followed the painful stress of self-injury which serves an emotional regulation function. The current animal model exposes animals to both types of stressors to see how they interact to alter pain tolerance and the underlying neurochemical mechanisms.

Results indicate that prior exposure to chronic non-painful stressors, alters an organism's response to later exposure to painful stressors. This result is discussed in the context of NSSI and potential neurochemical mechanisms that initiate and potentiate self-injury.

MICROWAVE ASSISTED ORGANIC SYNTHESIS (MAOS) OF NOVEL ARYLPHOSPHONIUM SALTS (APS) AND POLYMERIC BIOCIDES

Ursula Brandl, *Department of Physical Science*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Complications due to nosocomial infections caused by resistant strains of bacteria and biofilm formation are an ever present problem in healthcare institutions. Surfaces made of materials that are toxic to microbes have the potential to reduce the spread of infection. Our research involves synthesizing polymeric biocides that can be used to produce biomedical devices. Previously, our group synthesized novel arylphosphonium salts (APS). APS are bioactive and well known to exhibit antimicrobial activity. The cationic phosphorus of APS causes adhesion to negative charges on the surface of bacterial cells, and lipophilic ligands allow diffusion to the cytoplasmic membrane. Disruption of the cell membrane is believed to cause lysis of the bacterial cell, leading to cell death. Currently, we are quarternizing polymer bound triphenyl phosphine with alpha-bromo-p-toluic acid using microwave assisted organic synthesis (MAOS) to produce APS-toluic acid. Toxicity of APS-toluic acid can be increased by increasing the lipophilicity. We have successfully added an eight carbon alkyl chain to APS-toluic acid via esterification with octanol. Furthermore, any compound containing a hydroxyl functional group can be added to the polymer bound APS-toluic acid. The progress of the reactions was followed by IR spectroscopy and mass increases of the polymer were calculated. Physical properties were determined by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). The final product and intermediates were analyzed using MALDI-MS.

CONSTRUCTION OF A NULL ALLELE OF THE HFQ GENE OF THE DISSIMILATORY METAL REDUCING BACTERIUM SHEWANELLA ONEIDENSIS

Christopher Brennan, Christina D'Agostino, Matthew Goulet, Zachary Sexton, Brian Tjaden, 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, including both soluble and solid heavy metals. Our primary 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. We are particularly interested in those Shewanella sRNAs that may regulate the process of metal reduction.

The highly conserved RNA chaperone protein Hfq, which aids in both sRNA folding and mRNA target recognition, has been widely implicated in bacterial sRNA function. To better understand the mechanisms of sRNA function in Shewanella, we have constructed a putative null allele of the S. oneidensis hfq gene. Consistent with previously described phenotypes of hfq mutants in other bacteria, our preliminary data suggest that the S. oneidensis hfq mutant has a delayed growth phenotype and reduced survival in stationary phase. We are currently further evaluating the growth and survival of the Shewanella hfq null mutant. In addition, we are investigating the mutant's sensitivity to a variety of physical stresses and evaluating the capacity of the hfq mutant to reduce Cr (VI) and Fe(III) under anaerobic conditions.

BOTTOM-UP EFFECTS ON BARNACLE SURVIVAL AND RECRUITMENT ON NORTHEASTERN ROCKY SHORES

Caitlin Brisson, Marcy Cockrell, Heather Leslie, *Department of Ecology & Evolutionary Biology & Center for Environmental Studies*, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

We investigated the combined effects of variation in air temperature and primary productivity on the acorn barnacle, Semibalanus balanoides. Recruitment and survival were measured at four sites nested within each of three estuaries: Casco Bay (ME), Narragansett Bay (RI), and Long Island Sound (CT/NY), for a total of 12 sites. Within each estuary, sites were designated a priori as high or low productivity based on their proximity to urbanized areas. The north to south distribution of estuaries created a natural variation in air temperature. From February 2010 onward, we estimated barnacle survival from marked plots at each site, and measured recruitment via replicate 100 cm2 artificial settlement surfaces.

Barnacle survival and recruitment varied through time and among the estuaries. Recruitment peaked in February in Narragansett Bay and Long Island Sound, and in March in Casco Bay, suggesting an important role of temperature (or other factors varying with latitude). Overall, the magnitude of recruitment in Narragansett Bay > Long Island Sound > Casco Bay in 2011. Based on data from March and May 2011, barnacle recruitment and survival were positively correlated with chl a concentration, a proxy for primary productivity. These findings highlight the considerable spatial and temporal heterogeneity in survival and other proxies of fitness in this species on a regional scale, and highlight the importance of bottom-up effects on rocky shore populations.

EFFECTS OF BISPHENOL A (BPA) AND ESTRADIOL PERINATAL EXPOSURE ON HEPATIC ATP-BINDING CASSETTE (ABC) TRANSPORTER EXPRESSION IN MICE

Thomas Carbone, *Department of Biology*, Providence College, Providence, RI; Ajay Donepudi, Angela Slitt , *Department of Biomedical and Pharmaceuticals Sciences*, University of Rhode Island, Kingston, RI; Cheryl Rosenfeld, *Department of Biomedical Sciences*, University of Missouri, Kansas City, MO

RI-INBRE Summer Undergraduate Research Fellowship Program

BPA is a byproduct in plastic manufacturing. BPA is a known endocrine disruptor that activates estrogen-receptors α/β. Multiple studies in rodents illustrate developmental exposure to BPA results in insulin resistance, adipogensis, and behavioral and physiological changes through multiple mechanisms, including epigenetic modifications. However, none of these studies document how developmental BPA exposure can affect liver function, which is crucial in determining circulating hormone concentrations and chemical detoxification. In rodents, BPA undergoes biotransformation to BPA-glucuronide and is excreted into bile, most likely through the ATP-binding cassette (Abc) transporter, Abcc2. Abcc2 is involved in the of efflux conjugated organic anionic compounds, particularly glucuronide conjugated compounds. Our preliminary studies illustrate that developmental BPA exposure alters drug transporter expression in liver, specifically Abcc2. The study was performed to illuminate the effects of perinatal BPA exposure and compare them to developmental Ethinyl Estradiol (EE) treatment. Female a/a (C57BL/6) mice were exposed to BPA diet (50 μg/kg diet/50 mg/kg diet), EE 0.1ug/kg diet, or AIN93 Control diet from 2 weeks pre-mating with male Avy/a mice. BPA exposure was maintained during breeding and lactation period. Pups were weaned on postnatal day (PND) 21 and maintained on AIN93 control diet. Livers were collected from offspring after PND 135, total RNA was isolated, and Abcc 2-4, Abcg2, and Slc10a1 were analyzed by qPCR. BPA (both concentrations) and EE perinatal exposure decreased the hepatic expression of Abcc2 and Abcg2. BPA decreased Abcc3/4 expression at the dietary concentration of 50 mg/kg, whereas EE elicited a marked decrease in both Abcc3/4. Developmental BPA and EE exposure caused a similar decrease in Abc transporter mRNA at all concentrations. This data suggests that BPA may work via similar pathways as EE greatly affects the expression of key liver transporters involved in BPA excretion, as well as, other conjugated hormones and xenobiotics.

CREATING A PROTOCOL FOR ISOLATING POSTNATAL MOUSE BRAIN TISSUE

Kristina Carrero,Edward Hawrot, *Department of Molecular Pharmacology, Physiology and Biotechnology*, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

There are existing protocols for isolating different areas of the brain for long term studies. However there is not a protocol that enables scientists to culture medial habenula (MHb) cells long term (long term meaning weeks or months). The medial habenula is an area of the brain with an abundance of nicotinic acetylcholine receptors (nAChR's) which allow specific molecules to permeate cell membranes. Cultures provide a look into the activity and functions of neuronal cells, so that we can have a better understanding of the effects of disorders associated with the brain. The MHb has been linked to disorders such as Alzheimer's and schizophrenia as well as many other functions of the central nervous system. MHb tissue was cultured along with the hippocampus and cerebellum as a guideline to help determine the optimal conditions for the MHb. A standard protocol of cerebellum tissue was used as a starting point and was altered throughout the experimentation process. The tissues were exposed to a variety of conditions such as different digestive enzymes, enzyme media, and culture solutions to achieve the best results. Culturing MHb cells for duration of a week was successful, however the there was a lack in abundance. Further experimentation is needed to find the optimal conditions for culturing MHb cells long term.

S-ADENOSYL-L-METHIONINE ENHANCES THE VIABILITY OF THE PROBIOTIC YEAST, SACCHAROMYCES BOULARDII UNDERGOING PROGRAMMED CELL DEATH

Vincent Cascio, Daniel Gittings, Kristen Merloni, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Saccharomyces boulardii yeast is a probiotic agent prescribed to prevent and treat gastrointestinal disorders and combat the antibiotic-associated diarrhea caused by Clostridium difficile infections. To be effective as a probiotic, S. boulardii must maintain viability within the acidic environment of the gastrointestinal system. In this paper we provide evidence that S. boulardii undergoes Programmed Cell death in a manner similar to S. cerevisiae. We also show that S. boulardii survival is greater in a simulated gastric environment as compared to Saccharomyces cerevisiae yeast strains, and may have anti-apoptotic mechanisms unique to HCL-induced death. We provide evidence that S-adenosyl-L-methionine (AdoMet), a commercially available dietary supplement, is effective in enhancing the viability of S. boulardii by preventing PCD. Together, S. boulardii and AdoMet may act as a more effective treatment for gastrointestinal disorders than S. boulardii alone.

MODELING INTERCELLULAR CALCIUM DYNAMICS WITH THE INCORPORATION OF NOISE

Kaitlin Chambers, Lindsay Coates, Sandor Kadar, *Department of Chemistry*, Salve Regina Univrsity, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In cells, calcium ions act as secondary messengers for certain cellular processes, including the release of neurotransmitters, such as dopamine. The signal to release dopamine is sent through the oscillating calcium concentrations within the cytosol. By creating a mathematical model, it is possible to simulate the oscillating intracellular calcium concentration. The model used is the combination of two models, the Cuthbertson-Chay model and the Borghans-Dupont-Goldbeter model, applied for a two-cell configuration, where the first cell will provide a physiologically viable signal for the second cell to give a better representation of the real system. The mathematical model that is validated with experimental data can be leveraged to predict how the cell functions under varying conditions.

Environmental white noise affects chemical processes, which leads to random fluctuations of dynamical variables, such as concentrations, in biological systems. The presence of noise is known to promote signal transmission in biological systems through the mechanism known as Stochastic Resonance (SR). SR occurs when a weak, otherwise undetectable signal is amplified into a detectable signal. The Gaussian-distributed noise with zero average can be incorporated into the model as additive or multiplicative noise, impacting dynamic variables and/or concentrations. By introducing noise to the cytosolic calcium concentration in this model, it is possible to see the effects it will have on the calcium oscillations. The signal strength was measured with a Signal to Noise Ratio of the Fast Fourier Transform of the calcium traces. It was found that noise does amplify the signal that the cytosolic calcium produces. Preliminary comparison of the theoretical results are in line with existing experimental data.

The computational environment is based on MATLAB with Parallel Distributed Server, Systems Biology Toolbox, and the Amazon Elastic Computational Cloud. Upon completion of the task, the results are downloaded for post-processing in MATLAB and Excel.

MODELING INTRACELLULAR CALCIUM DYNAMICS WITH THE INCORPORATION OF NOISE

Lindsay Coates, Kaitlin Chambers, Sandor Kadar, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In cells, calcium ions act as secondary messengers for certain cellular processes, including the release of neurotransmitters, such as dopamine. The signal to release dopamine is sent through the oscillating calcium concentrations within the cytosol. By creating a mathematical model, it is possible to simulate the oscillating intracellular calcium concentration. The model used is the combination of two models, the Cuthbertson-Chay model and the Borghans-Dupont-Goldbeter model applied for a two-cell configuration, where the first cell will provide a physiologically viable signal for the second cell to give a better representation of the real system. The mathematical model, that is validated with experimental data, can be leveraged to predict how the cell functions under varying conditions.

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DIFFERENTIATING THE ROLES OF SKATE AHR1 AND AHR3 IN THE EMBRYONIC STAGES OF THE LITTLE SKATE

Nicole Cote, Jessica Fernandes, Rebeka Merson, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Planar halogenated hydrocarbons (dioxins) and polychlorinated biphenyls are highly toxic byproducts of industrial processes. Evidence shows that exposure to these toxins causes a variety of birth and developmental defects, and many are currently considered probable human carcinogens. The aryl hydrocarbon receptor protein gene, a ligand-specific member of the basic-helix-loop-helix family of transcription factors, mediates toxicity of these compounds. The protein gene appears to have dual physiological roles; however, its role in normal embryonic development is currently unclear. While humans only have one AHR gene, other organisms bear many. The purpose of this study was to use Whole Mount in Situ Hybridization in order to study the temporal and spatial expression of AHR3 and AHR1 in little skate, Leucoraja erinacea. The divergence of AHR function in skates will be able to provide a more accessible model in studying the receptors' many utilities, which appear to be merged in the single human AHR. Embryos representative of different developmental stages (as determined by morphological traits) were chosen and treated with a DIG-labeled probe in order to localize the expression of AHR3. In order to target AHR1, PCR was done to obtain products for probe synthesis and are currently in the process of cloning to ultimately localize the expression of AHR1.

IDENTIFICATION OF SRNA GENES IN THE DISSIMILATORY METAL-REDUCING BACTERIUM SHEWANELLA ONEIDENSIS

Christina D'Agostino, Matthew Goulet, Zachary Sexton, Nicholas Vincent, Brett Pellock, *Department of Biology*, Providence College, Providence, RI; Brian Tjaden, *Department of Computer Science*, Wellesley College,

RI-INBRE Summer Undergraduate Research Fellowship Program

Bacterial small, non-coding RNAs (sRNAs) positively or negatively regulate the expression of other genes in response to changing environmental conditions. We have used a computational approach to predict the existence of 159 sRNA genes in the bacterium Shewanella oneidensis, a member of a class of bacteria known as the dissimilatory metal-reducing bacteria (DMRB). When grown under anaerobic conditions, S. oneidensis can utilize a wide variety of extracellular substrates as terminal electron acceptors, including both solid and soluble heavy metals. Reduction of some soluble heavy metals [e.g. U(VI) and Cr(VI)] converts them into insoluble forms. Thus, it is of interest to explore the mechanisms that control this potentially bioremediative function.

Our goals are to identify the environmental conditions that regulate the functions of the Shewanella sRNA genes and determine how the sRNAs function in bacterial metabolism. Our approaches include constructing sRNA gain-of-function and loss-of-function strains to test hypotheses regarding predicted sRNA target genes and to evaluate the influence of individual sRNAs on metabolic processes. Ongoing research projects in the lab include 1) identifying S. oneidensis sRNA genes that are differentially regulated under conditions permissive for reduction of Fe(III) or Cr(VI), 2) investigating the S. oneidensis sRNA homolog of the Escherichia coli spot42 sRNA gene, and 3) characterizing several S. oneidensis sRNA genes that are strongly expressed during exponential growth in standard, defined medium.

CHARACTERIZATION OF ANTI-OXIDANT ENZYMES IN THE CAM PLANT KALANCHOE DIAGREMONTIANA

Diano Denio, Lonnie Guralnick, *Department of Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Kalanchoe diagremontiana is a CAM plant characterized by net nighttime CO2 uptake, an increased titratable acidity (malate) and stomatal closure during the day time. The malate stored at night is used as a CO2 source during the light periods. Stomatal closure during these light periods results in an increased oxygen concentration in the leaf. The conditions of high light and oxygen result in increased oxygen radical production. This has the potential to decrease photosynthesis by damaging the cell membranes. CAM utilization would increase the cell's need for antioxidant protection which differs from C3 plants which use exogenous CO2 uptake. At night the production of the PEP for CO2 capture causes increased respiration rates. This electron flow may also damage membranes. Previous research with CAM plants has shown that anti-oxidants and their associated enzymes may increase in activity during the imposition of water stress. Our research is being done to study the role of anti-oxidant systems during the circadian rhythm of CAM in this plant and to see if their activity changes during the imposition of water stress. Some preliminary work has shown that the anti-oxidant protective system may show a circadian rhythm in conjunction with its CAM rhythm. PEPCase activity showed a typical CAM rhythm with high activity in Phase I and IV while Gluthtione reductase showed high activity in Phase I. Superoxide dismutase showed highest activity in Phase II and III during the time of stomatal closure during periods when Rubisco activity is low. The results indicate that different enzyme systems may show different rhythms when compared to the CAM circadian rhythm. Further work is being done to study long term drought effects on these systems.

PROBLEMS WITHIN A MULTIMEDIA METHODOLGY: INTERNET BASED ADDICTION COUNSELOR EDUCATION STUDY

Jennifer Desjarlais, Jackie Morgan, Holly Cekala, Robin Montvilo, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In the field of addiction recovery different methods of provider education are utilized. Many behavioral health practitioners, including addiction counselors, are exposed to evidence-based practice through only infrequent experiences with continuing education events, such as forums, seminars or workshops. In order to determine the usefulness of the Internet as an optional tool in continuing education, we implemented an online tutorial using Qarbon's Viewlet 6.0 software. The online tutorial was based on a conference presentation given by Dr. Frank Sparadeo entitled "The Neuropsychology of addiction in adolescence: Changes in the adolescent brain and nervous system brought about chemical dependency." Information on the online tutorial was made available to substance abuse treatment providers via email and U.S. Postal Service. These providers were located both locally and nationally, including Alaska and Hawaii. Due to several factors such as software issues and participant response rates the data collected have been unusable to date. Currently we are seeking alternative ways to implement the same tutorial while eliminating some of the problems encountered. These alternative methods include utilizing new software and targeting a broader range of participants within the substance abuse treatment field. We hope to find appropriate methods for effective online learning, in order to better allow the utilization of the information within the substance abuse treatment field.

DOES THE NUMBER OF DISCRETE TRIAL TEACHING SESSIONS PER DAY INFLUENCE THE PERCENTAGE OF CORRECT RESPONSES?

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Therapy for children with autism is labor intensive and these children have so many therapy goals that time has to be used optimally. Consequently it is important to determine if there is a significant advantage to having a specific number of sessions per day. In other words, does the child's performance continue to improve with each successive session or does it reach a plateau or decrease in quality? The purpose of this study was to examine if there is a significant advantage to increasing Discrete Trial Teaching (DTT) sessions per day? Here advantage is defined as an increase in the percentage of correct responses across sessions.

In this study, we examined the response patterns of five children with autism who participated in an intensive applied behavior analysis program. Each child received three DTT sessions per day devoted to establishing the ability to follow an adult's eye gaze. Each session involved ten discrete trials. The quality of the child's performance was defined as the number of correct responses per session. We examined the daily pattern of responding across sessions attempting to determine if the child's performance tended to improve over the course of the three sessions per day.

We found that although there was a significant difference found among students in terms of their performance ($F(4,167)=13.27$, $p<0.001$), there was still a main effect for the number of sessions per day ($F(2,167)= 3.33$, $p=.038$). There was no interaction found between the number of sessions per day and the participant ($F(4,168)= 0.84$, $p=.50$). A follow-up t-test indicated that the third session of the day was significantly lower than the first and second session implying that one or two sessions per day might be optimal.

MODELING INTRACELLULAR CALCIUM DYNAMICS

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Calcium is an important second messenger in cellular processes such as mitosis, ATP synthesis, oxidative phosphorylation, and cellular signaling. The process modeled in this work is the intracellular calcium concentration oscillations triggering the release of dopamine. To study the dynamics in the calcium oscillations, a combined model comprised of the Cuthbertson-Chay and the Borghans-Dupont-Goldbeter models, was used. The model was then extended to include a second cell which provides a better representation of an actual system as the signal received by the cell would be a natural one, not artificial like the first. The sole purpose of this first cell is to generate a physiologically viable signal for the second cell. Environmental white noise may improve signal transmission in biological systems through the phenomenon of Stochastic Resonance. In the model the noise is applied to the rate that reflects the agonist binding to the cell, which carries the signal to which the dynamics is the most sensitive. This phenomenon happens when a weak signal that would not be able to carry information in the cell becomes a detectable signal in the system.

Analyzing the Signal to Noise Ratio obtained from the Fast Fourier Transformation showed whether the support for calcium signaling were mathematically significant. Noise does amplify a normally not viable signal up to an optimal level after which further increase in noise level corrupts the signal. A comparison of the experimental and theoretical signal strengths and frequency of the cytosolic calcium oscillation can validate the model which in turns improves our understanding of the biological system.

The parallel computing environment is based on MATLAB with the Distributed Computing Server and the Systems Biology Toolbox and the Amazon Elastic Computational Cloud. With this highly scalable computational power we can incorporate more aspects of the dynamics into this model than before.

SIZE DISTRIBUTIONS AND SEX RATIOS OF NEAR-SHORE HOMARUS AMERICANUS POPULATION OF NEWPORT NECK

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Distribution of the American lobster (Homarus americanus) ranges from Labrador, Canada to North Carolina. Lobsters generally inhabit rocky and sandy ocean bottoms from depths ranging from the shoreline to approximately 600 meters deep. American lobsters are particularly abundant in Narragansett Bay of Aquidneck Island and are one of Rhode Island's most important commercial fisheries. However, annual commercial landings and biomass indicate the inshore Rhode Island American lobster resource is in a state of decline due to several suspected factors, such as the North Cape oil spill of 1996, increased predation, pollution, and overfishing. Near shore marine environments are relatively under-sampled by commercial fisherman because of the rocky reefs and navigational challenges and therefore a particularly important nursery. In the summer of 2011 we surveyed distributions of the American lobsters near-shore (<15m) of Newport Neck, Rhode Island and obtained a measurement of size and age for each lobster captured and released. Data analysis demonstrates that there are significant differences in average sizes between some of the sampling locations and a significant male sex bias. The strong sex-biased ratio of relatively young (small-sized) males to females in near shore marine environment suggests that more information is needed on the patchy distribution of egg laying females and protection of coastal environmental to accommodate sea level rise consistent with climate change projections.

BEAVER PONDS AS POTENTIAL SOURCES OF GREENHOUSE GAS EMISSIONS

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Due to laws regulating trapping and a new abundance of forage and habitat, beaver populations have risen significantly over the past century, allowing the animals to rapidly repopulate forested wetlands throughout North America. Along with beavers' repopulation, their dams have caused a new alteration of the landscape's hydrologic regime. Beaver ponds slow water flow and cause organic matter accumulation, creating an anaerobic environment. This new anaerobic nature of the ponds has the potential to alter the flux of greenhouse gases – particularly methane and nitrous oxide. These are both potent greenhouse gases with global warming potentials of 25 and 298, respectively, when compared to carbon dioxide. We hypothesized that beaver ponds represent a hotspot for greenhouse gas emissions that can change the net assessment of greenhouse gas flux within forested landscapes. We collected sediment cores from three different beaver ponds and used mesocosm studies to assess the flux of greenhouse gases. Our focus was to understand i) the range of greenhouse gases in beaver ponds, ii) which greenhouse gas dominated emissions and, finally, iii) the characteristics that controlled the rate and type of greenhouse gas flux. After running samples on a gas chromatograph and completing statistical analysis, wide variation between ponds was found. One of the ponds was found to be a major greenhouse gas contributor. Overall, methane was found to dominate emissions in all three sites. Given the high levels of methane flux, this study strongly suggests the need to further investigate the factors that control methanogenesis in these beaver pond ecosystems.

PHOBIAS: DISCRIMINATED PUNISHMENT VS. AVOIDANCE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Traditionally, phobias have been identified with learned fears of objects that are unusually difficult to eliminate even when the fears are unrealistic. Experimental models of phobia have focused on the avoidance paradigm that can be summarized as indicating that responses that prevent aversive stimuli tend to be repeated. Thus, avoidance conditioning explains the increase in the occurrence of a particular behavior because, in the past, that behavior successfully prevented an anticipated danger. Avoidance models have been heavily criticized for being to far removed from the human phobic situation. It's been asserted that whereas phobics actively avoid a conditioned stimulus, laboratory animals avoid the unconditioned stimulus. Early attempts to train experimental animals to consistently avoid warning have been unsuccessful. This project attempts to address this weakness in animal models of phobia by implementing a discriminated conditioned punishment approach. The discriminated conditioned punishment model argues that phobias are to be identified with learned fear stimuli that are produced by, and thus punish, some otherwise adaptive behavior. The increase in an alternative behavior is not solely because the behavior “avoids” the phobic stimulus, but is due to conditioned punishment of goal seeking behavior, not avoidance conditioning per se.

Animals trained under the discriminated conditioned punishment paradigm have typically switched in response to the first stimulus in the sequence of warning lights, did so on nearly 100% of the trials. Each of these findings have been rarely seen in avoidance models of human phobia and has led many to suggest that human phobias can not truly be captured via animal models. The current experiment answers many of those criticisms and suggests that perhaps animal models of human phobia would be well served to shift the focus from avoidance to one of discriminated punishment.

FARMING WAMPUM: ELUCIDATING THE MECHANISM FOR PIGMENT DEPOSITION IN MERCENARIA MERCENARIA

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Wampum refers to saki beads made from a unique, purple substance – which is also referred to as wampum – found in the shells of the hard clam or quahog, Mercenaria mercenaria. The beads have great cultural significance to the Eastern Woodlands Native American tribes, and they were used as tangible manifestations of a spiritual exchange. Recently, the local Mashpee Wampanoag tribe has expressed interest in shellfish farming, but they only want to grow quahogs that will have heavy pigmentation. In order to address the Tribe's desires, this study seeks to elucidate the currently unknown chemical composition of the pigment, the mechanisms by which quahogs synthesize and deposit wampum in the shell, and the biological role of the wampum; this summer's research aimed to address the biological aspects of wampum. Quahogs were collected from various sites in Rhode Island: the Roger Williams University Mt. Hope Bay shoreline (Bristol); Nanaquaket Pond (Tiverton); The Cove/Old Orchard Cove (Portsmouth); Bissel Cove (North Kingstown); and Sheffield Cove (Jamestown). Sediment samples were also acquired from each site and are pending analysis for determination of major components with inter-site comparison for investigating sediment type influence on pigmentation. The interior of each shell half was photographed, and the images were subsequently analyzed using MatLab (Student Version) with wampum-specific programs (written by Dr. Liese Siemann). The programs evaluate the RGB mean pixel coloration of the shells and, based on color threshold values and user interface, yield percentage assessments of non-pigmented, lightly pigmented, and heavily pigmented shell. Further research will continue to survey wampum deposition in quahogs across various sites as well as revisit the investigation of the chemical characteristics of the pigment to understand its physiological role and the mechanism for stimulating its production.

BUTANOL – MEMBRANE PARTITIONING AS A FUNCTION OF LIPID SATURATION AND CHARGE

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The idea of cheaper and more environmentally friendly biofuels has stimulated a renewed interest in producing biobutanol by, for example, Acetone-Butanol-Ethanol (ABE) fermentation. With recent research showing microorganisms such as Clostridium Pasteurianum, that can utilize biodiesel byproducts such as crude glycerol as a growth medium the process has now been designed to be more cost effective. The only problem left with the process is butanol's toxic effect on the cells at a concentration greater than 20 g/L, causing a leaky cell membrane and eventually apoptosis.

To ferment butanol in the most efficient manor, microorganisms must be genetically engineered to produce a more rigid cellular membrane that can withstand higher concentrations of butanol without fluidizing and becoming leaky. Therefore the goal of this research was to determine how lipid saturation and charge affects the fluidity and butanol partitioning of liposomes used as model cell membranes. To do so, liposomes were made using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG) at ratios of 3:1 and 1:3 DPPC:DOPC and DPPC:DPPG. The method used to make the liposomes was thin film hydration followed by sonication and then extrusion at 200 nm to yield a homogeneous size distribution. The research focused primarily on using Dynamic Light Scattering (DLS) to measure liposome size as a function of increasing butanol concentrations through heating and cooling scans. This along with further research will eventually lead to a new genetically engineered cell membrane that can withstand much higher butanol concentrations than that of the current microorganisms. When comparing the results with other research DLS proved to be a very useful tool in determining the partitioning coefficient of butanol. The results showed strong evidence that a higher unsaturated to saturated lipid ratio (3:1) significantly reduces the fluidizing effects of butanol.

EFFECTS OF SIZE ON THE SURVIVAL AND GROWTH OF ACROPORA PALMATA FRAGMENTS TRANSPLANTED FOR RESTORATION

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RI EPSCoR Summer Undergraduate Research Fellowship Program

In response to the decline of Acropora spp. in many Caribbean reefs over the past two decades, restoration efforts have been made to bring back these beautiful corals that formerly thrived in shallow areas. Scientific studies on transplanting methods are important for determining the best way to restore corals. Our study focuses on Acropora palmata, known as the elkhorn coral because of its branching morphology. Branches often break during storms and the resulting fragments can be moved to new sites and reattached to the reef for restoration. We wanted to determine whether the size of fragments affects the success of transplanting, so we analyzed the growth and survival of 459 fragments, ranging in size from 4-1200 cm2, used for a restoration project in the British Virgin Islands. We found that fragments suffer high mortality in the first year after transplanting, and that the risk of mortality declines with increasing fragment size. Although larger fragments experience lower mortality, the growth rate of survivors is highly variable and unrelated to their initial size. When time and resources are limited, practitioners should select larger coral fragments for restoration where possible.

OVEREXPRESSION OF BCP1 IN SELECT KNOCKOUT STRAINS INVOLVED IN DNA DAMAGE REPAIR

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RI-INBRE Summer Undergraduate Research Fellowship Program

The ability to repair DNA damage is essential for maintenance of genomic integrity. Many proteins are involved in this pathway, from transcription factors to kinases. Any defect in the pathway leads to a decrease in the ability to repair DNA damage. As a result, there is an increase in mutation, sensitivity to damaging agents, and cell death. In humans, the protein BCCIP is a tumor suppressor that is involved in cell cycle regulation, DNA repair, and cytokinesis. Its fungal homolog in Saccharomyces cerevisiae, Bcp1, is an essential protein. However, there is not much known about its function or interactions in the cell. Learning more about Bcp1 would shed more light on its human homolog. To determine if there is any genetic interaction between genes in the DNA damage pathway and Bcp1, it was overexpressed in the yeast knockout strains Pph3, Rad9, and Sgs1. Although Bcp1 was successfully overexpressed in these strains, there was not any significant difference in growth rates or cell morphology. Focusing on the transformed ACX-Pph3 strain, there was also no difference compared to its parental knockout in terms of anaerobic growth or response to osmotic stress. Sensitivity to DNA damaging agents is currently being determined. Future studies will look into genetic interactions between proteins of a different pathway, such as the cell cycle pathway.

WHO IS THE NEXT IN LINE TO THE THRONE?

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Social ants, bees and wasps of the order Hymenoptera are comprised of two female castes, workers and queens. Sociality in these groups varies from highly eusocial, where queens and workers are born with a predetermined morphology; to primitively eusocial, where there is no morphological distinction and every member of the colony is reproductively capable. Hymenopteran insects are characterized by a haplodiploid sex determination system where females are diploid and males are haploid. This type of reproductive system could cause levels of relatedness to be significantly higher than in the traditional diploid-diploid system. Consequently, the range of relatedness triggers conflicts of interests among the castes (ie. who gets to reproduce, sex allocation and division of labor). Polistes, an example of primitively eusocial wasps represents an interesting case of how those genetic conflicts are ultimately solved. The purpose of our study is to determine the role of relatedness in the decision of queen succession in the event that the queen is no longer in the nest. In order to answer this question, we collected nests of Polistes erythrocephalus, from the tropics, from which we experimentally removed the queen. We used two different laboratory techniques, molecular markers and microscopy. The genetic markers helped us to obtain the genotypes of all colony members and to assign maternities. Stereomicroscopy allowed us to assess ovarian development of queens and workers, and the confocal microscopy allowed us to establish the presence of sperm in queens. With the knowledge of within colony genotypes and the state of reproductive development of colony mates, we will be able to answer if close relatives are more likely to take the queen's throne when she dies.

AMINO ACID SEQUENCE OF SNAKE HEMOGLOBIN BII CHAIN FROM SINDHI KRAIT (BUNGARUS SINDANUS SINDANUS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Hemoglobin is a respiratory protein present in the erythrocytes of all vertebrates. Typically, it consists of two identical pairs of α and β globin chains. Our investigation on snake Sindhi Krait hemoglobin is being conducted with the intent to characterize and better understand the interspecies relationships among various snakes at the molecular level. The snake is part of the family Elapidae and is further classified into two species: Common Krait (Bungarus caeruleus) and Sindhi Krait (Bungarus sindanus sindanus). It is further characterized into the subspecies Northern Punjab Krait (Bungarus sindanus razai) from Pakistan. The hemoglobin was isolated from the washed RBCs with physiological saline and the globin was further isolated by treatment in cold acidified acetone. The globin chains were separated by reversed-phase HPLC. The corresponding βII globin chain was oxidized and digested with TPCK treated trypsin. Peptides were separated by RP-HPLC, and homogeneity of the peptides was checked by MALDI-TOF mass spectrometry. The amino acid sequence of βII chain was deduced by Edman degradation of the globin chain and of the purified tryptic peptides in an automated protein sequencer. The primary structure of the βII chain of the Sindhi Krait was aligned to the only reported Elapidae Indian Cobra (Naja naja) βII chain, and it was found to be highly conserved.

GRAFTING ARYL PHOSPHONIUM SALTS ONTO COTTON AND POLYMER-BOUND PHENOL VIA MICROWAVE-DRIVEN ESTERIFICATION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Previous studies have shown that aryl phosphonium salts (APS) are a class of lipophilic cations that have antibiotic properties. With the growing antibacterial biomaterial industry, and the subsequent prevalence of resistant biofilm formation on in vivo prosthetics and tubing, new polymers for medical devices that can suppress biofilm formation are in high demand. The end goal of this research is to develop an efficient and high yield technique to esterify APS onto cotton and polymers using microwave accelerated organic synthesis (MAOS) and test the toxicity of various the various APS analogs with the streak plate method. Production of the APS is a relatively simple process involving the MAOS of α-bromo-p-toluic acid and triphenylphosphine (TPP) or on of its analogs in a solution of DMF. IR Spectra show a strong C=O peak at ~1700cm-1(COOH) and C-O peak at ~1100cm-1(OH) indicating successful formation of salt. The grafting of APS onto cotton or polymers has been problematic with initial experimentation utilizing thionyl chloride (TC) to form an acyl chloride on the salt and reacting with MAOS to attach to cotton. The high reactivity of the TC led to the formation of HCl and degradation of the cotton. Pyridine was implemented to push the reaction forward and prevent excess hydrogen from forming acid, however, IR spectra showed no signs of esterification despite further refining of the technique. It was then decided to esterify the cotton before forming the salt by reacting 4-bromomethyl benzoyl bromide directly with the cotton in a DMF/Pyridine solution using MAOS. Preliminary results were excellent, with IR peaks at ~1715cm-1 showing esterification, however, 4-bromomethyl benzoyl bromide is extremely reactive to moisture, and slight degradation of this reactant has shown to inhibit ester formation during MAOS. Handling techniques have been refined and future studies into the APS analogs are underway.

DOES THE TIME OF DAY INFLUENCE THE QUALITY OF PERFORMANCE OF CHILDREN WITH AUTISM DURING A DISCRETE TRIAL TEACHING SESSION?

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Therapy for children with autism is labor intensive and these children have so many therapy goals that time has to be used optimally. Consequently it is important to determine if there is a significant advantage to running a session at a particular time of the day. In other words, does the child's performance depend on the time of day in which the session is being performed? The purpose of this study was to examine if there is a significant advantage to performing a Discrete Trial Teaching (DTT) session at a specific time of day. Here advantage is defined as an increase in the percentage of correct responses across sessions.

In this study, we examined the response patterns of thirteen children with autism who participated in an intensive applied behavior analysis program. Each child received three DTT sessions per day devoted to establishing the ability to follow an adult's eye gaze. Each session involved ten discrete trials. The quality of the child's performance was defined as the number of correct responses per session. We examined the daily pattern of responding across sessions attempting to determine if the child's performance tended to change throughout the day. For this analysis, the times of day were divided into three categories: 1= 9:00-10:59, 2= 11:00-12:59, 3= 1:00-3:00.

We found a significant difference among the students ($F(12,925)=5.239$, $p<0.001$) but not for the time of day. A follow-up t-test indicated that there was no significant difference between the three sections of divided time ($t(597)=0.556$, $p=.05784$),($t(450)=0.5664$, $p=0.5714$),($t(394)=1.1203$, $p=0.2633$). This implies that the time of the day does not seem to influence the outcome of the session.

BLOOM SYNDROME PROTEIN INTERACTIONS

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RI-INBRE & RI EPSCoR Summer Undergraduate Research Fellowship Programs

Background: A predisposition to cancer is a characteristic of a rare genetic disorder called Bloom Syndrome. Bloom Syndrome is caused by a mutation in the Bloom Syndrome Protein (BLM) gene, a member of the RecQ helicases. The RecQ helicases are highly conserved enzymes that unwind abherrant DNA in the 3' to 5' direction. BLM protein is thought to operate in the homologous recombination (HR) repair pathway. HR repairs DNA double strand breaks in G2 or S phase as well as restoring a collapsed replication fork. Protein interactions have been shown between NBN and Fen1, two proteins involved in DNA processing. Methods: Protein expression plasmids corresponding to the Proquest Yeast Two-Hybrid System from Invitrogen were generated. Previously recombinant BLM fragments were shown to self activate in the Y2H system when used as the bait protein, therefore NBN and Fen1 cDNA were fused to the GAL4 DNA binding domain and fragmented BLM DNA was fused to the corresponding activation domain (prey protein). Proteins were analyzed by several different screening methods of Y2H, Results: The kit controls were successfully screened using the Y2H method. Fen1 and NBN bait tests for self-activation showed each to be suitable bait protein, as neither activated expression of the reporter when cotransformed with the prey empty vector. The experimental screen, however, reported no interaction between the BLM fragments and either Fen1 or NBN. Conclusion: The interactions between BLM and NBN or Fen1 may be too weak to be screened by Y2H system, literature reports for both proteins used co-immunoprecipitation analysis. Additionally, the BLM fragments as prey protein may be problematic if their interaction with DNA is stronger than their interaction with partner proteins, effectively diluting the available prey protein to promote cell growth.

ISOLATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS FROM RED MAPLE (ACER RUBRUM) LEAVES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Plants from the maple genus (Acer) have been widely used in traditional systems of medicines. The Red maple (A. rubrum) species is native to North America and widely regarded for its sap which is used for the production of maple syrup. There have been previous phytochemical investigations into the constituents of maple syrup but limited data is available about its leaves. In the current study, a methanolic extract of A. rubrum leaves was subjected to a series of chromatographic isolation procedures including MCI resin, C-18 medium pressure liquid chromatography (MPLC) and LH-20 column chromatography, as well as semi-preparative and analytical high performance liquid chromatography (HPLC). Chemical structures of three isolates were established by their nuclear magnetic resonance (1H-NMR and 13C-NMR) and mass spectroscopy (MS) data and identified as ginnalin A (1), ginnalin B (2) and ginnalin C (3). Further work will be done to assess the bioactivity of these compounds.

EARLY COMPREHENSION OF NOUNS AND VERBS

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RI-INBRE Summer Undergraduate Research Fellowship Program

English-speaking children produce nouns earlier and more frequently than verbs. These production data suggest that learning words for actions may be cognitively more complex than learning words for objects. Comprehension of nouns and verbs, however, has been more difficult to test in infants and toddlers, who are unable or unwilling to point, choose objects, or act out commands.

This study compares two measures of word comprehension that circumvent the limited behavioral repertoire of young children: parent report and the preferential looking task (PLT) with eye tracking assessment. Participants were children 14 to 18 months of age. Parents completed a standardized vocabulary checklist. During the PLT, children were seated in front of an eye tracker monitor. Half of the children were tested for comprehension of 12 nouns and half for comprehension of 12 verbs. During the noun condition, children viewed color photos of two objects (e.g., hat /bowl) before (baseline) and after (test) one of the images was labeled. During the verb condition, children viewed video recordings of two actions (e.g., throw/bite), performed by actors before and after one action was labeled. Children's visual attention to the two images during baseline and test trials was recorded by the eye tracker monitor. An increase in visual attention to the labeled object or action during the test trial indicates comprehension.

Data analysis indicates a moderately strong, statistically significant relationship ($r = .61$, $p = .02$) between children's comprehension on the PLT and parent report for the 12 nouns and verbs. Parents who reported that their children comprehended more of the 12 target nouns or verbs had children with higher visual attention to those target words during test trials on the PLT. However, when parent report and PLT measures differed, parents tended to overestimate children's comprehension of nouns and underestimate their comprehension of verbs.

ALTERNATIVE DNA STRUCTURES FORMED BY DNA RENATURATION IN PET-RP1B PLASMIDS CONTAINING A TRINUCLEOTIDE REPEAT INSERT

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Trinucleotide repeat regions of the human genome have been identified as the source of Huntington's disease, Fragile X syndrome, and additional human disease. In the process of causing illness, trinucleotide repeat regions of DNA have been found to pathologically expand in the genome, ultimately resulting in grossly aberrant proteins that cause cell death. One hypothesis is that that genome replication enables short regions of single stranded DNA to form, and these are able to form stable, alternative DNA structures that interfere with appropriate DNA replication. This hypothesis has resulted in research demonstrating the occurrence of alternative DNA structures inside trinucleotide repeat inserts of a pUC19 plasmid vector after DNA renaturation. In an attempt to expand this research, we have created variations of the pET-RP1B plasmid vector containing a trinucleotide repeat insert and subjected these plasmids to renaturation conditions.

MERCURY IN THE SEDIMENTS OF THE NARRAGANSETT BAY ESTUARY (RHODE ISLAND, USA): CONTAMINATION FROM A HISTORICAL AND SPATIAL PERSPECTIVE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Estuarine and marine sediments are repositories for heavy metal contaminants, including mercury (Hg), and thus provide a historical record of contaminant inputs into aquatic ecosystems. This study presents stratigraphic profiles of total Hg concentrations measured in sediment cores collected from the Narragansett Bay (Rhode Island, USA). From June 2008 to November 2010, sediments were collected at 12 sites across an anthropogenic gradient in the Bay using a push piston corer (maximum depth = 0.6-1.4 m). Sediment sub-samples were taken continuously at 2-cm increments from each core and analyzed for total Hg content (ppm dry weight) using automated combustion atomic absorption spectroscopy. Irrespective of spatial location in the Bay, sediment depth profiles indicate total Hg concentrations were low in the deeper portions of the cores (mean Hg = 0.013 ± 0.12 ppm), which coincides with the pre-industrial time period. Conversely, each core demonstrated a mid-depth maximum in Hg content (mean Hg = 1.52 ± 1.82 ppm) that presumably resulted from contaminant inputs that began during the industrial revolution of the mid-1800s and persisted into the mid-1900s. Finally, the initiation of the Clean Water Act in 1972 is apparent in cores, as total Hg concentrations decreased in the surface samples (mean Hg = 0.60 ± 0.04 ppm). Sediment total Hg concentrations also varied spatially throughout the Bay, with concentrations greatest in the upper reaches of the estuary and decreasing southward to Rhode Island Sound (range Hg = 0.26-6.59 ppm). Sediment Hg concentrations were particularly elevated in the developed regions of the Bay, including the Providence and Seekonk Rivers, hence suggesting that these areas are the initial centers of industrial growth and the dominant sources of historical and recent contaminant inputs.

NEUTROPHIL STRENGTH: HOW STIFF A SUBSTRATE CAN A NEUTROPHIL DEFORM?

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As the initiators of the immune system's inflammatory response, neutrophils must exudate the vascular system and traverse interstitial tissue to reach sites of injury or infection. Interstitial tissue in humans ranges in elasticity from less than 1 kPa in the brain to greater than 100 kPa in bones, where kPa are units of Young's modulus. Previous work has demonstrated morphological and chemotactic differences between neutrophils on substrates of different stiffnesses. We study the relationship between substrate stiffness and substrate deformations caused by neutrophil chemokinesis. Experiments were done in vitro using the chemotactic agent fMLP to induce neutrophil chemokinesis on polyacrylamide gels that were coated with the extracellular matrix protein fibronectin. The extent to which neutrophils deform their substrates was measured using traction force microscopy. In particular, we tracked the displacement of fluorescent beads on the gel surface. The relationship between average deformation and substrate stiffness was determined. Deformation did not depend on stiffness for the softer gels (10-40 kPa), but decreased to zero on stiffer gels (above 70 kPa), indicating an upper limit on the traction force a neutrophil can apply to its substrate. Since disease and inflammation have been shown to substantially change tissue stiffness, these results have implications for understanding neutrophil activity in both healthy and pathological tissues.

SICKLE CELL DISEASE: THE EXPLORATION OF A GENETIC DISORDER

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Sickle cell disease (SCD) is an inherited homozygous hemolytic anemia where RBC's block the blood flow, causing pain and/or organ damage. 1 in 12 African Americans have the trait and 1 in 400 have the disease.

At 4 months of age, symptoms initiate and include: jaundice, dizziness, coldness in the hands and feet. Sickle cell crisis is characterized by acute pain, however chronic pain occurs as well. Both pulmonary hypertension and acute chest syndrome develop as the disease progresses. This project involved the development of two teaching modules of SCD using high fidelity medical simulators. In the first scenario, a METI pediatric simulator is used to illustrate a 5-year-old child experiencing pain from a vaso-occlusive episode. This is a lesson in how to deal with the pain associated with the disease and reduce the risk of the disease progression. The second patient was a 20 year old with acute chest syndrome and acute respiratory distress syndrome as a result of SCD progression. The METI adult HPS was programmed for this simulation. This was a complex scenario where the patient had fallen ice-skating 6 days prior to admittance to the hospital. She developed severe vaso-occlusion with an unfavorable outcome. Upon admittance into the hospital her vitals were: Temp 96.3°F, HR 130 bpm, BP 155/93mmHg, RR 24, and O2 SAT 76%. These vital signs are typical of end stage of the disease and are challenging for students to interpret.

I chose to research sickle cell disease because I have the trait and there is a possibility that I could pass it on to any child I may have. This project gave me the opportunity to expand my knowledge base about the disease while giving me the opportunity to pass this knowledge on to other students that may utilize these sickle cell scenarios.

KIN DISCRIMINATION AND AGGREGATIVE PATTERNS AMONG ENTAMOEBA VARIETIES

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Kin recognition helps the evolution of cooperation in animals, but its relevance in microorganisms and their behavior toward relatives remains unclear. The Entamoeba lineage constitutes an ideal model to determine the behavioral and signaling cues needed for kin preference. Chemical cues have been reported for E. histolytica but not E. dispar, suggesting cell-communication between con-specifics and behavioral differences with relevance for disease. Entamoeba varieties have been reported as 'morphologically undistinguishable'. Two strains of E. invadens have been isolated from different hosts (VK-1: NS - lizards and IP1-snakes) but were classified within the same clade. Trophozoites of each strain aggregate only with members of their own variety suggesting they are able to associate based on behavioral and chemical communication. Adaptations to different environments and horizontal gene exchange could have influenced diversification of each lineage. Measurable aggregation and behavioral cues in fluorescence micrographs of Entamoeba varieties suggest that these characteristics should be included in phylogenetic studies.

DISTRIBUTION AND ABUNDANCE OF NON-NATIVE CRABS ALONG NEWPORT NECK

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RI EPSCoR Summer Undergraduate Research Fellowship Program

An invasive species is a non-indigenous plant or animal that inhabits areas becoming the most dominant species. Their dominance has the potential to affect the ecosystem, environmentally, ecologically, and economically. This biotic disturbance can negatively affect native species through competition for food, space, and/or limiting nutrients, or through predation or parasitism. The crabs native to Rhode Island include the blue crab (Callinectes sapidus), spider crab (Pyromaia tuberculata), rock crab (Cancer productus), and long-clawed hermit crab (Pagurus longicarpus), are becoming less common along Newport Neck. Asian shore crab (Hemigrapsus sanguineus) is far more abundant in many locations in comparison to the native crabs. In the summer of 2011, we sampled various sites along Newport Neck. This sampling included identifying and counting species of crabs to determine their abundance at each location. The invasive Asian shore crab was one invasive species found at all locations. The invasive green crab (Carcinus maenas) was found in a few locations where the Asian shore was found but not at all locations. The native species are significantly more common at Green Bridge site 7 and Green Bridge site 8 than the invasive Asian shore and green crab. The invasive Asian shore and green crabs were significantly more common at Second Tunnel site 4 than the native species. Climate change is thought to be one reason why certain species are disappearing and others are appearing. Climate change may further impact the abundance and the diversity of native species while forcing invasive species to relocate and dominate their non-indigenous habitat.

CHARACTERIZATION OF THE EFFECT THAT VARIOUS METAL IONS HAVE ON THE ENZYMATIC ACTIVITY OF THE SECRETORY LIPASE FROM LEISHMANIA DONOVANI

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RI-INBRRE Summer Undergraduate Research Fellowship Program

Leishmania donovani, a protozoan parasite, is the causative agent of the often fatal disease visceral leishmanisis. Lipases are enzymes that have been shown to aid in the growth, development, and virulence of several pathogenic organisms such as Candida albicans, Mycobacterium tuberculosis, and Staphylococcus warneri; however, not much information is known about the role of lipases in Leishmania species. Applying what is known about lipases to Leishmania, we can hypothesize that this enzyme may play a large role in their survival within the human host as well as the pathogenesis of the parasite. The goal of this study was to determine if metal ions affected the enzymatic activity of a purified episomally expressed HA-tagged lipase (LdLip3) produced and secreted by transfected L. donovani cells. The eluted fractions were tested for enzyme activity by performing assays with McIlvaine's buffer pH 4-8 at 26°C, 37°C, and 42°C using 4-methylumbelliferyl (4-MU) palmitate as a substrate. Our results showed that there was an average 33X increase in enzymatic activity when comparing the purified protein to the 1x supernatant samples from L. donovani tranfectants. Metal ions such as Mg+2 and Co+2 are known to be cofactors of some enzymes, greatly affecting their activity; therefore, in the current study several metal ions were tested to determine their effect on the activity of purified LdLip3. Results showed that ZnSO4, KCl, NaCl, MgCl2, and CoCl2 all had variable effects on the enzymatic activity, whereas the addition of MnCl2 produced a strong inhibitory effect. In continuing studies, a chelating agent (EDTA) is being used to determine if the effect of these metal ions on enzyme activity, whether activating or inhibitory, can be reversed. Taken together, our results support the idea that metal ions in general have a great effect on the activity of the secreted LdLip3 from Leishmania donovani.

DO RHESUS MONKEYS HAVE ARYL HYDROCARBON RECEPTOR 2?

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RI-INBRE Summer Undergraduate Research Fellowship Program

Aryl hydrocarbon receptor (AHR) proteins regulate the expression of certain genes in animals exposed to toxic chemicals such as polychlorinated biphenyls. Many fish, such as zebrafish (Danio rerio), have multiple AHR genes, but humans, mice, and rats are known to have only one. Scientists once thought that all mammals had only one AHR gene, but some mammals, such as cattle, have a predicted AHR2-like protein-encoding gene, which is identified in the genome. The ultimate goal of this work is to determine if Rhesus monkeys (Macaca mulatta) have the second AHR gene, AHR2. We spent some time browsing genomes online through ensembl.org and ncbi.nlm.gov, looking for synteny among primate genomes. I grew Rhesus monkey kidney epithelial cells (MK2) in culture, isolated ribonucleic acid (RNA) from them, and used it for reverse transcription and polymerase chain reaction, with primers that we designed using a predicted AHR2-like gene sequence. I performed agarose gel electrophoresis with the products and isolated the deoxyribonucleic acid (DNA) from the bands that were consistent with the desired product mass. I prepared these DNA products for sequencing this week. Part of human chromosome 21 and part of Rhesus monkey chromosome 3 are homologous. The predicted AHR2 gene is expected to be next to the PRMT2 gene on chromosome 3 in Rhesus monkeys. PRMT2 is found at the end of human chromosome 21. If an AHR2 gene is confirmed, that means that some primates do have AHR2, even though humans do not. Apparently, a human ancestor lost the AHR2 gene at some point. The next step might be to analyze DNA from cells from other primates to assess their phylogenetic relationships.

CO-EXPRESSION OF A1 AND B3 SUBUNITS OF THE HUMAN N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL (CAV2.2) INTO XENOPUS LAEVIS OOCYTES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Voltage-sensitive calcium channels are involved with a plethora of physiological functions and serve as receptors for a variety of pharmaceuticals and environmental toxicants. The N-type voltage-sensitive calcium channel (Cav2.2) is highly expressed in the presynaptic nerve terminal and regulates Ca2+-dependent neurotransmitter release. Expression of this channel in heterologous expression systems like, Xenopus laevis oocytes, allow for the elucidation of channel gating characteristics that give rise to its physiological function. In oocytes, the expression of the human Cav2.2 is dependant on both the pore forming α1 and the β3 regulatory subunits. The goal of this research was to establish the experimental conditions to express human Cav2.2 into Xenopus laevis oocytes. Individual plasmids containing either the human α1 and β3 subunits were transformed into E. coli and the resulting plasmids purified using a Qiagen Midi Kit followed by ethanol precipitation. Individual clones were verified in a series of digestion reactions using unique restriction enzymes. Validated plasmid cDNAs for α1 and β3 subunits were then successfully linearized and used as a template to synthesize cRNA with the mMESSAGE mMACHINE T7 Ultra Kit in vitro transcription kit. Synthesized cRNA was then injected into Xenopus oocytes at a concentration of 2.5 ng/cell for α1 and 1.2 5ng/cell for β3 and incubated for 3-5 days. Expression of Cav2.2 α1 and β3 subunits in oocytes was confirmed using two-electrode voltage clamping. Preliminary experiments indicate that this channel was blocked with ω-conotoxin-GVIA, a specific N-type voltage-sensitive calcium channel blocker. Successful expression of human Cav2.2 in Xenopus laevis oocytes will allow for the assessment of the effect of a variety of pharmaceuticals and environmental toxicants on the current characteristics of this channel.

CRYSTALLIZATION OF DNA-SMALL MOLECULE COMPLEXES

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Previous work in the laboratories of Dr. Karen Almeida and Dr. John Williams has shown that arylphosphonium salts (APS) interact with DNA. This data includes thermodynamics from isothermal titration calorimetry (ITC), melting curve shifts, electrophoresis, and the blocking of DNA amplification in vitro. Previous studies have shown that APS has both antibacterial and anticancer activity in culture and in vivo. Both properties show the same SAR's, and the APS/DNA interactions strongly correlate with bacterial toxicity. While these interactions between DNA and arylphosphonium salts have been demonstrated, the relative mechanism to which the end result is achieved remains unknown. In order to further study the specific reactions of APS with DNA, crystalline structures have been isolated from DNA and APS solutions using the hanging drop method for analysis via x-ray crystallography. Experiments were run with both genomic DNA from salmon testis and "designer" DNA with the specific dodecomer sequence 5'-CGCAAATTTGCG . The DNA was placed in a homogenous mixture with APS and various other solutes from the Sigma crystallization kit for DNA. 443 experiments were conducted and many yielded crystals. Crystals were isolated and collected using solute surface tension within cryo-loops, and flash frozen before storage in -80°C. The X-ray crystallography of the DNA-APS complexes will reveal structures that will provide insight into the interactions and APS in vivo. Further studies of the various APS analogs are already underway, and more information on the effects of various functional groups with alternate stereochemistry will become available.

DISTRIBUTION OF THE COMMON EIDER IN RELATION TO SUBSTRATE-SPECIFIC INVERTEBRATE POPULATIONS ALONG AQUIDNECK ISLAND'S NEWPORT NECK

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Aquidneck Island's Newport Neck hosts wintering migratory seabird, sea duck, and shorebird species that forage upon intertidal and near-shore food resources. Invertebrate fauna constitute the bulk diet of the Common Eider with Blue Mussels (Mytilus edulis) meeting their major nutritional and energetic requirements. This investigation explores relationships between distributions of Common Eiders along Newport Neck and the mussels upon which they feed. Sampling sites were established along Newport Neck and used both for shorebird and invertebrate observations. Shorebirds were surveyed weekly along the 3.5 mile cliff walk covering six sample sites, during low tide (+/- 2 hr) and during favorable weather conditions when foraging was likely to be most active. Blue Mussels were surveyed using 0.5 m² quadrats placed at six 2-meter intervals along the waterline and mussels were sized as small (2-10mm), medium (10-25mm) or large (>25mm). Substrate percentages were surveyed using 0.5 m² quadrats placed at ten 2-meter intervals along the low-tide, mid-tide, and high-tide lines (n=30 per location). Substrate percentages were assigned as either mud, sand, gravel, cobble, small boulder, large boulder, bed rock, concrete, or other manmade . During winter surveys (October 2010 – March 2011), Common Eider abundance was greatest at Site 5 (Ledge Road). Quadrat surveys in summer 2011 revealed that Blue Mussel abundance is greatest at Site 6 (Reject's Beach). The Land's End section of Newport Neck (sections 5 and 6) appear to have the highest abundance of this avian shellfish predator and their prey, Blue Mussel. Mussel abundance is consistent with substrate composition. While additional sampling is required to establish more complete invertebrate and avian profiles for each site, the current study suggests that Eiders forage most heavily where prey is more abundant.

EVALUATING NITROGENASE GENE DIVERSITY AND EXPRESSION IN MARINE HYPOXIC ENVIRONMENTS

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Microbial communities responsible for N fixation in marine sediments are extremely diverse making it difficult to identify functional community members and controls on their activity. It has previously been documented that hypoxia may act to drive N fixation in the non-N limited sediments of Greenwich Bay, RI USA (S. Brown, unpublished data). Bay sediments were shown to exhibit a seasonal switch in N cycling with high rates of net N2 fixation, this shift appears to correlate with seasonal nutrient loading and resultant periods of hypoxia. The purpose of this study was to acquire additional data supporting hypoxia as a drive for N2 fixation in non-N limited sediments and to examine the relative diversity of active N fixing organisms during periods of hypoxia and non-hypoxia. To target microbes that are most likely responsible for the increase in N-fixation we followed expression of the functional nitrogenase gene, nifH. We identified sequences from organisms that express the nifH gene in bay sediments as relatives of Pelobacter carbinolicus and Desulfovibrio vulgaris, anaerobes which reduce sulfur and sulfate compounds, respectively. To determine the impact of changing oxygen profiles on these important anaerobic N-fixers we followed the microbes related to sulfur reducers and have detected the highest expression of nifH during hypoxia in the N rich surface sediments near a wastewater treatment plant. We hypothesize that N fixation in these bacteria is not sensitive to combined N and that the evolution of H2S from the reduction of sulfur inhibits nitrification in the coupled nitrification-denitrification pathway. This feedback thus expands the niche for sulfur and sulfate reducers to thrive.

Although evidence is supporting of hypoxia as a drive for N fixation, the N fixing capabilities of these organisms are still not entirely understood and require further investigation to elucidate specific controls on their activity.

TESTING THE OPTIMAL NUTRIENT RANGE ON SPINACH GROWTH RATE IN HYDROPONIC SYSTEMS

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Salve Regina University Sustainability Fellowship

Agricultural production is limited due to critical issues including land loss, global warming, land deterioration, depletion of limited natural resources, and increasing populations. These issues along with human health concerns and food security address the need to develop alternate methods to find a secure source of healthy and resourceful food production. Hydroponics is the growth of plants without soil, and is beneficial by reducing pesticides use, fertilizer runoff, and providing greater crop density and growth season. In replacement of soil hydroponics uses water and nutrient additives. The future of hydroponics requires knowledge and application of efficient growth to maximize yield and lower costs. Spinach (Spinacia oleracea) is important concerning human health because of its high nutritional content, including vitamins C and A, the carotenoid lutein, iron, folic acid, and magnesium, and its increasing consumer demand. The United States is the second to largest spinach producer and three percent of world output. We measured the different growth rates of spinach in the Tower Hydroponic System at either end of the optimal nutrient range (1260 and 1610 ppm). pH was maintained at 6.5, and the towers were maintained 1260 ppm and 1610 ppm. Spinach growth was recorded by the number of harvestable leaves within three weeks. After three weeks there was no significant difference between the two nutrient levels and their corresponding spinach production. Bolting was observed earlier and more common in the lower nutrient treatment than in the higher nutrient treatment. Further investigation can be taken into the bolting patterns in spinach related to nutrient levels. Based on this study, we conclude that spinach production can be achieved at the lower level of nutrients, reducing the amount of nutrients needed to maintain a hydroponic tower system and cost to produce the same amount of harvestable spinach.

VARIABLE LIGHT INFLUENCES BASIL AND PARSLEY GROWTH IN A HYDROPONIC SYSTEM

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Salve Regina University Sustainability Fellowship

Photosynthesis in plants varies due to wavelength and light frequency. Hydroponic systems aid in the rate of photosynthesis by controlling light as a variable. Increasing the length of time and frequency of light exposure is directly proportional to the photosynthetic rate. Hydroponics also allows plants to grow in a soilless, climate controlled, pest-free, and virus-free environment. Agriculturally, basil (Ocimum basilicum) and parsley (Petroselinum crispum) are in high demand for their extensive culinary use, and for their high nutrient value. Our goal was to determine the affect of light intensity on these plants when grown in an indoor tubular hydroponic system under fluorescent lights. We predicted that when provided significantly lower light (8500 lumens v. 22500 lumens) harvestable product would decline. After three weeks of growth there was no significant difference in both parsley and basil production under the different light intensities. Commercially available light fixtures for hydroponics are overbuilt for these herbs when grown in tubular hydroponic systems resulting in a waste of resources.

SENSITIVE ELECTROCHEMICAL IMMUNOSENSOR OF CANCER BIOMARKER PHOSPHORYLATED P53 BASED ON GOLD NANOPARTICLE (AUNP) DECORATED ELECTRODE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Despite recent advances in treatment, cancer still remains a major leading cause of death in the world. Rapid, specific early detection of cancer biomarkers proteins in serum is the only hope to change this fact. Such sensitive detection schemes are expected to greatly improve patient prognoses, treatment success, and even lead to cancer prevention. The broad long-term goals are to develop nanomaterial-based arrays to measure collections of early cancer biomarker proteins for specific forms of cancer. Herein, we report a sensitive electrochemical immunosensor for phosphorylated p53, a cancer biomarker protein in serum. This novel immunosensor features a glutathione protected gold nanoparticle modified electrode with captured immunological complex in a sandwich format. The antigen-antibody biorecognition event was optimized and monitored using catalytic reaction involving horseradish peroxidase conjugated to a secondary antibody. This approach provided a detection limit of 10 pg mL-1 in 10ul of calf serum. Our results compare favorably with the standard enzyme-linked immunosorbent assays (ELISA). Work is in progress to lower the detection limits using multi-label signal amplification strategy. These easily fabricated AuNP immunosensors show excellent promise for clinical screening of cancer biomarkers and point-of-care diagnosis.

EFFECTS OF IN-GROUP STATUS AND OUT-GROUP STEREOTYPES ON REWARD ALLOCATION TO AN OUT-GROUP WHEN OUTCOMES ARE CONTINGENT AND NON-CONTINGENT

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RI-INBRE Summer Undergraduate Research Fellowship Program

The Intergroup Relations Model (Malloy, 2008) predicts that when groups' outcomes are non-contingent (NC - gain by one does not mean a loss by the other), a high status group will favor a disadvantaged out-group over the in-group. If groups' outcomes are contingent (OC), a high status group will favor the in-group over the out-group. In contrast, a low status group will favor the in-group over a higher status out-group regardless of OC. False feedback about performance on a dot-estimation task (DOT) created high and low status groups. Out-group stereotypes were manipulated (capable or incapable students). Design was a 2 (high-low status) x 2 (positive-negative stereotype) factorial. Participants allocated practice trials (i.e., reward) on the DOT to in-group and out-group members. In Experiment 1 outcomes were NC; ANOVA revealed a main effect for in-group status. Low status members allocated more reward to the in-group; high status members allocated more reward to the out-group. Experiment 2 was identical except participants were told that some subjects would perform the DOT with feedback to improve performance. Groups were divided into ≥ the 50th percentile, or < the 50th percentile on the DOT. The group that improved the most had a chance to win $100.00 (contingent outcomes). Low status members showed in-group favoritism. High status members showed a 52% decline in out-group reward allocation compared to Study 1. Analysis of merged data from Experiments 1 and 2 showed that contingency moderated the status and stereotype effects. For an incapable out-group, high and low status groups showed no in-group or out-group favoritism. For a capable out-group, high and low status groups showed egalitarianism under NC, but in-group favoritism under OC.

PROBING THE CARCINOGEN-INDUCED CONFORMATIONAL HETEROGENEITY USING MINOR GROOVE DNA BINDERS

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RI- INBRE Summer Undergraduate Research Fellowship ProgramDepending on the local sequence context at or around the vicinity of lesion and the nature of the carcinogen, arylamine-DNA adducts exist in three distinct conformations: the “B type” in which the carcinogen is located in the major groove, the base displaced “S type” where the carcinogen is stacked between the two flanking bases, and the “W” conformation where the carcinogen is wedged in the minor groove. These lesion induced conformations in various sequence contexts have been characterized/probed in the past primarily by employing NMR, a powerful but tedious technique. In this study, we are trying to establish various minor groove binders as new probing tools to gain insight vis-à-vis the population ratios of the above conformers. Preliminary data were obtained from UV melting, circular dichroism, fluorescence and isothermal titration calorimetry experiments. The results show that these minor groove binding drugs are promising candidates for probing in various adduct-induced DNA conformations.

MERCURY BIOACCUMULATION IN ELASMOBRANCHS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant that bioaccumulates in fish tissues, including numerous marine species. Cartilaginous fish of the subclass Elasmobranchii are important ecological constituents of marine ecosystems, yet the fate of Hg contaminants in their body tissues is largely unknown. In this study, four species of elasmobranchs: little skate, winter skate, smooth dogfish, and spiny dogfish were collected from the Rhode Island/Block Island Sound, and the Hg content (ppm wet wt) of white muscle tissue was analyzed using automated combustion atomic absorption spectrometry. Diet and feeding habits for each species were also assessed by stomach content and stable nitrogen (d15N) and carbon (d13C) isotope analyses. Mean Hg concentrations differed significantly among species, with highest levels measured in smooth dogfish (0.68 ppm), followed by spiny dogfish (0.31 ppm) and skates (0.11 and 0.07 ppm for little and winter skate, respectively). The Hg concentration of skate muscle tissue did not vary by body weight, suggesting that Hg does not bioaccumulate in these species. Conversely, smooth and spiny dogfish both bioaccumulate Hg, although smooth dogfish have a higher Hg content relative to spiny dogfish. The elevated Hg concentration of smooth dogfish may be explained by their higher trophic level status, as determined from d15N signatures (13.29‰ and 12.09‰ for smooth dogfish and spiny dogfish/skates, respectively). The enriched d13C values of skates and smooth dogfish indicated benthic foraging (range of mean d13C = -16.39‰ to -17.42‰), which was further confirmed by the dominance of decapods and crustaceans in the stomach contents. Conversely, squid and butterfish were the principal prey of spiny dogfish, and the contribution of these pelagic prey was reflected in the depleted d13C signature (mean d13C = -21.97‰). Future work includes researching the effect habitat use and prey Hg to better understand bioaccumulation patterns in these species.

ESCHERICHIA COLI MG1655 COLONIZATION OF THE MOUSE INTESTINE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Commensal strains of E. coli colonize the mammalian intestine in the presence of an intestinal microbiota made up of at least 1000 species, most of them strict anaerobes. Evidence is mounting that E. coli strains evolve in the intestine to maximize their colonizing abilities. Previously, we found that the mouse intestine selects two better colonizing mutants of E. coli MG1655, E.coli MG1655 ΔflhD, which is deleted for the gene that regulates motility and chemotaxis and E. coli MG1655 mot-1, which has a missense mutation in envZ gene that regulates responses to high osmolarity. The two mutants differ in two major ways. In contrast to E. coli MG1655 mot-1, E. coli MG1655 ΔflhD is non-motile and E. coli MG 1655 mot-1 grows 30% faster than E. coli MG1655 ΔflhD on galactose as a sole carbon source. In the present study, when mice were fed 105 colony forming units (CFU) of E. coli MG1655 ΔflhD and 105 CFU of E. coli MG1655 mot-1, they co-colonized in equal numbers, suggesting they are equal in colonizing ability. When mice were fed 1010 CFU of E. coli MG 1655 mot-1 and 105 CFU of E.coli MG1655 ΔflhD, they remained 5 orders of magnitude apart in the mouse intestine, again suggesting that they are equal in colonizing ability. However, when mice were fed 1010 CFU of E.coli MG1655 ΔflhD and 105 CFU of E. coli MG1655 mot-1, E. coli MG1655 mot-1 grew from low to high numbers in the intestine, suggesting that it can colonize an intestinal niche unavailable to E.coli MG1655 ΔflhD. It is also shown that the second niche that E. Coli MG1655 mot-1 colonizes does not require it to be motile and nor be able to grow with galactose as a carbon source.

HABITAT EFFECTS ON MERCURY BIOACCUMULATION IN BLACK SEA BASS (CENTROPRISTIS STRIATA)

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury is a widespread environmental contaminant that bioaccumulates in fish muscle tissue, and therefore poses a risk to human consumers. Understanding the human risk from mercury exposure requires insight into species-specific mercury concentrations ([Hg]) and variability in fish Hg content as a function of life history characteristics. The specific objectives of this study were threefold: (1) measure total [Hg] in the muscle tissue of black sea bass (Centropristis striata) – a marine finfish of commercial and recreational importance; (2) analyze total [Hg] relative to fish length, thus assessing bioaccumulation rates; and (3) evaluate the effect of diet and habitat use on Hg bioaccumulation. Sea bass were collected from inshore (Narragansett Bay) and offshore (Rhode Island/Block Island Sound; RIS/BIS) habitats using trawls and hook & line. The white muscle tissue of sea bass (n=117) was analyzed for total Hg using automated combustion atomic absorption spectroscopy. Visual analysis of stomach contents was also performed to assess variation in diet across habitats, which could account for geographic differences in sea bass Hg contamination. Irrespective of habitat-type, the Hg content of sea bass muscle tissue was positively correlated with length, indicating the bioaccumulation of Hg. Sea bass collected from the Bay, however, had higher Hg levels at a given length than conspecifics from RIS/BIS. The cumulative results indicate that Hg concentrations in sea bass vary significantly over relatively small spatial scales (5 km), and site-specific Hg levels are correlated with the anthropogenic contaminant sources in the Bay. Finally, the diet of the inshore sea bass population was dominated by crabs, whereas RIS/BIS conspecifics fed on crabs, shrimp, and algae. Future work will include the analysis of sea bass stable isotope signatures to better define their trophic ecology, as well as examining the Hg content of bass preferred prey across habitats.

ALTERATIONS IN THALAMIC MORPHOLOGY FOLLOWING EMBRYONIC DAY 15 METHYLAZOXYMETHANOL TREATMENT IN THE RAT

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Malformations of neocortical development such as microgyria have been observed in the brains of language-disabled humans. Rats with microgyria show rapid auditory processing (RAP) deficits similar to acoustic deficits observed in some human language-learning impaired populations. Previous studies have also shown RAP and other learning impairments in rats with periventricular nodular heterotopia (PNH). Specifically, disruption to neuronal mitosis with methylazoxymethanol (MAM) treatment in rats on embryonic day (E) 15 resulted in PNH, cortical dysplasia and learning deficits similar to those seen in rats with neocortical microgyria. In an effort to identify potential etiological components of observed processing impairments, previous studies in microgyric rats examined changes in thalamic cell size and alterations in brain regional morphology. These studies found a shift in cell size in the medial geniculate nucleus (MGN) of the thalamus with microgyric subjects having more small and fewer large neurons as compared to controls. Importantly, shifts in thalamic cell size have also been observed in the brains of language learning impaired humans examined post mortem. The current study sought to investigate histometric changes in thalamic nuclei (dorsal lateral geniculate nucleus (dLGN) and MGN) in behaviorally assessed MAM treated rats previously shown to have auditory processing and learning impairments. These rats were also previously shown to have decreased cortical, hippocampal and corpus callosum volumes. In the present study novel evaluation of cell number, cell size and regional volumes in the dLGN and MGN were conducted in serial brain sections of MAM treated subjects. Results showed decreased volumes in thalamic sub-nuclei of MAM treated animals as compared to controls. These findings parallel previous research in microgyric rats showing histometric changes at the thalamic level. Convergent findings suggest that common factors may underlie seemingly divergent neurodevelopmental disruptions.

PEPTIDE-DOXORUBCIN CONJUGATES: SYNTHESIS, CELLULAR UPTAKE, AND ANTICANCER ACTIVITIES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Doxorubicin (Dox) is an anthracycline anticancer agent widely used in the treatment of leukemia, breast carcinoma, and other solid tumors. Dox binds to nucleic acids through specific intercalation of the planar anthracycline nucleus with DNA double helix. The highly hydrophilic nature of Dox causes rapid distribution and excretion and leads to low bioavailability of the drug. Furthermore, Dox is effluxed from some cancer cells efficiently after 1 hour. Thus, nuclear delivery and retention in the nucleus are critical for efficient activity of Dox. Herein, we report on the synthesis of Dox conjugates with linear peptides containing arginine, cysteine, and/or hydrophobic linkers that may act as cell-penetrating peptides (CPPs). The peptides, CRCRCRCR, RCRRRRRRC and R(CH2)11R(CH2)11K, were synthesized using standard N-(9-fluorenyl) methoxycarbonyl (Fmoc)-based solid phase chemistry. The N-terminal of two peptides, RCRRRRRRC and R(CH2)11R(CH2)11K, was conjugated with Fmoc-protected Dox containing a glutarate linker via an ester bond. After deprotection, the conjugates were purified by reverse-phase preparative high-pressure liquid chromatography to afford Dox-RCRRRRRRC, and Dox-R(CH2)11R(CH2)11K. The chemical structures of final products were confirmed by using high-resolution time-of-flight MALDI mass spectrometer (MALDI-TOF). The cellular uptake of the conjugates was examined in leukemia CCRF-CEM cell line by fluorescence activated cell sorter (FACS) and compared to both Dox alone and the corresponding physical mixtures. Ester conjugate Dox-R(CH2)11R(CH2)11K exhibited 1.5-fold higher cellular uptake versus that of Dox alone and the corresponding physical mixture of Dox with peptide. The retention of Dox was monitored by fluorescence microscopy. All the synthesized compounds were evaluated for inhibition of cell proliferation of human colon carcinoma (HT-29), breast carcinoma (BT-20), and leukemia (CCRF-CEM) cells. These data provide insights about the potential application of peptides for delivering of anticancer drugs as cargos.

THE ROLE OF BCP1 IN SACCHAROMYCES CEREVISIAE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Preserving the integrity of the genome is fundamental for the stability of cells and survival of species. Over time, species have developed mechanisms for DNA repair. BCP1 is an essential gene in Saccharomyces cerevisiae and is the fungal homolog of BCCIP in mammals. BCCIP acts as a tumor suppressor involved in cell cycle regulation and DNA repair. Studying BCP1 in budding yeast cold provide insight on BCCIP and be applied to cancer therapy. Previous data suggest a bcp1 temperature sensitive mutant strain is more resistant to DNA damage agents methyl methansulfonate (MMS) and hydroxyurea (HU) than the parental strain. The objective of this study was to determine the effect of Bcp1 overexpression in various knockout strains of S. cerevisiae lacking genes involved in the DNA damage response, Dun1, Tof1, and Rad6, and parental BY4741. A plasmid bearing Bcp1 under control of a galactose inducible promoter was transformed into each knockout strain. Transformed strains ACX-BY, ACX-Dun1, ACX-Rad6, and ACX-Tof1 were all observed to over-express Bcp1 when induced with galactose. They showed no difference in growth rate, morphology, budding, and stress tolerance when induced with galactose compared to parental strains. There did not appear to be any effect of over-expressing Bcp1 in the parental and three knockout strains tested. Future experiments will focus on other knockout strains.

PHENYL THIO-, PROPYL-, AND CARBAMOYL PYRAZOLINE COMPOUNDS AS ANTI-AMEBIC AGENTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica causes amebiasis in humans with 100,000 deaths per year, worldwide. The current treatment for amebiasis is metronidazole, with severe side effects. Alternative treatments for amebiasis are of great interest. The goal of this research was to determine the inhibition constant (Ki) of several inhibitors synthesized against an essential enzyme for the glycolytic metabolism of E. histolytica. These compounds show promising results: inhibitor 1, 3-(4-chlorophenyl)-1-(4-chlorophenyl thiocarboxamide)-2-pyrazoline; and inhibitor 7, 3-(3-bromophenyl)-1-(4-chlorophenyl thiocarboxamide)-2-pyrazoline. Two inhibitors were tested on their abilities to interrupt the growth of E. histolytica using the growth inhibition assay: Controls (no inhibitor added), inhibitors ([I]=60μM, 120μM and 240μM), DMSO and 20uL metronidazole were added to separate wells in each 48-well plate; then, 250 amoebas were added per well; finally, plates were incubated at 37 °C for 48 hours and 72 hours before performing a cell count. The kinetic assay was performed to calibrate the Ki value for both inhibitors: the rate of dehydrogenase (EhADH2) reaction with substrate acetaldehyde under the presence of each inhibitor was measured by spectrophotometer, using NAD+ μM/min as the unit of measurement; then, the Ki value for each inhibitor was calculated using Michaelis-Menten and Lineweaver-Burke kinetic equations. The growth inhibition assay indicated that the number of amoebas in each inhibited well (inhibitor 1 or 7) was significantly lower than in each controlled well. The kinetic assay showed the decrease in rate of the EhADH2 reaction with acetaldehyde with presence of inhibitor 1 and inhibitor 7. The Ki value for inhibitor 1 is 50.42±0.70, and the Ki value for inhibitor 7 is 44.05±0.71. In conclusion, Inhibitor 1 and 7 both exhibit are able to disrupt growth of E. histolytica and inhibit the EhADH2 alcohol dehydrogenase activity.

FINDING THE HIDDEN LINK: ANALYZING THE EFFECTS OF 1-OCTEN-3-OL ON MURINE BONE MARROW STROMAL CELLS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Stachybotrys is a genus of filamentous, spore-producing fungi that lives in damp, cellulose-rich environments and includes the species S. chartarum—one the most commonly isolated toxic black molds. S. chartarum is particularly prevalent in damp, poorly ventilated, water-damaged buildings. Human exposures occur indirectly by inhalation of the volatile organic compounds (VOCs) and mycotoxins S. chartarum secretes, not by direct contact. This environmental exposure is strongly correlated with numerous adverse health effects, including: increased respiratory distress and asthma; sick building syndrome; increased cancer rates (particularly liver and breast); higher susceptibility to bacterial and viral disease; higher mortality under general anesthesia; chronic fatigue, cough, and fever; and an overall decrease in general health. To date, the causal mechanisms underlying this correlation remains unclear. We hypothesize that the secreted aerosolized compounds have deleterious effects on immune cell survival and function, thereby contributing to the observed negative health effects. To test this hypothesis, we exposed murine bone marrow stromal (BMS) cells to concentrations of 1-octen-3-ol ranging from 0.001% - 0.05% for periods of up to 1 hour. We measured % cell survival for various exposure concentration/duration combinations using MTT dye cell proliferation assays. From these assays, we estimate that the LD50 of 1-octen-3-ol on BMS cells is 0.005% for 15 minutes. These results indicate that the compounds secreted by S. chartarum do have cytotoxic effects on immune cells. This resulting cytotoxicity can critically impair immune responses and may provide the missing link between environmental mold exposure and the observed adverse health effects.

CHARACTERIZATION OF NOVEL LIGANDS AS TOOLS TO STUDY SIGMA-2 RECEPTORS

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Sigma receptors are widely expressed in many different cell types across animal species. Unlike the sigma-1 receptor, the sigma-2 receptor has yet to be cloned. Sigma-2 receptors are highly expressed in tumor cell lines. Activation of sigma-2 receptors by agonists enables apoptotic cell death pathways in cancer cells. Furthermore, they have been shown to be biomarkers for tumor cell proliferation by non-invasive imaging technologies. Thus, sigma-2 receptor ligands have potential as both cancer therapeutic agents and cancer diagnostic tools.

In this study we investigated a series of novel sigma ligands from two different structural classes. A series of fluoroalkoxy benzamides was synthesized by Dr. Marcian Van Dort and colleagues (University of Michigan), and designed to be imaging agents. MV10-36A, MV10-30A, MV10-20A and MV10-19A were shown to have high to moderate affinity for sigma-2 receptors. To determine whether the compounds were agonists or antagonists, their ability to induce cell death in human neuroblastoma cells was examined using the MTT assay. Their antagonist nature was confirmed by the ability of 10uM and 30uM of the compounds to attenuate the ability of the sigma-2 agonist, CB-64D (10uM) to induce cell death.

A series of 4-fluorophenyl piperazine benzoxazalone analogs was synthesized by Dr. Christopher McCurdy and colleagues (University of Mississppi). CM-572 and CM-617 were designed to contain an isothiocyanate group that can covalently bind to the active site of the sigma-2 receptor. Both compounds exhibited affinity for sigma-2 receptors and were shown to bind irreversibly to sigma-2 receptors of rat liver membranes. To examine whether they function as agonists or antagonists, neuroblastoma cells were pretreated for 60 min with 1, 10, or 100uM CM-572 or CM-617 and cell viability examined using the MTT assay. Significant cell death was observed only at 100uM. This suggests that the compounds may be irreversible antagonists or partial agonists.

A BIOMIMETIC APPROACH TO THE OXIDATIVE CROSS COUPLING OF TYROSINE CONTAINING PEPTIDES

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Formation of the biaryl carbon-carbon bond is the quintessential step for the synthesis of cyclic biaryl peptides that exhibit antibiotic properties such as biphenomycin B and vancomycin. Contemporary methods for the cross coupling of aryl substrates rely heavily on the Suzuki-Miyaura reaction which, although it is often high-yielding, is far from ideal in terms of atom and step economy. The key drawback to Suzuki coupling lies within the functionalization of target amino acids. The halogenation and borylation of the phenol rings are both time consuming and produce environmentally hazardous by-products. To directly produce oxidatively coupled products, such as cyclic biaryl peptides, from peptide substrates, nature often uses iron-bearing enzymes. Inspired by this precedent, we propose two methods for chemically synthesizing biaryl cyclic peptides. The first will employ a Fe(O)N4Py complex, which has been previously shown to form amino acid radicals which may be capable of cross-coupling. A second approach will use an enzyme isolated from the root tips of the common horseradish plant, Horseradish Peroxidase (HRP), which contains an iron-heme active site, to couple the phenol rings of bis-tyrosine tripeptide substrates in the presence of hydrogen peroxide.

GROUND ARTHROPOD POPULATION DYNAMICS THROUGH ONE SEASON OF SUCCESSION IN ABANDONED URBAN LAND COVERS

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RI EPSCoR Summer Undergraduate Research Fellowship Program

In a world undergoing rapid urbanization, urban land cover is increasing. Although much land is being developed, in situations when land is abandoned or remediated, more natural ecosystems may have a chance to reestablish. Understanding how to promote the reestablishment of favorable arthropod populations is key to restoring ecosystem services such as pest control and nutrient cycling. In this study, we created a field experiment in Bristol, RI to evaluate differences in population dynamics of ground arthropods among plots in which management regimes changed after two years of management for common urban land covers (lawn, gravel and wood mulch). In lawn plots, mowing was stopped to allow more complex vegetation structure to develop. In mulched plots, turfgrass seed was applied after removing mulch to promote faster establishment of vegetation and arthropods. Unmanaged old field vegetation was included as a reference land cover. From these four treatments, each replicated four times, arthropods were sampled once a week for two-day intervals over six weeks (June-July) using two pitfall traps per 3m X 3m plot. It was hypothesized that arthropod population dynamics would differ among the treatments due to differences in their habitat structure complexity and composition, especially over time. Results show that arthropod numbers were initially smaller within previously mulched plots that lacked dense vegetation. Following a month of vegetation growth, arthropod numbers became more similar among the treatments. For example, on the first sampling date (June 11) ants were most abundant in old field plots, but on July 14, the treatments had similar numbers. This suggests arthropod recolonization of previously-mulched plots alongside short-term changes in vegetation structure. Information on the restoration process in urban land covers can offer valuable insights on how to promote the favorable reestablishment and management of arthropod populations to promote favorable ecosystem services.

SOLVENT DEPENDENT KINETICS OF THE IRON PENTACARBONYL-CATALYZED PHOTOISOMERIZATION OF ALLYL ALCOHOL

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RI EPSCoR Summer Undergraduate Research Fellowship Program

It has been shown that 60-90% of iron pentacarbonyl (IPC) molecules form a weak hexacoordinated complex with a single solvent molecule under ambient conditions in small alcohol, arene, and ether solvents. It is likely that the "pre-assembly" of the IPC-solvent complex hinders the IPC-catalyzed photoisomerization of allyl alcohol to propanal, provided that a stable solvent-substituted iron tetracarbonyl can form. Indeed, an ethanol-substituted tetracarbonyl species was observed in a recent UV pump - X-ray probe study of the ligand substitution dynamics of IPC in ethanol. Monitoring the relative areas of characteristic reactant and product peaks via proton NMR has indicated that the reaction proceeds more slowly in alcohols (ethanol, isopropanol, and 1-butanol) and THF, all of which are complexing solvents, than in the non-complexing solvents cyclohexane and nonane. Benzene, although a complexer, exhibits a rate similar to that of the non-complexers. Unlike ethanol, benzene is a poor π-accepting ligand and thus a stable benzene-substituted tetracarbonyl should be unlikely to form. Current efforts are focused upon detection of the key π-allyl hydride tricarbonyl intermediate, as this species should be more prevalent in solvents in which photoisomerization proceeds more quickly.

EFFECTS OF TRANS-2-OCTENAL ON IMMUNE CELLS

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RI-INBRE Summer Undergraduate Research Fellowship Program

The idea that mold produces toxic metabolites has raised questions about the potential negative health effects people experience after being exposed to indoor mold. Numerous studies have shown that mold exposure in humans can result in adverse respiratory effects, such as asthma, allergies, respiratory infections, and pulmonary bleeding, as well as some forms of cancer. Volatile organic compounds are among the many toxic metabolites secreted by toxic black molds which have been linked to severe illness. Trans-2-octenal is a volatile that is known to be secreted by many types of toxic mold species. It is a generally waxy chemical, and has a very strong odor, giving mold its musty smell. In this study, we examined the relationship between murine bone marrow cells and the toxic mold volatile trans-2-octenal. Murine bone marrow cells serve as precursors to immune cells and therefore if these cells are damaged, immune responses in the host may be altered which could lead to the numerous adverse health effects that have been reported. We exposed the volatile to the cells for certain periods of time in order to calculate the amount of cell survival using the MTT dye assay. The assay plate was divided into four sections: 5 minutes, 15 minutes, 30 minutes, and 60 minutes. Each of these sections contained 15 wells; each row contained certain dilutions of the trans-2-octenal mixed with media. After letting the volatile sit for the designated time, the volatile was removed from the wells and MTT dye assay was performed. Based on the data collected, we determined that the LD50 for BMS cells exposed to trans-2-octenal is 0.0005% for 25 minutes. These results provide evidence that immune cell function may be impaired when exposed to trans-2-octenal, resulting in possible health problems.

BUTANOL – MEMBRANE PARTITIONING AS A FUNCTION OF LIPID SATURATION AND CHARGE

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The idea of cheaper and more environmentally friendly biofuels has stimulated a renewed interest in producing biobutanol by, for example, Acetone-Butanol-Ethanol (ABE) fermentation. With recent research showing microorganisms such as Clostridium Pasteurianum, that can utilize biodiesel byproducts such as crude glycerol as a growth medium the process has now been designed to be more cost effective. The only problem left with the process is butanol's toxic effect on the cells at a concentration greater than 20 g/L, causing a leaky cell membrane and eventually apoptosis.

To ferment butanol in the most efficient manor, microorganisms must be genetically engineered to produce a more rigid cellular membrane that can withstand higher concentrations of butanol without fluidizing and becoming leaky. Therefore the goal of this research was to determine how lipid saturation and charge affects the fluidity and butanol partitioning of liposomes used as model cell membranes. To do so, liposomes were made using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG) at ratios of 3:1 and 1:3 DPPC:DOPC and DPPC:DPPG. The method used to make the liposomes was thin film hydration followed by sonication and then extrusion at 200 nm to yield a homogeneous size distribution. The research focused primarily on using Dynamic Light Scattering (DLS) to measure liposome size as a function of increasing butanol concentrations through heating and cooling scans. This along with further research will eventually lead to a new genetically engineered cell membrane that can withstand much higher butanol concentrations than that of the current microorganisms. When comparing the results with other research DLS proved to be a very useful tool in determining the partitioning coefficient of butanol. The results showed strong evidence that a higher unsaturated to saturated lipid ratio (3:1) significantly reduces the fluidizing effects of butanol.

INVESTIGATING THE EFFECT OF 8-OXOGUANINE IN REPLICATION-DEPENDENT EXPANSION OF CAG TRINUCLEOTIDE REPEATS

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Several genes linked to neurological diseases, including Huntington's Disease (HD), contain a CAG trinucleotide repeat motif. For HD, an individual that contains 5-35 repeats in exon 1 of the Huntingtin gene exhibits no disease characteristics and is not prone to developing the disease. However, there is an unstable length (36-40 repeats) where expansion of the repeat tract can occur, resulting in a mutant protein responsible for the disease phenotype. We have investigated a proposed mechanism by which CAG trinucleotide repeat expansion may occur during DNA replication.

CAG repeat primers and complementary CTG repeat templates of longer length were synthesized. These sequences were then annealed to form duplex DNA and incubated in vitro with the Klenow fragment (exo—) of DNA polymerase I from E.coli., which extended the CAG primer using the CTG template. Under certain conditions, the primer was extended to a longer length than the original template, indicating that trinucleotide repeat expansion occurred. Specifically, expansion was observed using a (CAG)10 primer, but not observed when the primer included a 10-base mixed clamp to fix it in place with respect to the template.

CAG primers containing the oxidized base 8-oxoguanine (8-oxoG) were also tested. Previous work has shown that this damaged base has a high propensity to form in CAG repeat regions. We found that primers containing 8-oxoG showed a greater degree of expansion than undamaged primers, which we propose is due to an increase in the dynamics of the system from 8-oxoG's destabilization of DNA duplex. This result is notable because it considers a DNA lesion that is known to occur, and links it to the formation of a diseased gene.

TIME WELL SPENT

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RI-INBRE Summer Undergraduate Research Fellowship Program

Since late March 2011, when an INBRE/IBACES orientation was held to introduce and assimilate new program candidates, our activities have focused on the addiction-recovery community. We hit the ground running continuing with a qualitative study in collaboration with the Rhode Island Department of Health that focuses on those facing substance abuse issues and concerns of HIV/Hepatitis-C status. Focus groups held at various treatment facilities throughout the state resulted in a plethora of information that was coded and reviewed for inter-rater reliability. Our goal is to use the results obtained from the focus groups to see how effective current recovery treatments are. Another area that was researched concerned the elderly and gambling behavior through the gambling studies project at Rhode Island College with Dr. George Ladd. This area of research brought us to the meetings of the Connecticut Council of Problem Gambling and also led us to attend the National Conference on Problem Gambling in Boston, Massachusetts. Additionally, our group helped to coordinate and facilitate the Rhode Island Department of Education's Safe and Drug Free Schools conference. We have also collaborated with the Institute of Addiction Recovery at Rhode Island College to showcase spirituality's role in addiction recovery. Additional areas in which we have worked include the use of TimeBanks of Rhode Island along with Respite Care. We are participating in meetings of the Women in Recovery Task Force, and the Elderly and Addictions Task Force. Our latest endeavors relating to our areas of research involve attending planning meetings for the Rally 4 Recovery which will culminate in the 10th Annual Rally 4 Recovery to be held September 10th, 2011 in Providence, Rhode Island. We are looking forward to the upcoming year bringing us new challenges and growth opportunities to incorporate in our research program.

SULFOTRANSFERASE EXPRESSION IN HUMAN LIVER DISEASE

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Sulfotransferases (SULTs) appear in multiple human tissues. Their function is to activate, deactivate, or mark substrates for removal. SULTs 2A1, 1E1, 1A3, and 1A1 are the major sulfotransferases that appear in human liver cytosol. They sulfonate DHEA, estradiol, dopamine, and phenols respectively. Disease of the liver has been shown to alter the expression of certain transcription factors which are thought to regulate the expression and activity of sulfotransferases. In this study, SULT 2A1, 1E1, 1A3, and 1A1 expression were measured in liver tissue from individuals with alcohol cirrhosis, diabetic cirrhosis, steatosis, diabetes and healthy liver using Western Blot technique. Primary antibodies directed against each of the four different SULTs, and a single secondary antibody attached to an infra-red (IR) marker were used. The intensity of the IR signal was measured, and these results were compared to SULT activity and mRNA measurements of the same samples. The results show that the levels of immunogenic protein of SULT2A1 and SULT1A3 in the liver cytosol correlate to the specific sulfotransferase activity.

MOLECULAR DIMERIZATION STATE OF NAMPT

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Nicotinamide Adenine Dinucleotide (NAD+) is essential for the balnced energetics required to maintain cellular balance. NAD+ acts as a coenzyme for redox reactions and as a major energy transfer molecule in cells, however cellular stores of NAD+ can be depleted under conditions of persistent DNA damage. Nicotinamide Phosphoribosyl Transferase, or NAMPT, is the rate-determining enzyme in the predominant pathway to regenerate NAD+ from Nicotinamide (NAM) and 5'-phosphoribosyl-1'-pyrophosphate. Previous studies have shown that NAMPT is a dimeric protein, meaning that two NAMPT molecules will readily form intermolecular interactions.

Purpose: The objective of this research is to determine the molecular state of NAMPT dimeric formation.

Methods: Yeast Two-Hybrid (Y2H) analysis of WT and mutant forms of NAMPT was used to screen the dimeric interaction. NAMPT (WT, Y188D and Y188F) cDNA was inserted into the bait (GAL4 DNA binding domain) and prey (GAL4 activation domain) DNA plasmids provided by the Proquest system (Invitrogen). An interaction between both the bait and prey is required for cellular growth to occur in selective media. Plasmids were verified by restriction enzyme digestion prior to transformation into the MaV203 yeast strain. Transformants were screened in 96-well plates on selective media.

Results: Control plasmids representing a strong protein interaction, a weak interaction and no interaction performed as expected. Yeast containing the NAMPT bait with prey empty vector control showed no self-activation and is therefore a suitable bait protein for further analysis. Cells containing both NAMPT bait and prey plasmids grew from twice to six times more then control cells, confirming that NAMPT is a dimeric protein.

Conclusions: NAMPT mutants will be screened next to determine if the phosphorylation state of the protein effects the dimerization.

HISTORICAL ABUNDANCE OF EARLY LIFE HISTORY SUMMER FLOUNDER (PARALICHTHYS DENTATUS) IN NARRAGANSETT BAY

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The summer flounder, Paralichthys dentatus, is a temperate flatfish that utilizes Mid-Atlantic estuaries during the juvenile, post-settlement stage. Recent anecdotal observations, however, have noted a northward shift in the distribution of these juveniles that now encompasses southern New England estuaries, e.g., the Narragansett Bay (RI/MA, USA). Moreover, the apparent geographic range expansion of juvenile flounder may be mediated by climate change. To this end, the objectives of this study were twofold: (1) determine if there has been a significant increase in the annual abundance of larval and juvenile summer flounder in the Narragansett Bay, and (2) identify if warmer winter water temperatures have contributed to such an increase. Temporal changes in flounder abundance in the Bay and surrounding coastal ponds was synthesized from current and historical data provided by the RI Department of Environmental Management and Roger Williams University field surveys. Moreover, information on long-term changes in winter water temperature in the Bay (January to March) was provided by the URI/Graduate School of Oceanography. The abundance of summer flounder larvae peaked during October and November, whereas juveniles (age-0 and age-1) were most abundant during the late spring and early summer. Historical information further revealed that there has been no change in the annual abundance of flounder larvae. Conversely, the annual abundance of the age-0 and age-1 juveniles has increased significantly in the Bay and surrounding coastal ponds since 1988 and 1993, respectively. Juvenile flounder abundance was also positively correlated with winter water temperature, as hypothesized, although this relationship was not significant at the $p < 0.0\ 5$ level. Accordingly, warm winter temperatures appear to reduce the overwintering mortality of flounder during the transition from plankton to post-settlement juveniles, yet a multitude of environmental factors likely contribute to the observed annual increases in abundance.

A CASE STUDY COMPARISON OF JOINT ATTENTION SKILLS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Autism is a neurodevelopmental disorder affecting communication, behavioral, and social development. The Center for Disease Control (CDC) has recently estimated that Autism affects approximately 1 in 110 children. This poster presents a comparison of a 12 month old infant with Autism and a cohort of typically developing infants of the same age. The developmental differences observed in the child with Autism reflect the diagnostic criteria for Autism. Differences were observed in several major developmental milestones. The observations of these behaviors were standardized using the Early Social Communication Skills (ESCS) assessment. As expected, one of these behaviors was joint attention, which includes eye gaze both initiated by the child as well as responding to an experimenter's eye gaze. Another behavior was social interaction. For example, if the experimenter rolls a ball a typically developing child is expected to direct attention towards the ball and the experimenter as an invitation to play. This observation also examined the child's turn taking ability. Behavioral requests were also examined. For example, when a wind-up toy stops working a typically developing child would be expected to direct attention to the toy and then back to the experimenter as a behavioral request to wind the toy again. These observations indicate that there are behavioral differences between a group of typically developing infants and an infant with Autism that are observable in several major developmental milestones as early as 12 months of age.

IDENTIFYING RNP1, RNP2, AND APP WITH THE HOPE TO GAIN AN INSIGHT INTO SYNAPTIC TRANSMISSION

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The squid optic lobes have very high densities of large nerve terminals from the photoreceptor cells of the retina, which can be biochemically prepared from homogenates of freshly dissected optic lobes. Referred to as synaptosomes, they are mainly comprised of the pre-synaptic terminals containing synaptic vesicles and mitochondria. They are prized in studies to identify and characterize proteins localized to the synaptic region. In the present, study we investigate by immunoreactive techniques the localization of the amyloid precursor protein (APP), commonly associated with the onset of familial Alzheimer's disease, and two classes of RNA binding proteins, identified by antibodies immunoreactive to the consensus sequences GFGFLSFQSCGSLFDKDK (anti-RNP1) and LFIGGLSYDTNEDTIKK (anti-RNP2). Synaptosomes were prepared by standard techniques and examined by electron microscopy, confocal microscopy, SDS-PAGE, and Western blots probed with the antibodies. Anti-APP, anti-RNP1 and anti-RNP2 showed distinct labeling patterns by immunofluorescence and confocal microscopy. On Western blots the RNP1 antibody labels molecular weights 130kD, 70kD, 60kD, and 35kD in the first supernatant. As the sample is purified ultimately to the P2 stage, however, the 130kD molecular weight band seems to decrease in intensity and ultimately cease to exist. RNP2, on the other hand, labels 65kD and 37kD bands in the synaptosome fraction as well as in the supernatant. A multitude of tests involving degradation, immunoprecipitation, and antibodies raised in a variety of animals were performed using primarily the supernatant and synaptosome fractions to study the proteins at hand to be able to show that APP, RNP1, and RNP2 are all present in these fractions. Looking towards the future, further tests will be done to see the exact role these proteins play in synaptic transmission.

LIPID-COATED MAGNETIC NANOPARTICLES FOR SIRNA TRANSFECTION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Nanoparticles offer new advances in the pharmaceutical community such as controlled delivery and release of drugs as well as therapeutic biological macromolecules. Our goal was to create lipid-coated magnetic nanoparticle (L-MNP) carriers for the efficient delivery of short interfering RNAs (siRNA) to mammalian cells. siRNAs are short (19-21 nucleotide) double-stranded RNA molecules that can be used to silence target gene expression. siRNAs hold great promise for targeted therapeutic strategies for several important diseases. Importantly, existing siRNA delivery systems are limited by an inability to accurately control the timing of siRNA delivery and by low cellular transfection efficiencies. The use of magnetic nanoparticles could circumvent both of these limitations by allowing magnetic guidance-mediated cell sorting post-delivery and controlled siRNA release.

L-MNP carriers were created by adding iron oxide (magnetite, Fe3O4) nanoparticles with diameters of 30 or 45 nm drop-wise into a cationic liposome solution containing a 1:1 ratio of DPPC (1,2-dipalmitoyl-glycero-3-phosphocholine) to cationic DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) while sonicating the solution at 50°C. Fluorescently-tagged siRNA were added to all delivery systems before transfection and were used to quantify the transfection rates and compare each carrier's performance. The cationic charge of the lipid nanoparticles was confirmed with zeta potential experiments. Dynamic light scattering was used to track L-MNP formation and the results suggest that the L-MNPs were polydispersed and consisted primarily of nanoparticle aggregates with an average diameter of X nm. Aggregation also likely occurred when the samples were left to sit over a strong magnetic field to separate L-MNPs from excess liposomes.

Transfection experiments were conducted in HeLa cervical carcinoma cells and inverted fluorescence microscopy was used to determine the efficacy of FAM-siRNA delivery. Several delivery systems were used including the L-MNPs, the DPPC/DOTAP liposomes used to prepare them, and the commercially-available transfection agent Lipofectamine 2000. Transfections were conducted with and without magnetic guidance. Preliminary results show that our cationic liposomes created worked well when compared to Lipofectamine 2000 and current experiments are being done to test and adjust the performance of our L-MNP carriers.

A NOVEL MOUSE MODEL FOR INVESTIGATING THE SIGNIFICANCE OF UFD2A ACTIVITY IN SKELETAL MUSCLE DEVELOPMENT AND DIFFERENTIATION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Ufd2a is a protein which ubiquitylates certain protein substrates, thereby signaling their degradation by the proteosome. Previously, a transgenic mouse model was utilized to examine the effects of inactivating Ufd2a in vivo. Ufd2a ubiquitylation activity was shown to be essential for cardiac muscle and neuronal development, since deletion of the catalytic domain of the Ufd2a gene was found to be lethal. In order to circumvent the limitations of this previous model, we have generated a line of mice in which the inactivation of Ufd2a is regulated by the expression of Cre recombinase in skeletal muscle. To accomplish this, we have obtained mice which have the exon coding for the catalytic domain (U-box) of Ufd2a surrounded by lox-p sites (ΔU) and bred these to mice which harbor the Cre-recombinase gene driven by the MyoD promoter (iCre-MyoD). This enables us to observe the effects of inactive UFD2a on skeletal muscle cells, while cardiac muscle and neuronal tissue to develop normally. Mice harboring the ΔU and iCre-MyoD alleles (which have been confirmed by PCR) are born at the expected genotypic ratios indicating that the inactivation of Ufd2a in muscle is not lethal. Preliminary data with small numbers of mice indicates that inactivation of Ufd2a does not affect overall weight from 10 days to 9 weeks of age. In order to determine the efficiency of recombination, individual skeletal muscle fibers were isolated and analyzed by RT-PCR and western blotting. Finally, we wish to determine the effects of Ufd2a inactivation on differentiation of primary myoblasts in culture. In order to do this, two primary muscle cell isolation and culturing protocols have been tested, one of which appears to result in increased myoblast numbers and more efficient cell fusion. This protocol will be used to isolate muscle cell precursors to assess differentiation efficiency.

HOLLOW CUS NANOPARTICLES FOR CANCER THERAPY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Photothermal ablation therapy has gained increasing attention in recent years as a minimally invasive alternative to conventional approaches for cancer treatment such as surgery and chemotherapy. In this study, hollow copper sulfide nanoparticles (HCuSNP) were developed as a new type of agent for photothermal ablation of cancer cells. HCuSNP were synthesized and conjugated with anti-cancer chemotherapeutic agent, cisplatin (CDDP). Human lung adenocarcinoma epithelial cells (A549) were used for uptake fluorescein-labeled HCuSNP for different incubation times. The photothermal effect in nude mice bearing A549 tumor 24 h following intravenous injection of HCuSNP was monitored by irradiation using a near-infrared (NIR) laser beam at 900 nm. HCuSNP-mediated destruction of A549 tumor cells was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. As a result, HCuSNP have an optical absorption band in the NIR range with a maximum absorbance at 1050 nm. Transmission electron micrograph shows HCuSNP are around 50 nm in diameter, with hollow interior and CuS crystal shell. The CDDP-loaded HCuSNP (HCuSNP-Pt) have about 20% of drug loading (Pt to Cu ratio). Fluorescent micrograph depicted intracellular uptake of HCuSNP in tumor cells in a time-dependent manner. Irradiation by a NIR laser beam resulted in an increase in the temperature of tumor up to 46oC in mice injected with HCuSNP-Pt or HCuSNP. MTT assay illustrated HCuSNP-Pt displayed anti-cancer effect with a profile similar to free CDDP. Owing to their unique optical property, small size, and low cost of production, HCuSNP are promising new nanomaterials for cancer photothermal ablation therapy as well as carriers for tumor specific delivery of anti-cancer drugs.

COMPETITION AMONGST ULVA SPP. IN NARRAGANSETT, RI

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RI EPSCoR Summer Undergraduate Research Fellowship Program

In order to fully understand Ulva bloom dynamics, we must determine how bloom species interact with one another. Ulva blooms in Narragansett Bay, RI, are dominated by two co-occurring species: U. compressa and U. rigida. Given their similar morphology and cohabitation, these species likely compete with each other for light and/or nutrients. Therefore, we examined the impacts of intra- and interspecific competition on U. compressa and U. rigida under mesocosm and field conditions. For both experiments, we assessed the occurrence of competition by determining whether Ulva growth decreased with increasing Ulva densities. In our mesocosm experiment, we varied light and nutrient levels to determine whether these factors are a limiting resource. In our field experiment, we examined the influence of herbivores by placing Ulva blades in cages of varying size mesh that differentially excluded herbivores. We found that both Ulva species are influenced by intra- and interspecific competition. Within the mesocosms, competition increased more under shade conditions, indicating light is a limiting resource for these species. In addition, U. rigida experienced greater interspecific than intraspecific competition under all mesocosm conditions. Interestingly, U. compressa showed greater interspecific competition in full sun, and intraspecific competition in shade, indicating it is a superior competitor at lower light levels. In the field, herbivory was greater in the amphipod dominated small mesh cages than in the mud crab dominated large mesh cages. In the large mesh cages, U. compressa showed decreased growth due to intraspecific competition, while U. rigida was more impacted by interspecific competition. However, in the small cages, competition was undetectable, likely due to preferential amphipod grazing on U. compressa. Overall, this work demonstrates that U. compressa is the superior competitor under all abiotic conditions, however, when U. compressa preferring herbivores are present, U. rigida can gain a competitive advantage.

GENOME REDUCTION IN YEAST INVOLVES PROGRAMMED CELL DEATH

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RI-INBRE Summer Undergraduate Research Fellowship Program

Genetic reduction is of great significance in many biological pathways, one of many examples being the production of gametes. After discovering that a tetraploid strain of Candida albicans undergoes significant cell death on reduction to a diploid state, we set out to determine the means of death and whether or not it was a general, genome-reduction-related process. To measure this cell death cells were grown on either pre-sporulation or sporulation media for tetraploid and diploid cells, respectively, of both Candida and Saccharomyces cerevisiae. Measurements of viability were made with a methylene blue stain, with tetraploid cells on average showing 23% survival by day two compared to 89% for a diploid control. To test the mechanism of the death, assays were done to measure ROS levels and caspase activity. Cells were stained with dihydrorhodamine 123 and then viewed under a confocal fluorescence microscope, with on average 52% of the tetraploid showing fluorescence whereas only 2% fluorescence was visible in the diploid control. A second assay was done using a FLICA protocol for caspase activity, with tetraploids showing around 53% fluorescent cells while only 2% of diploids showed fluorescence. These results indicate high ROS levels and high levels of caspase activity in the tetraploid, suggestive of programmed cell death via apoptosis. Similar experiments in diploid strains of Candida albicans, Candida lusitania, and Saccharomyces cerevisiae are being carried out to show that this programmed cell death is a retained feature of genome reduction in yeast in general. Further research will also be done with knockout strains of apoptosis-related genes to further understand the mechanism of death.

CHARACTERIZATION OF COFACTOR BINDING AFFINITY OF ENTAMOEBA INVADENS ALCOHOL DEHYDROGENASE E (EIADHE) IN STRAINS VK AND IP1

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Little is known about the kinetics of the enzymes Entamoeba invadens alcohol dehydrogenase E (EiADHE) and Entamoeba histolytica alcohol dehydrogenase 2 (EhADH2). These are key metabolic enzymes in the glycolytic alcoholic fermentation pathways of each lineage. These enzymes catalyze the conversions of acetyl-CoA to acetaldehyde and acetaldehyde to ethanol, using iron (II) and NAD+ as cofactors. EiADHE and EhADH2 are bifunctional enzymes consisting of two domains; the ALDH domain catalyzes the conversion of acetyl-CoA to acetaldehyde and the ADH domain catalyzes the conversion of acetaldehyde to ethanol. Many microorganisms and parasites have been shown to require iron for growth and survival, which has led to the proposal of iron chelators for antimicrobial treatment. This study focuses on determining the binding affinity of Fe2+ in EiADHE. The catalytic activity of the ADH domain for two isolated strains of E. invadens (VK and IP1) was observed under varying iron concentrations and the calculated data was compared to the known binding affinity for EhADH2 in addition to other kinetic data.

THE IDENTIFICATION OF UNIQUE CDNA MARKERS AMONG PATHOGENIC AND NONPATHOGENIC LEISHMANIA

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RI-INBRE Summer Undergraduate Research Fellowship Program

The purpose of this research was to identify cDNA markers that could be used to distinguish four species of pathogenic and nonpathogenic Leishmania: L. donovani, the causative agent of visceral leishmaiasis; L. mexicana, the causative agent of weepy cutaneous leishmaniasis lesions endemic in Central America; L. major ,the causative agent of dry shallow lesions endemic to the Middle East and L. tarentolae, a non pathogenic species. Results of cDNA-AFLP analysis using various selective primers revealed monomorphic, polymorphic and unique fragments among the Leishmania species. The identification of several polymorphic cDNA AFLP markers revealed that variations in gene expression (cDNA) profiles among these species exist. Therefore, cDNA-AFLP can be used to distinguish between pathogenic and non pathogenic, cutaneous and visceral, or between two different species that cause cutaneous leishmaniasis. These results show the highly sensitive cDNA-AFLP technique is a broadly applicable technology which can be used for identifying gene expression differences in the various clinical manifestations caused by several species of Leishmania. Polymorphic and unique AFLP fragments are currently being cloned and sequenced to identify the genes that are uniquely expressed by a specific species in order to determine how these morphologically and genetically similar species are able to cause such distinct clinical manifestations.

EPIGENETIC MODIFICATION OF FUNGI AND BACTERIA TO UNCOVER NEW SECONDARY METABOLITES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Epigenetics is the study of variations in gene expression caused by mechanisms other than the underlying DNA sequence. Some of these mechanisms include acetylation, phosphorylation or ubiquitinaton of histones, methylation of the DNA sequence, and other mechanisms that affect DNA storage. Many drug compounds directly or indirectly influence these mechanisms and can affect the epigenome of an organism, altering a cell's expression patterns. Studies of microbial genomes have indicated that there are typically many silent natural product biosynthetic pathways, inactive under laboratory growth conditions. We exposed various fungi and bacteria to several of these epigenetic modifiers in hopes of altering their gene expression to potentially increase the number of secondary metabolites they form. Fungi isolated by deep ocean drilling and sediment collection from the South Pacific Gyre were exposed to epigenetic modifiers for one week, and then the organic material from their supernatant was extracted and analyzed using LCMS. Additional experiments on a bacterial antibiotic producer were attempted as well. By altering microbial epigenomes, we aim to discover new secondary metabolites that may have antimicrobial or other therapeutic properties.

THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE LOCALIZES TO POST-SYNAPTIC TERMINALS AND TO THE SURFACES OF AXOPLASMIC ORGANELLES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Alzheimer's disease is a devastating neurodegenerative disorder that afflicts more than 26 million individuals worldwide, and the incidence of this disease is predicted to reach 100 million by 2050. It is well demonstrated that mutations in the Amyloid Precursor Protein (APP) lead to familial forms of this disorder; however, the functions of wild-type or mutant APP are unknown. Here immunocytochemistry coupled with confocal and electron microscopy was used to examine the distribution of APP in a variety of cell types. By confocal microscopy lamprey and goldfish spinal tissue labeled for APP, PSD-93, and the synaptic vesicle-associated marker SV2 demonstrated that APP and SV2 occupying close but distinct locations, while APP and the postsynaptic marker PSD-93 colocalize. Additionally, transmission electron microscopy on squid axoplasm showed that APP consistently localizes to the membranes of organelles, both attached and unattached to microtubules. These data suggest that APP localizes to the post-synapse rather than the pre-synapse, as well as to the membranes of axoplasmic organelles.

ACETIC ACID INDUCED CELL DEATH IN THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE, INVOLVES CALCIUM

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RI-INBRE Summer Undergraduate Research Fellowship Program

Acetic acid is a known inducer of programmed cell death in the budding yeast, Saccharomyces cerevisiae, which is associated with the generation of reactive oxygen species, the condensation and the cleavage of chromosomal DNA, and the activation of caspases. Several laboratories have proposed a link between programmed cell death in yeast and calcium, but the mechanism underlying this link remains unknown. To further explore the role that calcium plays in the cell death pathway in yeast, we have begun to characterize the cell death of different calcium mutants cultured in acetic acid. We have discovered that several calcium mutants including yeast cells lacking VCX1, CNB1, and CRZ1, have higher viabilities in acetic acid as compared to wildtype controls. Our initial studies with a calcium responsive (CDRE) lacZ reporter also suggest that acetic acid-induced programmed cell death is associated with the down regulation of calcium signaling pathways.

USE OF CIRCULAR DICHROISM FOR RELATING CARCINOGEN-INDUCED DNA BENDING AND NUCLEOTIDE EXCISION REPAIR EFFICIENCY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Aromatic amines are an important class of chemical carcinogens, which upon metabolic activation, attack DNA at the C8 position of guanine to form dG-adducts. These lesions exist in a mixture of three distinct conformations, major groove (B), stacked (S), wedged (W) and their population ratio largely depends on the type of lesion and its neighboring bases. Aminofluorene-modified E. coli NarI sequence (5'---GGCG*CT---3') has shown greater lesion flexibility over the G*CC sequence context. This 3'-next flanking T effect appears to be a general phenomenon. As such, 4-aminobiphenyl-modified duplex (CCATCG*CXACC) with the same G*CT context (X=T) exhibited a 2:3 mixture of B- and S-conformers whereas the isomeric duplex (X=A) exclusively yielded the B-conformer. It was found that arylamine lesions are more susceptible to undergo DNA repair in the GCA sequence context as compared to GCT. We hypothesize that the lesion-induced conformational heterogeneity in the GCA context significantly alters the overall DNA helical structure (i.e., bending) and is readily recognized by repair proteins. To prove this hypothesis we used circular dichroism (CD) to examine the secondary structures of the modified DNA. The results showed significant deviation in the CD pattern of arylamine-modified duplexes with the impact greater in the GCA sequence context, in good agreement with the repair efficiency data.

DISCOVERY OF NEW ANTIBIOTICS FROM DEEP SEA FUNGI

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RI-INBRE Summer Undergraduate Research Fellowship Program

The discovery of new antibiotics has not kept pace with rising drug resistance. Infections by methicillin resistant Staphylococcus aureus (MRSA) now account for more deaths in US hospitals than both tuberculosis and HIV/AIDS combined. Infections caused by Gram-negative bacteria are also increasingly more difficult to treat. Almost half of Pseudomonas aeruginosa infections are multidrug resistant. The incidence of carbapenem resistance encountered with the pathogen Acinetobacter baumannii is sharply rising also, leaving few therapeutic options. The long-term goal of this project is to discover novel antibiotics produced by strains of deep-sea fungi. To date, a collection of 118 marine fungi has been assembled from sediments collected in the South Pacific Gyre. Individual strains were cultivated on various marine agars and cryopreserved at -80 °C. Chemical extracts were generated by ethyl acetate extraction of 100 mL cultures conducted in marine broth. In total, 163 extracts were tested for antibiotic activity against MRSA, P. aeruginosa, and A. baumanii using disc diffusion methods. Additional assays were conducted against the bacteria Vibrio harveyi and Chromobacterium violaceum to test for the presence of quorum sensing antagonists. Overall, 34% of the extracts demonstrated activity against at least one bacterial target. Antibiotic activity was most often observed against MRSA. Future studies will focus on the purification and structure determination of the antibiotics produced by these deep ocean microbes.

DUAL GOLD NANOPARTICLES SCREEN PRINTED ELECTRODE ARRAY FOR IMMUNOELECTROCHEMICAL DETECTION OF TWO CANCER BIOMARKER FOR HEAD AND NECK SQUAMOUS CELL CARCINOMA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Protein arrays for measuring multiple protein cancer biomarkers in clinical samples hold great promise for reliable early cancer detection. Herein, we report a prototype 2- sensor electrochemical immunoarray based on dual gold nanoparticles screen printed electrodes arrays decorated with glutathione protected gold nanoparticles (AuNPs-GSH/AuNP) for the simultaneous detection of multiple protein biomarkers for head and neck squamous cell carcinoma (HNSCC) in serum. The prototype sensor design utilizes an electrochemical flow cell coupled to a dual sensor electrode array. Immunoarray procedures were designed to measure Interleukin-8 (IL-8) and interleukin-6 (IL-6) simultaneously in a single serum sample. All of these proteins are elevated in serum of patients with HNSCC with relatively similar levels of serum concentration. Horseradish peroxidase (HRP) was used as label on detection (secondary) antibodies in a sandwich immunoassay format. Biotinylated secondary antibodies (Ab2) that bind specifically to streptavidin-HRP conjugates provided 14-16 labels per antibody and gave the necessary higher sensitivity required for IL-8 and IL-6 detection at physiological levels. These results show great promise for real time multiplexed cancer biomarker detection for point-of-care diagnostics.

CHARACTERIZATION OF THE INTERACTION BETWEEN UFD2A AND P97/VCP HAS IMPLICATIONS FOR ITS ROLE IN INCLUSION BODY MYOPATHY WITH PAGET'S DISEASE AND FRONTOTEMPORAL DEMENTIA

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UFD2a is an E3 enzyme that adds ubiquitin to proteins that will then be sent to the proteosome to be degraded (Koegl 1999, Kaneko 2003, Mahoney 2002). We have recently discovered that striated muscle cells express a unique alternative splice form of this enzyme which we refer to as UFD2a-III but its function remains unclear. UFD2a is known to interact with the ATPase, p97/VCP, which binds to ubiquitylated proteins and has many essential regulatory functions in the cell which range from chromosome segregation to autophagy. Mutations in p97/VCP resulting in amino acid substitutions R155H or A232E cause diseases such as IMBPFD, a type of inclusion body myopathy which presents with an accumulation of autophagosomes (Ju, 2010). Since p97/VCP function appears to be regulated through its binding to a number of diverse molecules in a mutually exclusive manner, we sought to characterize the interaction between VCP and UFD2a proteins using quantitative reporter systems we developed for the Yeast 2-Hybrid (Y2H) assay. Analysis of the interaction between mutated forms of p97/VCP and wild type UFD2a have determined that the C-terminal flexible loop, the binding site for another p97/VCP cofactor, UFD3/DOA1, was essential for binding to UFD2a. In addition, UFD2a appeared to have the strongest affinity for p97/VCP A232E, the mutation which results in the most severe disease in humans and mice, while its weakest interaction was with wild type p97/VCP. Finally, the muscle specific isoform, UFD2a-III, displayed a very weak interaction. Currently, we are working to verify that all forms of p97/VCP and UFD2a are expressed equally in the yeast used for the Y2H assays. Since the interaction between yeast UFD3 and the p97/VCP yeast orthologue, cdc48, is known to be important for ribophagy (a form of autophagy), we might speculate that UFD2a regulates this process by competing for p97/VCP binding.

SYNTHESIS AND SIRNA COUPLING OF POLYETHYLENEIMINES

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We report herein the synthesis of two chiral polyethyleneimes (PEIs) derived from the cationic polymerization of chiral oxazolines, followed by basic hydrolysis of the formyl groups. These PEIs are intended to bind to a target siRNA to enable effective delivery to malignant cancer cells, where they will effectively silence a malignant gene. The two homochiral polymers formed use the R and S enantiomers of 4-benzyloxazoline as the monomeric unit. The oxazolines were, themselves, synthesized from the corresponding amino alcohol and dimethylformamide dimethyl acetal. The polymers were synthesized with either ten or thirty repeat units, which were determined by the ratio of oxazoline to the methyl triflate initiator. Now that these polymers have been successfully synthesized, we are studying their binding affinities with a sample siRNA.

BCP1 OVEREXPRESSION IN SACCHAROMYCES CEREVISAE LEADS TO REDUCED GROWTH IN A TRANSFORMED MDT1 KNOCKOUT STRAIN

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RI-INBRE Summer Undergraduate Research Fellowship Program

Saccharomyces cerevisiae, budding yeast, is a simple eukaryote that can be used in the study of cell cycle and protein interactions. S. cerevisiae also has many genetic similarities to humans. One of these genes is known as BCP1 in yeast and its human homolog is BCCIP. In humans, BCCIP has been shown to be involved in cell cycle regulation and functions as a tumor suppressor. In yeast, BCP1 is essential for survival, and in previous experiments using a temperature sensitive mutant, BCP1 inactivation led to greater tolerance to the DNA damaging agent, methylmethane sulfonate (MMS), compared to the wild-type strain. In this study, the objective was to overexpress BCP1 in yeast knockout strains with deletions in genes that code for key proteins in the yeast cell cycle, specifically, MDT1, PCL1, SWE1, PHO85, and CLB3 and to observe the effects of BCP1 overexpression by studying the viability and MMS sensitivity of the transformed strains compared to the original knockout. Four strains showed no apparent phonotypic effects of BCP1 overexpression; however, in the transformed MDT1 knockout strain, there was a dramatic decrease in growth compared to the parental knockout strain. Future work needs to be done to examine the interaction between BCP1 and MDT1, and how an MDT1 deletion along with BCP1 overexpression affects the cell cycle.

BAX-INDUCED CELL DEATH IN THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE, INVOLVES CALCIUM

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RI-INBRE Summer Undergraduate Research Fellowship Program

BAX is a proapoptotic member of the Bcl-2 family of proteins. Upon activation, Bax binds to the outer mitochondrial membrane, which ultimately induces programmed cell death in mammalian cells.Naturally Saccharomyces cerevisiae does not contain BAX; however, when mammalian BAX is expressed in yeast, it functions as it does in mammalian cells, inducing programmed cell death. Several laboratories have proposed a link between calcium and BAX function in mammalian cells. We are investigating the role of calcium in BAX-induced cell death in yeast by overexpressing human BAX in several yeast calcium mutants. We have discovered that mutants lacking genes important for calcium regulation including CRZ1, PMR1, PMC1, CNB1, CCH1, and VCX1 are all relatively resistant to BAX-induced cell death. We have preliminary data that suggests that BAX-induced toxicity leading to programmed cell death rises as calcium levels rise.

EFFECTS OF NEONATAL HYPOXIA-ISCHEMIA AND INTER-ALPHA INHIBITOR TREATMENT ON BRAIN WEIGHT

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RI-INBRE Summer Undergraduate Research Fellowship Program

Reduced oxygenation (Hypoxia) and blood flow (ischemia) to the brain in neonates can result from umbilical cord occlusion, prolonged labor, preterm birth or perinatal trauma. Following HI, a series of inflammatory responses persist exacerbating tissue damage. Inter-Alpha Inhibitor Proteins (IAIPs), have been shown to significantly reduce advancement of inflammation and tissue injury following infection in mice and adult stroke in rats. The current study investigated the effects of systemic IAIP administration on brain weights following induced HI in postnatal day (P) 3 and 7 rat pups. Separate HI and IAIP groups received ligation of the right common carotid artery to induce ischemia followed by 90 min of 8% O2. Sham surgery was performed on control subjects with 90 min of open room air exposure. Sham and HI rat pups received an intraperitoneal injection of ~0.1 cc NaCl, while IAI pups received 30 mg/kg IAIP treatment prior to hypoxia and 24 hours following HI. Brain samples were extracted and weighed 72 hours following HI induction. Results from a one-way ANOVA revealed a significant difference in brain weight between P7 groups. Post hoc analysis showed that HI pups had significantly lower brain weights as compared to sham animals. No brain weight differences were seen between sham and IAIP treated subjects. Further, no significant effects of brain weight were seen between the P3 groups. Our results suggest that IAI may prevent loss of brain tissue following P7 injury as evidenced by brain weight sparing. Preliminary data also suggest that the severity of HI injury or the effectiveness of IAIP as a treatment for neonatal HI may be differentially regulated across development. Future studies will assess the distribution and number of dying neurons and quantify the extent of injury in order to evaluate the efficacy of IAIP as a neonatal neuroprotectant.

FOCUS ON QUALITATIVE RESEARCH

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RI-INBRE Summer Undergraduate Research Fellowship Program

Considering that the current state of research largely focuses on quantitative methods, our use of qualitative methods provides an exciting alternative. In order to gather information in the field of chemical dependency, the qualitative method utilizing focus groups was implemented. This method allows the incorporation of information from evidence-based practice in the field of chemical dependency. Thus far we have made use of the focus group technique with two different populations: women in recovery and those facing substance abuse issues coupled with HIV and Hepatitis C concerns. The information gathered from the women in recovery resulted in a Rhode Island state policy change surrounding the issue of childcare for those women involved with DCYF. It also brought about discussion which led to state-wide policy reform concerning mothers' retention of healthcare after children have been removed by DCYF. While working with the second population, those facing substance abuse issues coupled with HIV and Hepatitis C concerns, focus groups were held at various methadone clinics as and residential treatment facilities throughout the state. The data gathered were coded and analyzed using inter-rater reliability, allowing us to pull out vital information that will be utilized by the Rhode Island Department of Health. Our latest endeavor involves working with the Rhode Island Department of Corrections' population of prisoners who are dealing with substance abuse issues. Working with this specialized population requires two separate IRB approvals, one from Rhode Island College and the other from the State of Rhode Island Department of Corrections. It also requires volunteer training at the RIDOC medium security facility and obtaining security clearance. We hope to begin the prison study in the fall and are looking forward to finding new studies to further hone our qualitative research skills.

SEQUENCE ANALYSIS OF AFLP FRAGMENTS IDENTIFY CDNA MARKERS FOR VARIOUS PATHOGENIC AND NONPATHOGENIC LEISHMANIA SPECIES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Several species of Leishmania, a trypanosomatid protozoan, are parasites of man while others are nonpathogenic. There are distinct differences in the clinical manifestations seen with various species of these organisms. For example, L. donovani causes a fatal visceral leishmaniasis whereas L. mexicana and L. major cause cutaneous forms of disease and L. tarentolae is not pathogenic to humans. Interestingly, genome studies revealed that the structure and sequence of the DNA among Leishmania species is highly conserved. Taking this previous data into consideration, it is hypothesized that differences in gene expression among Leishmania species may result in distinct clinical manifestation that present with each parasite infection. The goal of this study was to identify gene expression markers for each Leishmania species. cDNA-AFLP was performed using EcoRI and MseI selective primers with cDNA from Leishmania donovani, L. tarentolae, L. mexicana, and L. major. The resulting pool of selective AFLP fragments from each species was cloned. Colony PCR and gel electrophoresis were used to identify those colonies that contained plasmids with AFLP inserts. Plasmids were purified from positive colonies and were subjected to DNA sequencing. Sequences were analyzed using Sequencer software and Blastn searches available through NCBI databases to identify the gene associated with the cloned AFLP fragments. Results of the sequencing analyses thus far have revealed six different cloned AFLP products; two ribosomal RNA sequences, a hypothetical protein and three with no matches in the nucleotide databases. These sequenced fragment bp lengths are currently being compared to results obtained from cDNA-AFLP poly-acylamide gels to determined if they are monomorphic, found in all four species, polymorphic, found in two or three species or unique, found only in one species. A unique AFLP product found only in one species could serve as a potential cDNA marker for the pathogenesis associated with that species.

GENETIC CHARACTERIZATION OF THE MECHANISM OF ACTION OF SULFORAPHANE (SFN) IN THE YEAST, SACCHAROMYCES

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National Institutes of Health

Sulforaphane (SFN) is a member of a class of antioxidants known as isothiocyanates that are found in broccoli and other cruciferous vegetables. Reports from several laboratories have shown that SFN has anticancer and antimicrobial activity. However, the mechanism by which SFN acts in living cells remains elusive at this time. Recent studies that investigated the mechanism of SFN's chemotherapeutic effect in mammalian cells have suggested that SFN works by causing cell cycle arrest and/or initiating programmed cell death. To elucidate the mechanism of action of SFN, we have initiated studies of its effects on the budding yeast, Saccharomyces cerevisiae. We are now conducting a loss-of-function genetic screen of approximately 5000 knockout yeast in order to isolate mutants, which show sensitivity towards treatment with SFN. We have determined that at a concentration of 200 mg/ml SFN retards the growth of wild type S. cerevisiae by inducing programmed cell death. Interestingly, some of the knockout yeast displayed a heightened sensitivity to SFN as compared to the wild type cells. This sensitivity has facilitated our efforts to identify a genetic pathway through which SFN influences programmed cell death in yeast cells, a pathway that could be conserved in human beings. In our screen, we have already isolated several knockout yeast, in which the deletion of specific vacuolar proteins that are required for autophagy, including Vam3 and Vam7, results in sensitivity to SFN-induced programmed cell death. Moreover, we have discovered that Δatg1 yeast (lacking a kinase essential for autophagy) in a different strain background are also sensitive to sulforaphane, suggesting a possible role for autophagy in the mechanism of SFN-induced programmed cell death.

MICROTUBULE AND ACTIN-BASED MOTORS ASSOCIATE WITH MOTILE PIGMENT GRANULES IN THE SQUID PHOTORECEPTOR

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RI-INBRE Summer Undergraduate Research Fellowship Program

Within the squid photoreceptor, pigment granules migrate from the cell base to the distal tip in response to light and act as molecular sunglasses to shade the photosensitive components of the cell. Here, we study the movements of these granules and investigate their association with the cytoskeleton and their distribution relative to molecular motor proteins. By confocal microscopy, the granules are found in association with regions dominated by actin and appear attached to the actin network. By electron microscopy these granules are found in contact with the plasma membrane, perhaps through this actin interaction. EM micrographs reveal that the granules also associate with microtubules at discrete foci adjacent to electron dense patches internal to the granule membrane. In addition, the granules label for Kinesin-1, the dynein intermediate light chain, and myosin VI; however, they do not label for Kinesin-3 or by secondary antibodies. These data together suggest that pigment granule movement is likely to be microtubule-dependent and that actin and myosin also play a role in granule function.

INVESTIGATION OF COFFEE GROUNDS AS A POTENTIAL FEEDSTOCK IN POLYURETHANE PLASTIC FORMATION

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The price of crude oil is increasing without signs of coming down. Our research project's objective is to use a waste biomass product as an alternative to petroleum based chemical feedstock. Researchers are looking everywhere for alternatives to petrochemical components for plastics, such as castor oil and soy oil. These oils are chemically modified to serve as a polyol replacement in polyurethane synthesis. Although those other plant oils are promising alternative feedstocks, the oil extracted from waste coffee grounds provides an attractive alternative to soy and castor oils. This project looked at coffee grounds as a renewable option that can serve as a biomass derived polyol in order to make polyurethane foams. Our process investigated a variety of solvent extractions of the coffee grounds using the Advanced Solvent Extractor (ASE). Dichloromethane was selected as the solvent for ASE extraction with coffee oil average yields of 10% (mass/mass). Fatty acid methyl ester (FAME) analysis of the coffee oil showed close to 50% unsaturated fatty acid content. Hydroxylation of the coffee oil with hydrogen peroxide and formic acid were optimized for the preparation of polyol. The degree of hydroxylation was measured using the method of Roberts1. Using the hydrogen peroxide/formic acid oxidation a polyol with an average hydroxylation number of 8 mg NaOH/g was obtained. Additionally, hydroxylation was verified by FAME analysis using a gas chromatography-mass spectrometer. Test batches of polyurethane foam were prepared using the coffee oil derived polyol for future biodegradation studies.

1 Roberts, W.L. and Schuette, H.A. (1932) Ind. Eng. Chem., 4, 257.

DISTRIBUTION AND ABUNDANCE OF MARINE INVERTEBRATES ALONG NEWPORT NECK

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RI EPSCoR Summer Undergraduate Research Fellowship Program

As carbon levels rise as a result of human consumption, scientists have come to a consensus that the climate is changing. Marine organisms will be impacted as oceans absorb approximately 80% of the carbon added to the atmosphere, resulting in increased acidity. Warming global temperatures will increase sea level primarily through thermal expansion. Marine invertebrates are especially vulnerable during their larval stage to changes in oceanic pH, as well as to sea level rise which may limit vertical migration of species. Using long nets, 0.5m2 quadrats, crab searches and lobster traps, we were able to quantify the distribution and abundance of the coastal invertebrates and small fish at seven locations along Newport Neck in Newport, RI. As the first report of a five year study, we model the potential response of these organisms to a changing climate within their specific and varying habitats in Newport, RI. During this first year of study, we tested the hypothesis that the distribution of invertebrates relies on intertidal substrate composition, and that areas possessing a greater substrate diversity will possess greater diversity and abundance of marine invertebrates. Crabs, blue mussels (Mytilus edulis) and American lobsters (Homarus americanus) were equally distributed amongst the sites, whereas barnacles were found to be significantly more abundant at site 3 (Carey Beach) than the other sites. Long-nosed spider crabs (Libinia dubia) were found to be significantly more abundant at site 6 (Reject's Beach) than sites 4 (Second Tunnel), 5 (Ledge Road), and 8 (Green Bridge – The Point). Species richness was highest at sites 3 (Carey Beach) and 6 (Reject's Beach). Substrate composition is consistent with marine intertidal diversity. Future sea level rise will change the substrate composition of Newport Neck, and change the abundance and distribution of these species that serve as an important nursery to commercial fisheries.

MEASUREMENT OF INTRACELLULAR CALCIUM DYNAMICS IN MOUSE NEOCORTICAL CELLS EXPOSED TO PESTICIDES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroids and DDT have been shown to modify the gating characteristics of different voltage-sensitive ion channels (e.g., voltage sensitive sodium channels). Studies conducted by Cao et. al., 2011 have shown that the pyrethroid, deltamethrin, increases calcium influx in a concentration dependent manner under resting conditions. Furthermore, this increase in calcium was blocked by tetrodotoxin suggesting that the calcium influx was a secondary action to the activation of voltage-sensitive sodium channels. Pyrethroids are use dependent insecticides and their toxicity is enhanced under depolarizing conditions. The purpose of these experiments is to investigate the effect of deltamethrin and DDT under resting and depolarized conditions using a fluorescence assay with fluo-3. To conduct these experiments, the cortices of 18-day embryonic mouse were triturated in culture media, plated on poly-D-lysine coated 96-well plates and then incubated at 37°C. Nine to ten day old cultures were loaded with fluo-3 AM in Locke's buffer, washed and assessed for changes in fluorescence. Preliminary results indicate that fluo-3 was successfully loaded into the mouse neocortical primary cell cultures and changes in calcium were detected in cells treated with deltamethrin and DDT. Thus these experiments confirm that pyrethroids modify calcium homeostasis of neocortical neurons.

A SURVEY OF HEAVY METAL CONCENTRATIONS IN WATER AND SILT SAMPLES COLLECTED FROM MELVILLE POND

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RI-INBRE Summer Undergraduate Research Fellowship Program

In recent years the land use pattern of Melville pond and the surrounding area have changed from a storage site for underground fuel tanks to a camp ground and recreational hiking area. Possible contamination of the area due to residual metals deposited from the storage tanks is a concern since recreational exposure to individuals may be unhealthily. The goal of this research is to survey Melville Pond and the surrounding area to assess the concentration of heavy metals in silt and water samples. To do this, individual water and silt samples were collected from the area and heavy metals extracted using microwave digestion. Metal concentrations in samples were determined using inductively coupled plasma-mass spectrometry (ICP-MS). Preliminary results indicate that silt samples throughout the pond contain concentrations of metals above the threshold values set by the Toxicity Characteristic Leaching Procedure and Characteristic Wastes (TCLP) criteria for hazardous waste determination and the Rhode Island and Providence Plantations Department of Environmental Management (RIDEM) regulations for dredged material. Nickel was found above the chronic level of toxicity in the water sample near the inflow and lead was found above the chronic level in one of the silt samples from within the pond compared to limits set by RIDEM Ambient Water Quality Criteria Guidelines and regulations for dredged material. Copper was found above the chronic and acute levels in the water sample from near the outflow and zinc was found above the chronic and acute levels in the water sample from near the outflow and zinc was found above the chronic and acute levels in another water sample from within the pond. The preliminary results show that the Melville Pond area has high concentrations of these heavy metals above the chronic and acute threshold limits.

BEAVER PONDS AND TRANSIENT HEADWATER STREAMS: HIDDEN HOTSPOTS OF BIOGEOCHEMICAL CYCLING

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Soil microbial biomass, an indicator for biogeochemical cycling, is the living portion of the soil organic matter excluding plant roots and soil animals larger than .005 cubic um. Soil microbial biomass accounts for less than 5 percent of the total organic matter in soil, yet is the center of biological activity. Microbial biomass aids in the degradation of pesticides, heavy metals, and other pollutants. Microbial biomass serves as both a source and sink for carbon, nitrogen, phosphorous and sulfur. Areas high in soil microbial biomass are deemed "hotspots" for nutrient cycling and chemical degradation. Since beaver ponds and impoundment pools in transient headwater streams are areas with high retention times and an accumulation of organic matter, we hypothesized they would be hotspots of biogeochemical cycling. We analyzed microbial biomass carbon levels in sediments from three beaver ponds and nine impoundment pools from three transient headwater streams in Rhode Island. The chloroform fumigation extraction method was performed, which lyses microbial cell walls, releasing labile pools of carbon from the organisms. Elevated microbial biomass values were observed in impoundment pools in two out of three streams and one out of three beaver ponds. Elevated microbial biomass values ranged from 205 to 1,390 mg C/kg soil, exhibiting considerable variation between sites. The highest microbial biomass values were found within all impoundment pools of one stream. Forested, transient streams obtain fresh pools of carbon – as indicated by high microbial biomass – due to routine additions and flushing of organic matter. Organic matter in beaver ponds accumulates more slowly with less turnover. While we observed variability, beaver ponds and impoundment pools in streams can be substantial hotspots for biogeochemical cycling and more research is warranted to investigate their nature and rate of nutrient cycling, pollutant removal potential and capacity for greenhouse gas generation.

CASPIN FUNGIN INDUCES CASPACE INDEPENDENT PROGRAM CELL DEATH IN WILD TYPE SACCHAROMYCES CEREVISIAE CELLS WHILE FKS1 DELETED MUTANT STRAINS EXHIBIT RESISTANCE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Caspin Fungin is the first drug of the new antifungal echinocandin class. It operates by inhibiting beta {1-3} D glucanase in the cell the wall of target cells. Caspin fungin has already been found to be effective against infectious yeast strains such as Candida and Aspergillus. We show that in budding yeast strain Saccharomyces cerevisiae Caspin fungin induces the generation of ROS (reactive oxygen species), mitochondria fragmentation, and cellular membrane degradation through caspace independent mechanism. Strains that have a the beta {1-3} D glucanase catalytic site deleted (Fks1 mutant) showed resistance to Caspin Fungin through cellular wall integrity. These mutants however still demonstrated ROS generation and mitochondrial fragmentation, which leads us to believe that another mechanism which Caspin Fungin induces PCD might be at play.

ASSEMBLY AND ANALYSIS OF ARYL HYDROCARBON RECEPTOR GENES FROM LITTLE SKATE

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RI-INBRE Summer Undergraduate Research Fellowship Program

To understand factors controlling gene expression, it's necessary to identify regulatory elements and transcription factors binding sites (TFBS). TFBS are nucleic acid sequences often called motifs and are usually well conserved for specific transcription factors. Little is known about the regulation of the single aryl hydrocarbon receptor (AHR) gene in humans, and nothing is known about the multiple paralogous genes present in most vertebrate groups. We used bioinformatics methods and genome databases to assemble four AHR genes from little skate for the purpose of finding TFBSs to better understand AHR gene regulation. Further, this information will allow us to think about the divergence of function of different species with orthologous genes, including human. Genomic contigs were identified using a combination of cDNA cloning, EST sequences, and transcriptome sequencing through the Little Skate Genome Project. Genes were then assembled from the de novo assembly and a query of upstream and downstream protein-coding sequence was used to order the contigs into complete genes. Approximately sixty contigs used to make up these "supercontigs". Next we used Alibaba 2.1, Patch 1.0, and P-Match 1.0, which all access the TRANSFAC TFBS database, to identify potential conserved TFBS and regulatory elements in the promoters of the 4 assembled genes. Several promising sites were detected, including sites for binding two NFkB subunits. Further exploration by phylogenetic footprinting is likely to provide insight on conserved regulatory elements in these AHR genes.

EXPRESSION, PURIFICATION, AND STRUCTURAL ANALYSIS OF THE BIOFILM PROMOTING PROTEIN YJDK

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Biofilms are communities of bacterial cells that are enclosed in a self-produced polymeric matrix. They strongly adhere to surfaces and negatively impact humans in many ways. Biofilms can form on the surfaces of medical devices such as catheters and implants and are highly tolerant to antibiotics. This antimicrobial tolerance has been linked to the existence of persisters, cells that are characterized by slowed growth. Since many presently established antibiotics act against rapidly dividing cells, persister cells are able to remain unaffected. Microarray studies have shown that certain genes are upregulated in persister cells, among these are toxin:antitoxin pairs. Under normal conditions, the antitoxin binds and suppresses its toxin. However, the antitoxin is extremely labile. Upon its degradation the toxin is free to exert its function in either repressing DNA replication or translation. Expression of the toxin YjdO in E. coli cells leads to a dramatic decrease in bacterial growth while co-expression with its antitoxin, YjdK, restores normal growth. We are using nuclear magnetic resonance (NMR) spectroscopy to determine the 3D structure of YjdK, which will give insight into its function. A protocol for the expression and purification of YjdK from E. coli has been established. A variety of NMR experiments ([1H,15N]-HSQC, 15N-NOESY, HNCA, CBCACONH, and CCCONH) have been recorded for the purpose of completing the sequence-specific backbone assignment of YjdK as a first step in structure determination. Knowledge of the structure of this biofilm promoting protein will give insight into the mechanism of antibiotic tolerance, which will lead to the development of antibiotics that can target proteins responsible for generating persister cells.

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