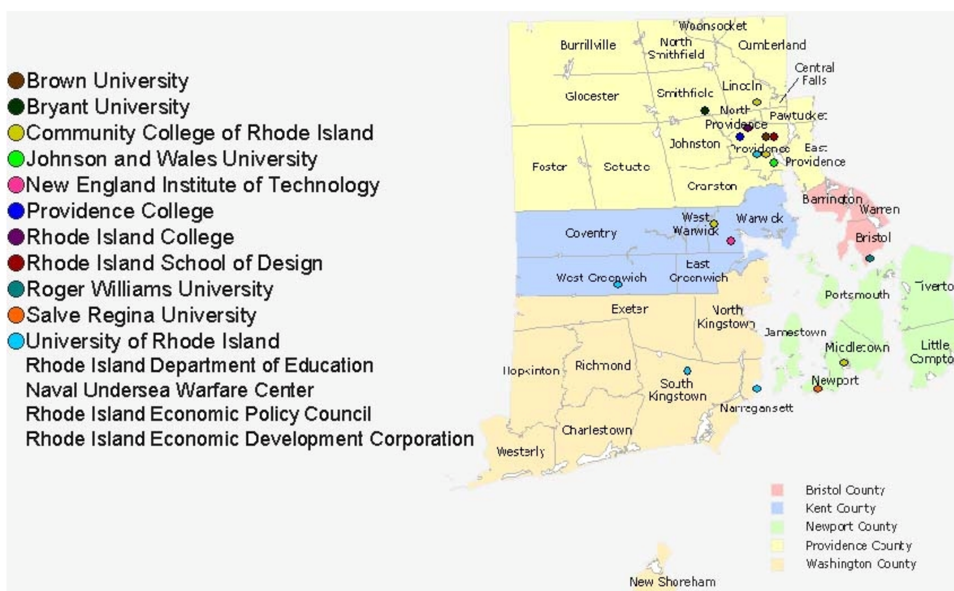
2008 SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE



Monday, August 4, 2008
9:00 AM

THE RYAN CENTER, UNIVERSITY OF RHODE ISLAND

Supported by

RI-INBRE & RI EPSCoR

Summer Undergraduate Research Fellows Conference

Monday, August 4, 2008

Ryan Center Concourse

University of Rhode Island, Kingston

9:00 – 9:45	***Continental Breakfast & Group A Poster Set-Up***
9:45 – 10:00	***Welcome***
	President Robert Carothers, University of Rhode Island
	Dr. Zahir Shaikh, RI-INBRE Program Director
	Dr. Jeffrey Seeman, RI EPSCoR Project Director
10:00 – 11:30	***Summer Undergraduate Research Fellows Poster Session***
	Group A (Poster #'s 1 – 41)
11:30 – 12:30	***Lunch & Group B Poster Set-Up***
12:30 – 2:00	***Summer Undergraduate Research Fellows Poster Session***
	Group B (Poster #'s 42 – 81)

LIST OF STUDENT POSTERS

***Please note that the poster numbers listed in the following tables also correspond with the page numbers in the abstract book. When reading the individual abstracts, the Summer Research Fellow is underlined.*

University of Rhode Island

Poster #	Summer Fellow	Mentor
21	Delia Daza	David Rowley, Ph.D.
22	Audrey DiRaimo	Paul Cohen, Ph.D.
78	Catharine Donahue	Carol Thornber, Ph.D.
32	Andrea Hodgson	Matthew Stoner, Ph.D.
35	Jacqueline Jeha	Kerry LaPlante, Pharm. D.
79	Caroline Killian	Navindra Seeram, Ph.D.
37	Fred Kolling IV	Niall Howlett, Ph.D.
39	Christopher Lee	Peter Paton, Ph.D. Elizabeth DeCelles, Ph.D.
40	Jessica Lehmann	Keykavous Parang, Ph.D.
41	Sara Linden	Zahir Shaikh, Ph.D
42	Wendy Lopez	William Euler, Ph.D.
46	Melissa Martin	Robert Rodgers, Ph.D.
47	Kevin McConeghy	Kerry LaPlante, Pharm. D.
52	Jolene Octavius	Bethany Jenkins, Ph.D.
58	Amy Rabideau	Geoffrey Bothun, Ph.D.
59	James Rebello	Fatemah Akhlaghi, Ph.D.
61	Melissa Rotunno	David Nelson, Ph.D.
62	Nathan Rubien	Dina Proestou, Ph.D. Maria Gomez-Chiarri, Ph.D.
67	Fabian Sierra	Gregory Otto, Ph.D.
69	Leanne Stover	Abraham Kovoor, Ph.D.
72	Erica Tow	Angela Slitt, Ph.D.
74	Yasah Vezele	Stanley Barnett, Ph.D.
75	Amy Viveiros	Carol Thornber, Ph.D. Brian Wisor, Ph.D.

Brown University

Poster #	Summer Fellow	Mentor
9	Joanna Bernhardt	Heather Leslie, Ph.D.
11	Lauren Bowers	Gilad Barnea, Ph.D.
17	Jason Chan	Derel Stein, Ph.D.
18	Laura Chartier	Heather Leslie, Ph.D.
26	Caitlin Feehery	Casey Dunn, Ph.D.
27	Julia Geehring	Gary Wessel, Ph.D.
43	Daniel Ludwig	G. Tayhas Palmore, Ph.D.
51	Megan O'Brien	David Rand, Ph.D.
66	Elizabeth Schroeder	Susan Gerbi, Ph.D.
73	Kristen Uhl	Carmen Marsit, Ph.D.
60	Sol Maria Reyna	Mark Zervas, Ph.D.

Providence College

Poster #	Summer Fellow	Mentor
36, 76	Andrew Bagdasarian	Yinsheng Wan, Ph.D.
6	Wesley Beaulieu	Jack Costello, Ph.D.
15	Yi Cao	Nicanor Austriaco, O.P.
29	Erik Gravel	Nicanor Austriaco, O.P
33	Helene Hosinski	Nicanor Austriaco, O.P.
36	Becky Kivlin	Yinsheng Wan, Ph.D.
45	Katheryn Marks	Elizabeth Aravelo, Ph.D.
48	Cailin McDeed	Nicanor Austriaco, O.P.
56	Nathan Pinches	Nicanor Austriaco, O.P.
68	Brad St. Martin	Nicanor Austriaco, O.P.
70	John Sullivan	Nicanor Austriaco, O.P.
76	Brittany Wallin	Yinsheng Wan, Ph.D.

Rhode Island College

Poster #	Summer Fellow	Mentor
1	Aaron Acquisto	John Williams Jr., Ph.D.
2	Amanda Albanese	Rebeka Merson, Ph.D.
8	Krystal Bergeron	Karen Almeida, Ph.D.
12	Liam Burke	John Williams Jr., Ph.D.
8	Yvonne Chekaluk	Karen Almeida, Ph.D.
20	Lisa Chin	John Williams Jr., Ph.D.
81	Marissa Delpico	Sarah Spinette, Ph.D.
8	Priscila Falcao	Karen Almeida, Ph.D.
80	Nicole Gadbois	Sarah Spinette, Ph.D.
30	Sean Hersey	Rebeka Merson, Ph.D.
31	Jacob Hicks	John Williams Jr., Ph.D.
8	Eileen Murphy	Karen Almeida, Ph.D.
64	Joseph Salisbury	John Williams Jr., Ph.D.
77	Tyler Zalobowski	Rebeka Merson, Ph.D.

Roger Williams University

Poster #	Summer Fellow	Mentor
3	Jessie Alden	
4	Christopher Amrich	Marcia Marston, Ph.D.
19	Marissa Checca	Avelina Espinosa, Ph.D.
28	Ashley Goss	Lazaros Kochilas, Ph.D.
		Kerri Warren, Ph.D.
34	Natalie Huey	Karin Tammi, Ph.D.
53	Jennifer Linehan	David Taylor, Ph.D.
53	Eric Payne	David Taylor, Ph.D.
54	Danielle Perley	Marcia Marston, Ph.D.
55	Monichan Phay	Avelina Espinosa, Ph.D.
57	Maria Piraino	David Taylor, Ph.D.
63	Erica Ryke	Avelina Espinosa, Ph.D.
71	Samantha Taylor	Marcia Marston, Ph.D.

Salve Regina University

Poster #	Summer Fellow	Mentor
5	David Augustowski	Bernard Munge, Ph.D.
7	Amy Beltramini	Sandor Kadar, Ph.D.
10	Amanda Borges	Sandor Kadar, Ph.D.
13, 14	Mark Byrne	Alison Shakarian, Ph.D.
16	Natasha Catlin	Steven Symington, Ph.D.
23	Brittany Dodson	Alison Shakarian, Ph.D.
24	Richard Dowd	Bernard Munge, Ph.D.
25	Michael DuLac	Steven Symington, Ph.D.
14	Christine Ganim	Alison Shakarian, Ph.D.
38	Colleen Krause	Bernard Munge, Ph.D.
44	Peter Maricic	Bernard Munge, Ph.D.
23, 49	Brigid McEwan	Alison Shakarian, Ph.D.
50	Lines Millord	Bernard Munge, Ph.D.
65	Deanna Salter	Steven Symington, Ph.D.

Evaluating Cationic Lipophilic Monomers and Polymers for Antimicrobial activity

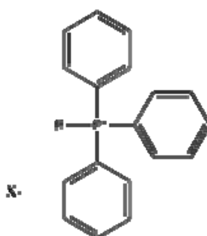
Acquisto, A., Williams, J.C., and LaPlante, K.L.

Physical Sciences Department, Rhode Island College

RI-INBRE Summer Undergraduate Research Fellowship Program

OBJECTIVE: There is a critical need for new antibiotics to combat evolving bacterial pathogens. Arylphosphonium salts (APS) are a general class of lipophillic cationic compounds that are shown to have anti-parasitic, anti-cancer and some antibacterial activity in vivo. In depth investigation of these compounds against drug resistant bacteria have not been conducted, therefore, this project aims to evaluate the activity of APS compounds against a range of drug resistant pathogenic bacteria.

METHODS: A library of Arylphosphonium Salts (APS) and APS synthesized derivatives were tested against numerous gram-positive and gram-negative bacteria. Antibacterial activity was determined using basic micro-dilution and time-kill assays. Drug resistant strains of *Staphylococcus aureus* (MRSA, MSSA), *Enterococcus faecalis* (EF), *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) bacteria were tested.



RESULTS:

APS	MRSA MIC range in mg/l n=2	MSSA MIC range in mg/l n=2	EF MIC range in mg/l n=2	EC MIC range in mg/l n=2	KP MIC range in mg/l n=2
APS – 426.32	>128	128	128	128	≥ 128
APS – 389.27	>128	128	128	128	≥ 128
APS – 342.80	>128	128	128	128	≥ 128
APS – 414.92	16	4	64	128	≥ 128

CONCLUSION: The proposed in vitro study will provide investigations into the activity of Cationic Lipophilic Monomers and Polymers as antimicrobial agents against multi-drug resistant pathogenic bacteria. Future studies are necessary to determine the utility of these agents in clinical settings.

Quantification of Cytochrome P450 1A (CYP1A) mRNA expression in little skate (*Leucoraja erinacea*)

Albanese, A., Merson, R. R.

Department of Biology, Rhode Island College

RI-INBRE Summer Undergraduate Research Fellowship Program

The aryl hydrocarbon receptor (AHR) is an intracellular transcription factor that regulates many genes, including cytochrome P450 1A (CYP1A), in response to environmental contaminants. Known AHR agonists, including polychlorinated dibenzo-*p*-dioxins and polychlorinated biphenyls (PCBs), are persistent, globally-distributed pollutants that are highly toxic and linked to deleterious health effects in humans and wildlife. We use the benthic marine cartilaginous fish little skate (*Leucoraja erinacea*) to investigate evolutionary conservation of AHR signaling, determine susceptibility of cartilaginous fishes to dioxin-like compounds, and test skates as an environmental model for toxicology. To describe the responsiveness of skates exposed to AHR agonists, CYP1A mRNA levels were quantified by real-time quantitative PCR of liver, stomach, and intestine cDNA synthesized from skates exposed the -naphthoflavone, a polycyclic aromatic hydrocarbon (PAH) type AHR agonist, and skates captured in Boston Harbor, Massachusetts, at a site polluted with PCBs and PAHs. We show that the CYP1A expression is highly variable among individuals captured at the same site and among the tissues from individual skates. Expression of CYP1A was highest in liver from one skate captured at Deer Island Flats; interestingly, this animal had lowest stomach CYP1A mRNA. Despite this variation within the sampling location, the expression levels in the skate treated with BNF and skates from Deer Island Flats were higher than the skates captured at Cape Cod Bay, the “clean” reference site. These findings agree with other studies in cartilaginous fishes that provide evidence of a responsive AHR pathway and support that skates may be susceptible to the toxic effects of environmental pollutants. In conclusion, our study reveals that exposure to AHR agonists induces the expression of CYP1A in little skate, supporting the notion that cartilaginous fishes are sensitive to PCBs and PAHs, and provides important information about the impacts of environmental contaminants on this group of organisms that are ecologically and economically important. Future studies will include additional sampling and controlled exposure experiments to determine the utility of skates as sentinels of chemical pollutants in marine habitats.

Species richness of neotropical Dictyotales marine algae collected from Caribbean and Pacific Panama inferred from DNA barcoding of the *Cox1* gene

Alden, J. and Wysor, B.

Department of Biology and Marine Biology, Roger Williams University

RI-EPSCoR Summer Undergraduate Research Fellowship Program

Identifying marine algae using traditional taxonomic classifications is extremely complex due to the morphological similarities of species. When separating species of a particular genus, two specimens may appear to have all of the same morphological features, yet are actually two species. Alternatively, two specimens whose morphological characteristics seem to differ may reveal to be members of the same species upon molecular analysis. This research focused on initiating a DNA barcode study of the cytochrome oxidase subunit 1 gene (*Cox1*) for the Dictyotales family of marine brown algae in Panama to help resolve the species divisions. DNA from select specimens collected by Dr. Brian Wysor in 1999 was extracted using the Qiagen DNeasy Plant Mini Kit and the *Cox1* gene was amplified by polymerase chain reactions. After several failed amplification attempts, methods of extraction and PCR were adjusted. However, even after adjustment, sequences from only three specimens were successfully produced. While in Bocas del Toro, Panama in July 2008, approximately 100 new Dictyotales specimens were collected and identified by morphological characteristic analysis. DNA from select specimens of this collection was extracted using a phenol/chlorophorm extraction protocol made available by Dr. Amy Driskell of the DNA Barcode for Life Project and the Smithsonian Institute. This extraction produced successful PCR products where all other extraction attempts failed. At this time, there are no sequences from this collection, but with the success of the new protocol, samples from both the 1999 and 2008 collections will now be able to be sequenced and analyzed. Once sequence data is produced from all available samples, distinctions between the Pacific and Caribbean coast populations of Dictyotales will be investigated based on the molecular data.

Genetic analysis of tail fibers in marine viruses

Amrich, C., Marston, M.

Department of Biology, College of Arts and Sciences, Roger Williams University

RI-INBRE Summer Undergraduate Research Fellowship Program

Marine virus communities are genetically diverse and their populations are dynamic, influencing the cyanobacterial host (*Synechococcus spp*). Only a few cyanophages have been completely sequenced and these genomes show interesting features, including diverse tail fibers that potentially influence host specificity. Previously, the diversity of cyanophage communities has been characterized by basic structural genes at the community level and no research has been done on characterizing cyanophage by tail fiber genes. This study examines the genetic diversity of tail fiber gene (g101) within a community of phage. Five PCR primer sets were developed for various segments of the Myoviral tail fiber gene using the complete genome sequence for a Synechococcus cyanophage: Syn9. Syn9 isolates from Narragansett Bay as well as additional Myovirus isolates from Rhode Island waters were tested via PCR for the presence of Syn9-like tail fiber sequences. The genes from isolates that gave positive amplification were sequenced and compared. All of the g101 primer sets amplified tail fiber segments from the Syn9 isolates. At least one of the primer sets can amplify tail fiber genes from some, but not all, of the other Myoviruses tested. By understanding the variations in tail fiber genes, the viral community can be further characterized and the evolutionary history can be determined for these diverse genes.

DNA Molecular Interactions Studies using Quartz Crystal Microbalance (QCM) for Nanopore DNA sequencing Application

Augustowski, D. and Munge, B.

Department of Chemistry, Salve Regina University

RI-INBRE Summer Undergraduate Research Fellowship Program

Sequencing a person's genome is far beyond affordability for anyone. Many diseases are linked to genetics, but not much is understood about them. In order to understand how these diseases work at low cost and high speed, new methods for DNA sequencing are required. Salve Regina University is working in collaboration with NABsys Inc. to develop DNA sequencing technology called Hybridization-Assisted Nanopore Sequencing (HANS). In order for DNA sequencing to occur, the DNA must pass through a nanopore at just the right speed. The nanopore requires a surface that interacts with the DNA enough to regulate the translocation speed which is critical for successful DNA sequencing. We are using Quartz Crystal Microbalance (QCM) to study these DNA molecular interactions with various thiol compounds including 3-Mercapto-1-Propanol, 6-Mercapto-1-Hexanol, Cysteamine Hydrochloride, 2-Propene-1-thiol, 1-Thiol-β-D-glucose sodium salt, and o-(2-mercaptoethyl)-methyl-hexa(ethylene glycol). The work done so far has allowed us to come to the conclusion that 6-mercapto-1-hexanol has stronger interactions with DNA than 3-mercapto-1-propanol.

Predator-prey interactions of the hydromedusa *Aequorea victoria*

Beaulieu, W.T., Regula, C.M., Colin, S.P. and Costello, J.H.

Department of Biology, Providence College

RI-EPSCoR Summer Undergraduate Research Fellowship Program

Previous research has shown that there is a strong relationship between jellyfish morphology and their feeding ability. The hydromedusa *Aequorea victoria* is an oblate cruising predator that entrains prey in vortices at the bell margin which are created via bell contractions during swimming; however, it can also behave as an ambush predator by remaining still with its tentacles extended. In addition, it is well known that this medusa positively selects for soft-bodied prey such as other hydromedusae and larvaceans, while negatively selecting for hard-bodied prey such as copepods. In this study, we observed the feeding behavior of this medusa with various prey in the laboratory in order to provide a mechanistic explanation for this phenomenon. The quantities we measured were capture efficiency (number of prey captured on tentacle/number of prey entrained in flow), retention efficiency (number of prey ingested/number of prey captured) and percent of time spent swimming. We found that capture efficiency was significantly higher with larval ctenophores of the species *Mnemiopsis leidyi* (88.0%) than with the crustacean *Artemia salina* (59.6%). This suggests that at this stage in the predator-prey interaction, soft-bodied prey are more likely to be captured. Data on other variables and prey are still being collected and analyzed. We hypothesize that retention efficiencies will be similar between soft and hard bodied prey or favor soft bodied prey, and also that prey type may affect swimming behavior and hence, mode of foraging.

Development of a Comprehensive Model with Biphasic Regulation for Intracellular Ca^{2+} Dynamics

Beltramini, A., Borges, A., and Kadar, S.

Department of Chemistry, Salve Regina University

The purpose of this project is to create a comprehensive model that combines existing models with the representations of known cellular processes in order to account for more than any of the models do alone. There are two general models that have been used in the past. One describes the G-protein cascading mechanism the other describes the Ca^{2+}-Induced-Ca^{2+} Release (CICR) from the Endoplasmic Reticulum (ER). Neither of these models account for the biphasic regulation of the IP_3 receptor (IP_3R) by Ca^{2+}. The model used in this project integrates all of the aspects of the cellular functions that have been previously included in models as well as the biphasic regulation of the IP_3R. This model has allowed for the reproduction of experimental data that the other models are unable to replicate.

Bloom Syndrome Protein: A Study of Protein Partnerships

Bergeron, K.L., Chekaluk, Y.I., Falcao, P.B., Murphy, E.L., and Almeida, K.H.

Department of Physical Science, Rhode Island College

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Bloom syndrome is a rare recessive disorder characterized by premature death due to a predisposition to a wide array of cancerous states. An increase in genomic instability is used to diagnose Bloom Syndrome however; there is no known cure. Bloom Syndrome is caused by mutations of the BLM gene. The Almeida lab focuses on the partnerships of the BLM protein known to influence genomic stability**. Method:** The N- and C-termini of BLM may affect stability by partnering with the homologous recombination repair protein Rad51. Therefore, a systematic set of deletion mutants has been generated for each terminus. DNA corresponding to each polypeptide fragment was cloned into Gateway entry vectors, sequenced and recombined into destination vectors for expression in *E. coli*. The oligopeptide fragments contain both a 6x His epitope tag on the N-terminus and a Flag epitope tag on the C-terminus. **Results:** Each fragment was enriched to near homogeneity on Nickel affinity columns. The remaining impurity should not affect interaction studies, as the impurity is endogenous to the *E. coli* background. **Conclusions:** Co-Immunoprecipitation analysis will determine the strength of each fragment's partnership with Rad51.This study will clarify the role of BLM in maintaining genomic stability.

Rocky bench habitats create thermal refuges for intertidal organisms.

Bernhardt, J., Bromberg, K., and Leslie, H.

Department of Ecology and Evolutionary Biology, Brown University

RI-EPSCoR Summer Undergraduate Research Fellowship Program

Gradients of thermal stress are known to determine the biological zonation of rocky shores, and are thus important in understanding the distributions of intertidal organisms. The thermal landscape of a rocky shore is influenced by local climate and tidal cycle, as well as by the orientation and slope of the substrate at any given microsite. One aspect of the thermal landscape that is potentially important, but understudied, is the size of the substrate. To examine the effects of rock size and resulting thermal stress on the distribution of intertidal organisms, we surveyed the upper vertical limit of the acorn barnacle *Semibalanus balanoides* on cobbles, boulders and benches across Narragansett Bay, RI. We also transplanted cobbles to bench substrates and used concrete to manipulate effective rock size. We found the upper limit of barnacles to be highest on benches, and lowest on cobbles. Barnacle survivorship on cobbles was increased experimentally by increasing effective rock size. Thus, larger rocks, via their thermal buffering capacity, increase the survivorship of *Semibalanus*, and thereby influence its distribution across the rocky shore. The effects of substrate size are likely to be widespread and scale up to the community level because *Semibalanus* is a dominant and important intertidal species. These results suggest that rocky bench habitats may provide a thermal refuge for barnacles and other intertidal organisms; and, consequently, might be an effective target for conservation in the face of global climate change.

An exploration of a comprehensive mechanism for intracellular calcium oscillations

Borges, A., Beltramini, A., and Kádár, S.

Department of Chemistry, Salve Regina University

A comprehensive model of calcium dynamics was developed to account for the short comings in two existing models that represent the mechanism of intracellular calcium oscillations. The two models that are studied are the receptor-operated model by Cuthbertson & Chay [1], and the Ca^{2+}-induced Ca^{2+} release (CICR) model of Borghans, Dupont, & Goldbeter [2]. Both models are robust enough to model fundamental characteristics of calcium dynamics; however do not comprehensively discuss the overall process. Once combined, various aspects of the model behaved independently, however their relationships were key factors to the dynamics of the model. Existing data was used to test the effectiveness of the combined model. The combined model realistically reproduces the experimental procedure and data, and as a result, can be used to further predict processes regulating calcium signaling in the cell.

Axonal guidance mechanisms of olfactory neurons

Bowers, L., Tsai, L., and Barnea, G.

Department of Neuroscience, Brown University

RI-INBRE Summer Undergraduate Research Fellowship Program

There are over 1000 different olfactory receptors expressed in the mouse olfactory epithelium, with each individual olfactory sensory neuron expressing only one of these receptors. Olfactory sensory neurons that express a particular receptor are distributed randomly within zones of the olfactory epithelium, but their axons all project to topographically fixed glomeruli within the olfactory bulb. Research conducted by Barnea et al. (2004) and by Feinstein and Mombaerts (2004) reveal that changes in the amino acid sequence of an olfactory receptor perturb the neuron's ability to project to the glomerulus, suggesting that the receptors themselves play a role in axonal guidance within the olfactory system. In order to further investigate the role of the olfactory receptor in axonal guidance, we are assembling a targeting construct to alter the sequence of the citronellal receptor, C6, in mice. Using this construct, we will be able to alter important binding sites on the C6 receptor to determine their role in axonal guidance to the C6 glomerulus. The construct will also introduce GFP through an internal ribosomal entry site inserted after the C6 coding sequence to allow us to visualize the effect of each specific change on axonal guidance in the olfactory bulb.

Synthesis and derivation of Tamoxifen analogues utilizing the Wittig reaction

Burke, Liam P. and Williams, J. C. Jr.

Department of Physical Sciences, Rhode Island College

RI-EPSCoR Summer Undergraduate Research Fellowship Program

Tamoxifen, a selective estrogen receptor modulator (SERM), is an important compound used in the treatment and prevention of breast cancer. Other SERMs may prove to be pharmaceutically useful because of their interactions with the estrogen receptors that may inhibit carcinogenesis in breast tissue while minimizing other, undesirable, effects. The goal of this research is to use phase-transfer catalysis as part of the Wittig reaction to synthesize di and triaryl ethene Tamoxifen analogues with variable functional groups at the position of the dimethylaminoethylphenolic ether in Tamoxifen. These derivatized molecules will be screened for inhibitory interactions with estrogen receptors, correlation of QSAR variables with activity, and docking to estrogen receptors by computational modeling with a program using structures from the Protein Data Bank. The compounds will also be screened for activity against human breast cancer cell lines and Hep2G cells, as well as for activity towards the aryl hydrocarbon receptors.

Determining the Copy Number of *Ld*LIP3 Transcripts in *Leishmania* Promastigotes Using Real-Time PCR

Byrne, M. and Shakarian, A.

Department of Biology and Biomedical Sciences, Salve Regina University

RI-INBRE Summer Undergraduate Research Fellowship Program

Lipases are enzymes that break down ester bonds in fat molecules. It is hypothesized that lipases play a major role in the biology of the protozoan parasite, *Leishmania spp*. *Ldlip3* is a candidate gene in *Leishmania* which codes for a secretory lipase. To determine the number of copies of LdLIP3 specific transcript in *Leishmania* promastigotes, real time reverse transcriptase PCR was used to synthesize cDNA from RNA and display the amount of PCR product while the reaction occurred. Standard curves were constructed of cloned *Ldlip3* genes of known concentration to determine the amount of *LdLIP3* transcribed. *β*-tubulin, a housekeeping gene which is expressed constitutively by the parasite, was used as a control for the real time reverse transcriptase PCR reactions. The relative amount of transcript expressed from the *Ldlip3* gene of *Leishmania tarentolae* was determined using serial dilutions of RNA samples subjected Real time reverse transcriptase PCR with gene specific primers. The number of copies of *LdLIP3* specific transcript per each cell was determined. In continuing studies levels of transcript will be compared between pathogenic and non-pathogenic strains of *Leishmania.*

A Biochemical Characterization of Recombinant Lipases Secreted by *Leishmania*

Byrne, M., Ganim, C., and Shakarian, A.

Department of Biology and Biomedical Sciences, Salve Regina University

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Lipases catalyze the hydrolysis of fats to form glycerol and fatty acids. Secreted lipases have even been implicated as virulence factors of some pathogens. Experimental evidence to support the biological role of secreted lipases in *Leishmania* remains to be elucidated. The goal of this project was to express the putative secretory lipase gene *LdLIP3* from the human pathogen *Leishmania donovani* and determine it's lipolytic activity using 4-methylumbelliferone fatty acid substrates.

Methods: Culture supernatants of *Leishmania* promastigotes grown in M199 medium without serum were harvested, concentrated and incubated with various 4-methylumbilliferone fatty acids substrates in McIlvaine's buffer buffer at a pH range of 3 - 8 for 30 min at 26°C, 37°C or 42°C. Results indicate that supernatant from transfected cell lines produce an increase of lipolytic activity when compared to control cells under optimal assay conditions using 4 methylumbelliferone-fatty acids as substrate.

Results: We have identified lipase activities from *in vitro* culture supernatants of *Leishmania* using a range of 4-methylumbilliferone substrates from acetate to dodecanoic acid. It was determined that the optimal activity was obtained at 37°C in McIlvaine's buffer at pH 4.0-5.5.

Discussion and Conclusions: Thus, our data indicate that the *LdLIP3::HA* lipase was successfully expressed. This achievement will allow us to more closely investigate the physical and biochemical properties of the secreted lipase and define its possible role in the life cycle of *Leishmania*.

Characterizing the Responses of Different Cell Types of the Yeast *Saccharomyces cerevisiae* to Ethanol-induced Program Cell Death

Cao, Y. and Austriaco, N.

Department of Biology, Providence College

RI-INBRE Summer Undergraduate Research Fellowship Program

Apoptosis or programmed cell death is a form of cell suicide that occurs when cells are exposed to stressful conditions. High levels of ethanol, a by-product of yeast fermentation, have been shown to induce apoptosis in the budding yeast *S. cerevisiae*. Just as the human body consists of several cell types from muscle cells to liver cells, yeast also occurs in several cell types. These include the haploid **a** and **α** cells, the diploid **a/α** cells, and the pseudohyphal invasive cells. While most of the yeast strains used in laboratories worldwide are domesticated strains unable to undergo filamentation, we are using a wild type strain that is capable of dimorphic shift from the ∑1278b strain background to investigate the responses of the different cell types to ethanol-induced apoptosis. We have shown that while haploid cells have a similar response after exposure to 22% ethanol, the filamentous form of the haploid has greater tolerance for the ethanol. We are continuing to characterize the behavior of diploid yeast cells in 22% ethanol. While investigating the effects of ethanol on butanol-induced haploid invasive cells, we discovered that the stress from butanol and ethanol together gives a higher death rate than ethanol alone.

Pyrethroid inhibition of the mammalian T-type voltage-sensitive calcium channel ($Ca_v3.2$)

Catlin, N., Mutanguha, E., and Symington, S.B.

Department of Biology and Biomedical Sciences, Salve Regina University

RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroids are widely used insecticides in both agricultural and vector control programs. Given the widespread use of pyrethroids for the control of insect vectors of devastating human and animal diseases (particularly in urban environments) and additional exposure via dietary uptake, human consumption is virtually certain. The purpose of this research was to determine the effects of pyrethroid insecticides on the current characteristics of a human t-type voltage-sensitive calcium channel ($Ca_v3.2$). Human $Ca_v3.2$ cDNA was transcribed into cRNA using the mMessage mMachine *in vitro* transcription kit and injected into defollicated *Xenopus* oocytes. Human $Ca_V3.2$ currents were characterized using two-electrode voltage clamping with Ba^{2+} as a charge carrier. Pyrethroids effects on the overall peak current, voltage-dependent activation and inactivation, and steady-state activation and inactivation tau were determined following perfusion with various concentrations of bioallethrin, cypermethrin, deltamethrin, fenpropathrin, and permethrin. Deltamethrin appeared to be the most potent pyrethroid examined and reduced the overall peak current in a concentration-dependent manner with an approximate EC_{50} of 10^{-13} M. Furthermore, 40% of $Ca_v3.2$ peak current was inhibited by 10^{-7} M deltamethrin, a concentration that elicits a maximum response, compared to $Ca_v3.2$ expressing oocytes treated with DMSO (solvent control). In other preliminary experiments, 1R-cypermethrin (a toxic enantiomer) inhibited peak current in a similar fashion as deltamethrin. However, the non-toxic, 1S-cypermethrin had no effect on $Ca_v3.2$ peak current compared to DMSO control. Preliminary results using a suite of pyrethroids (bioallethrin, cypermethrin, deltamethrin, fenpropathrin, and permethrin) indicate that the action of pyrethroids on $Ca_v3.2$ is structurally related.

Dendritic DNA analysis using nanofluidic channels and solid-state nanopores

Chan, J., Jiang, Z., Ren, Y., Stein, D.

Department of Physics, Brown University

RI-EPSCoR Summer Undergraduate Research Fellowship Program

Nanopores and nanofluidic channels are powerful tools to analyze and manipulate single DNA molecules. Until now, nanopores and nanofluidic channels have never been used to analyze dendritic molecules. We have synthesized a three-pronged "Starfish" DNA molecule through a series of standard ligations, which will be analyzed using nanopores and nanofluidic channels. A nanopore is a tiny hole with a diameter of 2-30nm, approximately 10,000 times thinner than a human hair. While passing through a nanopore, individual DNA molecules can be detected as dips in the current that flows through the pore. "Starfish" will be driven across a nanopore on a silicon chip to test whether a solid-state nanopore can identify a physical feature along single molecules, namely the three-arm junction of "Starfish". On the other hand, a nanofluidic channel is a transparent, rectangular-shaped device that is typically a few millimeters long, 2-100μm wide and 50-500nm deep. Fundamental scaling theories for linear polymers by de Genne and Odijk have been extensively tested by driving λ-phage DNA molecules through nanochannels filled with ionic solution (Stein 2006). We will introduce "Starfish" into nanochannels to test the scaling theories of de Genne and Odijk for star-shaped polymers.

Barnacle growth rates vary spatially and temporally at sites with differing productivity

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Past studies have shown that primary productivity can have large effects on organisms living in the intertidal zones. Areas with higher primary productivity seem to provide organisms with greater rates of recruitment and growth (Sanford and Menge 2001).

We compared the growth rates of the intertidal barnacle, *Balanus glandula*, at two capes: one of which has relatively high productivity, while the other has relatively low productivity. Building off of previous research done at the capes, we analyzed photos of barnacles to determine the growth rate of barnacles at three sites within each cape.

Barnacles at the high productivity cape grew to twice the size of those at the low productivity cape. This study provides additional evidence of a powerful bottom-up effect occurring in this region of Oregon. Previous studies have shown that barnacles also have greater larval production in areas of higher productivity than compared to areas with lower productivity (Leslie et al. 2005). Thus, greater reproductive potential and higher growth rates are linked and are mutually affected by ocean conditions. Both of these factors are important precursors for setting up marine protected areas.

Characterization of a novel bifunctional dehydrogenase enzyme in *Entamoeba invadens* and its importance in finding anti-amebic compounds

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RI-INBRE Summer Undergraduate Research Fellowship Program

The protozoan parasite, *Entamoeba histolytica,* infects fifty million people and causes one hundred thousand deaths per year. Amebiasis is the third leading cause of death from parasitic diseases and is primarily treated with metronidazole. Toxicity and drug-resistance concerns have prompted the search for alternative therapeutic agents. A promising approach is the development of novel anti-metabolites. *Entamoeba* is an ancestral protozoan organism that evolved shortly after the branching of prokaryotes and eukaryotes and lacks mitochondria, deriving its energy from the fermentation of glucose to ethanol. An important enzyme in this pathway is, *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2), which possesses both alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities. EhADH2 expression is required for the growth and survival of *E. histolytica* trophozoites. We have identified a new member of the ADHE family in *Entamoeba invadens*, an amoebic parasite found in several different reptile taxons which causes amebiasis and derives a similar type of colon pathology to that of the *E. histolytica* pathogen found in humans. *E. invadens* is a useful model system because the homologous bifunctional enzyme *Entamoeba invadens alcohol dehydrogenase E* (EiADHE) is more tolerant to oxygen than *E. histolytica* and is a better model for enzymatic analysis. The *E. invadens* genome has 60% sequence identity with *E. histolytica,* including proteins implicated in amoebic virulence and drug resistance. By creating mutations in the genetic sequence that encode essential amino acids in the catalytic site(s) of EiADHE, it will be possible to analyze their effect on both enzymatic activities. A comparison between EhADH2 and the *E. invadens* novel protein will provide new insights in their structure and function. Cycloalkanols, amines, halides, tertiary alcohols are expected to be non-toxic to humans at the concentrations required to eliminate *E. histolytica* trophozoites.

Green Chemistry to Make Toxic Compounds; II

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RI-INBRE Summer Undergraduate Research Fellowship Program

A redesign of the synthesis of a series or arylphosphonium salts for toxicology studies of their anti-bacterial, and cytotoxic activity is underway. The solid-state reactions are done in a dental amalgamator at temperatures around 100°C from the kinetic energy produced by the shaking of the stainless steel capsule and ball pestel. The reactions' progress is monitored by melting points of the reaction mixtures and the final products are isolated and recrystallized from hot water or ethanol. This reaction can also be done using a mortar and pestle and muscle power. The salts formed from alpha-bromotoluic acid and triphenylphosphine are isolated nearly quantitatively and a series of compounds using substituted triarylphosphines are also isolated in high yield. We have observed structure-reactivity relationships, where both electronic and steric factors affect the yields. A solid-state Fisher esterification of the toluic acid salts using Dowex as the catalyst has also been accomplished.

Priming of Inflammatory Responses in Microglial Cells by Lipopolysaccharides from Pathogenic Marine Vibrios

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RI-INBRE Summer Undergraduate Research Fellowship Program

Lipopolysaccharides (LPS) are cell wall components of Gram-negative bacteria and serve as endotoxins during infections of other organisms, particularly humans. When in the central nervous system, LPS can aggravate brain microglia and prime the release of neuroinflammatory mediators, including tumor necrosis factor-α (TNF-α), superoxide anion (O_2^-) and thromboxane B_2 (TXB_2). LPS can cause microglia to become chronically active and overproduce these pro-inflammatory chemicals, leading to the injury of surrounding tissues. LPS are currently suspects in the onset of neurodegenerative diseases, such as Alzheimer's and Parkinson's.

Vibrio vulnificus is a marine pathogen that causes infections in humans, generally after a person has eaten contaminated seafood. *V. vulnificus* can also infect through open skin wounds. A recent study found that *V. vulnificus* LPS was ten-fold less potent than *E. coli* LPS at priming rat brain microglia for release of O_2^-, TXB_2, and TNF-α. *V. vulnificus* capsular polysaccharide had no effect on the brain microglia, consistent with LPS being the primary trigger for enhancing the pro-inflammatory responses. The aim of this project is to explore the variability in LPS toxicity among other pathogenic marine *Vibrio* species. A panel of pathogenic marine vibrios, including V*. harveyi*, V*. vulnificus*, and V*. anguillarum*, will be cultivated on marine media, and the resulting LPS will be extracted and purified using an optimized method. LPS from the various species will then be tested for enhanced production of O_2^-, TXB2, and TNF-α and lactate dehydrogenase in rat brain microglia.

Escherichia coli Nissle 1917 Uses L-Arabinose but not D-Ribose to Colonize the Mouse Intestine in Mice Pre-Colonized with *E. coli* MG1655

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RI-INBRE Summer Undergraduate Research Fellowship Program

Why *Escherichia coli* colonizes the mammalian intestine relates to its symbiotic relationship with the host: the host creates an environment favorable for *E. coli* to grow and *E. coli* creates an anaerobic environment favorable for the anaerobes that are so important for intestinal health. However, little is known about the nutrients *E. coli* uses to grow in the intestine. Moreover, recent evidence suggests that two different strains of *E. coli*, *E. coli* MG1655 and *E. coli* Nissle 1917, isolated from different humans, can colonize the mouse intestine simultaneously and that each uses different nutrients to do so. Our interest is in identifying those nutrients. In the present study, mice were pre-colonized with *E. coli* MG1655 and10 days later were fed either *E. coli* Nissle 1917 and an *E. coli* Nissle 1917 Δ*rbsK* mutant, unable to use D-ribose for growth or *E. coli* Nissle 1917 and an *E. coli* Nissle1917 Δ*araBAD* mutant, unable to use L-arabinose for growth. *E. coli* Nissle 1917 and the *E. coli* Nissle 1917 Δ*rbsK* mutant grew from low to high numbers at the same rate in the mouse intestine and colonized the mouse intestine thereafter in equal numbers. In contrast, although the *E. coli* Nissle 1917 Δ*araBAD* mutant grew from low numbers to high numbers at the same rate as *E. coli* 1917 in the mouse intestine for 3 days, over the next week the numbers of *E. coli* Nissle1917 remained constant, whereas the numbers of the *E. coli* Nissle1917 Δ*araBAD* mutant dropped about 4 orders of magnitude. Therefore, while it appears that *E. coli* Nissle1917 uses neither D-ribose nor L-arabinose to grow from low to high numbers in mice pre-colonized with *E. coli* MG1655, once high numbers are reached, it uses L-arabinose, but not D-ribose, to maintain itself.

Identification and Characterization of the Gene Encoding a Secretory Lipase in the Human Pathogen *Leishmania Donovani*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background: The cell and molecular biology of *Leishmania* are investigated as models of human parasitism. Emphasis is placed on characterizing their secreted proteins toward defining the roles of these constituents in parasite survival and development. We hypothesize that the lipase activity released by *Leishmania* may be involved in the alteration of host cell membrane architecture, suggesting that lipase plays an important role in the biology of *Leishmania.*

Methods: A PCR approach was used to clone and sequence the *L. donovani* homologue of one of these lipases, LdLIP3. Southern hybridization of gDNA from *L. donovani* was used to determine the structure and copy number of the LdLIP3 lipase gene loci.

Results: Sequence analysis of LdLIP3 revealed an ORF of 927bp and a protein of 308aa with a predicted molecular mass of 33.0kDa. Further analysis showed a putative 24aa signal peptide, and the absence of an anchor motif, both consistent with this lipase being secreted. A conserved serine-lipase active site (LVTGHSV) was identified at position 162-171. Southern blot analysis revealed the presence of more than one copy of LdLIP3 in the *L.donovani* genome.

Discussion and Conclusions: Our data supports the hypothesis that lipase activity is encoded by *L. donovani.* Results should provide the tools we need to further characterize this gene and its activity which will lead to a better understanding of the role of lipase activity within the biology of the important group of human pathogens.

Sensitive Electrochemical Immunoassay for MMP3 Using Gold Nanocatalyst Amplification Strategy

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RI-INBRE Summer Undergraduate Research Fellowship Program

Matrix Metalloproteinase-3 (MMP3) is a peptidase and a member of the larger MMP family. MMP2 and MMP9 are known biomarkers for prostate cancer, and MMP3 is an activator of both of these. The early detection of cancer biomarkers can greatly improve patient's prognosis, treatment success and may even lead to cancer prevention. In order to achieve accurate and ultra-sensitive detection of cancer biomarkers, we have developed a novel hybrid method using an electrochemical sandwich immunoassay based on single-walled carbon nanotube platform and gold nanocatalyst label. The analytical signals are derived from mediated nitrophenol reduction catalyzed by multiple Au nanoparticles loaded on a polymeric bead for signal amplification. Preliminary results indicate a remarkable low detection limit in the Zepto Molar range. The combination of gold nanocatalysts and carbon nanotubes seems to be an incredibly powerful tool for ultra-sensitive detection of protein biomarkers and offers great promise for a rapid, simple, cost effective method for clinical screening of cancer biomarkers and point-of-care diagnosis.

Cloning of a mouse T-type voltage sensitive calcium channel ($Ca_v3.1$) into *Xenopus* expression vector

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RI-INBRE Summer Undergraduate Research Fellowship Program

Previous attempts to express a mouse t-type voltage-sensitive calcium channel ($Ca_v3.1$) in *Xenopus* oocytes have failed even though cRNA has been successfully transcribed *in vitro* and injected into oocytes. The likely reason for no expression is due to the lack of the 5'untranslated region of the *Xenopus* globulin gene, a region that improves expression of a variety of genes in this expression system. The goal of this research project was to incorporate the mouse $Ca_v3.1$ gene into the *Xenopus* expression vector that contains the 5'untranslated region of the *Xenopus* globulin gene. The mouse $Ca_v3.1$ gene was PCR amplified with homologous primers that also possessed the ClaI and AflII restriction sites, ligated into a TOPO expression vector, and cloned into *E.coli* TOP10 cells. The newly cloned $Ca_v3.1$ was isolated, digested with ClaI and AflII and gel purified. The *Xenopus* expression vector, which has been used successfully to express ion channels in oocytes, was also digested using the same restriction enzymes. The purified vector and $Ca_v3.1$ were then ligated and cloned into *E.coli* TOP10 cells and the newly constructed plasmid utilized to synthesize $Ca_v3.1$ cRNA. It is expected that the newly synthesized cRNA will be successfully expressed in *Xenopus* oocytes.

Resolving old questions in mollusk phylogenetics with phylogenomics

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

The protostome tree of life was recently reworked using the relatively new science of phylogenomics, where phylogenies can be created using large-scale sequencing of ESTs (expressed sequence tags) using new sequencing technologies. Many old relationships were solidified and some surprising discoveries were made, but even though the overall picture has become more definitive, the phylum Mollusca still has many old, unresolved questions that must be answered. There is little resolution within various morphological and molecular studies, so this project has been designed to use phylogenomics to address this problem and answer some, if not all, of those questions about the evolutionary history of Mollusca and the relationships within and between the classes. Samples of mollusks from all over the world are being collected and sent to the Dunn lab where RNA is extracted out of the tissue. The mRNA from this total RNA is then extracted and a cDNA library is created. As of this point, five samples have been used to create cDNA libraries, which have been sent off to the Broad Institute at Harvard to be sequenced. A new sequencing technology developed by Solexa is being used to sequence the cDNA to create numerous ESTs, and once this data comes back to our lab, sophisticated analysis of the data will begin to create the most comprehensive mollusk phylogeny to date. This will finally let scientists know what the internal relationships of gastropods are, whether the "true shell" of mollusks evolved once or multiple times, and various other answers to the mysteries of one of the most diverse phylums on the planet.

Germline Segregation, Morphology, and Cloning in Echinoderms

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

While mechanisms of fertilization and early development in Echinoderms has been deeply studied, the process by which these organisms develop from larvae to adults has yet to be thoroughly examined. The limited recent examination of these developmental stages has revealed that select organisms have demonstrated the ability to clone in the presence of an abundance of resources (nutrition and habitat) or in the presence of predators. It has been previously demonstrated that Echinoderms use an epigenetic developmental mode meaning that there is clear differentiation of germ line post establishment of primordial major organs. The timing of cloning in these organisms has been noted to take place after the establishment of the germ line. By examining various species of Echinoderms from fertilization to adulthood, morphological features and molecular development of their germ line through tracking of germ line specific proteins vasa and nanos was examined; the focus of the project was on whether the clonal bud originates from cells of the segregated germ line or from determined somatic cells. Additionally, these species will be further examined and compared to various species from taxon Cnidarian which share highly conserved vasa and nanos genes to those in Echinoderms. These studies will hopefully determine evolutionary significance of cloning and germ line segregation among species within and between taxa as well as reveal more about the mechanisms of Cnidarian development.

HDAC1 is required for cardiac development in zebrafish

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Histone Deacetylase 1 (HDAC1) catalyzes the removal of acetyl groups on DNA-bound histone proteins, changing local chromatin conformation and gene expression. Zebrafish lacking HDAC1 develop abnormally, revealing a role for HDAC1-assisted gene regulation in the nervous system, cartilaginous structures and the cardiovascular system. Characterization of the neural defects in the mutant *hdac1*$^{hi1618Tg/Tg}$ has been published; however, the cardiovascular defects are only briefly noted in the literature. We have found that HDAC1 deficiency leads to malformed, poorly performing hearts and regional vascular defects.

The first aim of this project was to determine the embryonic *hdac1* gene expression pattern. Wholemount insitu hybridization revealed that *hdac1* mRNA is present in the heart and in vascular regions sensitive to loss of HDAC1 function. Because some *hdac1* transcript appears to be present in the *hdac1*$^{hi1618Tg/Tg}$ mutants, we are currently examining expression of genes that may serve as downstream targets of HDAC1 regulation.

The second aim was to characterize the cardiac functional defects in the *hdac1*$^{hi1618Tg/Tg}$ mutant. Here we report that HDAC1 is required for normal cardiac tube morphology and heart rate from the onset of rhythmic beating. Heart rates in the *hdac1* mutant are lower than the wild type sibling at all stages. We also found that the mutant heart rate is insensitive to downward temperature shift on early day one (27 hours post fertilization, hpf) but develops proportional sensitivity by day 2 (48 hpf). We are currently examining the HDAC1-dependent components of cardiac pace making via pharmacological channel block.

Characterization of the Autophagy Gene *ATG16* in a *Saccharomyces cerevisiae* Model of Crohn's Disease

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RI-INBRE Summer Undergraduate Research Fellowship Program

Crohn's Disease (CD) is a chronic inflammatory bowel disease that can cause stricturing and fistula formation in the gastrointestinal track in addition to other symptoms such as abdominal pain, bleeding and malnutrition. Recent genome-wide association scans have associated two autophagy genes, ATG16L1 and IRGM, with CD. Autophagy is an important process that allows cells to regulate size, damaged proteins and recycle old cellular components. The autophagy process is carried out by the formation of double-membrane vesicles around the cytoplasm, which enclose the target for digestion by lysosomes. Current research has demonstrated that autophagy is essential to cell maintenance as well as defense against environmental pathogens. A great deal is still not known about the autophagy process and the connection between autophagy and the pathology of CD. To begin to explore this question, we are developing a model system using *Saccharomyces cerevisiae* to better understand the relationship between autophagy and CD. We hope to demonstrate that yeast cells with a defective autophagic pathway, because of a null mutation in the *ATG16* gene, are more susceptible to yeast viral pathogens. Our preliminary data suggests that ∆*atg16* yeast cells are more prone to MI viral infection than wild type strains. We are continuing to test the experimental protocol for better data in an attempt to prove autophagy plays an essential role in cellular defense against gut bacteria, and hence a role in the pathology of CD.

Functional and localization divergence of three shark AHRs

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RI-INBRE Summer Undergraduate Research Fellowship Program

Planar halogenated aromatic hydrocarbons (HAH) and some polycyclic aromatic hydrocarbons (PAH) are persistent environmental pollutants and are toxic in organisms with intact aryl hydrocarbon receptor (AHR) pathways. Organisms possessing AHR proteins with low affinity to HAH and PAH agonists are relatively resistant to their toxic effects. Mammals possess one *AHR* gene while other vertebrates have multiple AHR paralogs that differ in their structure as much among each other as they do among distantly related species. We investigate the structure and function of *Squalus acanthias* (spiny dogfish shark) *AHR*s to identify role partitioning that may be present among the genes. Through cDNA cloning and sequence analysis we've discovered that sharks possess three *AHR* genes, two of which appear to be orthologous to the *AHR1* and *AHR2* of other vertebrates. Our goal was to test the ability of the three AHRs to be activated by typical AHR ligands and induce expression of a reporter gene driven by dioxin response elements. We report that the shark AHRs differ in their ability to activate the reporter with addition of PCB126, a HAH agonist, and 3-methylcholanthrene, a PAH agonist. AHR2 and AHR3 can activate the reporter, whereas AHR1 does not. To investigate cellular localization and response to agonists, we use GFP-fusion proteins and transient transfection in mammalian cell lines. Consistent with the reporter assays, AHR2 and AHR3 can locate to the nucleus while AHR1 is unresponsive, remaining in the cytoplasm. We are currently performing dose-response experiments and ligand binding assays to rigorously test these results, and investigating the localization of the three AHR proteins in the *S. acanthias* embryonic stem cell line (SAE) cells. Our results have thus far supported a distinct divergence in the localization and function of the three paralogs. Future research includes examining the role for these AHRs in pathways outside of the adaptive toxic response.

Click Chemistry Synthesis of Triazoles for Screening Against Estrogen Receptors

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RI-INBRE Summer Undergraduate Research Fellowship Program

Tamoxifen and GSK-4716 are potent selective estrogen receptor modulators (SERMs). Tamoxifen, a prominent treatment option for breast cancer, inhibits estrogen binding in breast tissue. However, this is frequently accompanied by an increase in estrogenic activity in other tissues, sometimes resulting in various deleterious effects. Synthesis of triazoles is being conducted in order to prepare a library to screen against estrogen receptors. These compounds are structural analogs of Tamoxifen. Diphenylacetylene from Sigma-Aldrich, or as synthesized by using methods from a standard laboratory text, was used in "Click" chemistry reactions with aryl or benzyl azides to form the triazoles. Reactions were monitored by thin layer chromatography (TLC) and infrared spectroscopy (IR), isolated by column chromatography and confirmed by IR and mass spectroscopy (MS).

Synthesis and *in vitro* screening of novel anti-estrogens

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RI-INBRE Summer Undergraduate Research Fellowship Program

The estrogen receptor (ER) is a nuclear receptor expressed from two different genes, alpha and beta; and expression levels of ER vary throughout the body. Breast cancer cells express ERα and ERβ and breast tumor growth often depends on the mitogenic effects that are the result of estrogen binding to ER. One way to stop breast cancer cell growth is to use direct-acting anti-estrogens that block estrogen binding to ER. Multiple new chemical entities (NCEs) were synthesized in the laboratory of a collaborator (Dr. John Williams, Rhode Island College) and were screened for putative anti-estrogenicity in the hormone-responsive breast cancer cell line ZR-75-1. ZR-75-1 cells were transiently transfected with $p(ERE)_3$-TATA-Luc (an estrogen-responsive reporter plasmid) and luciferase assays were performed with the NCEs alone or in co-treatment with estrogen. Results show that at least one compound (RIC#26, identity blinded) significantly inhibited estrogen-induced luciferase activity. Additionally, RIC#26 inhibited also the estrogen-dependent upregulation in ZR-75-1 cells of the endogenous gene TFF1 (PS2). Subsequent chemical syntheses will be performed based on the collated results of these screens with the goal of designing more potent and selective anti-estrogens.

Characterization of the Human Aging Gene, *klotho*, in the Yeast, *Saccharomyces*

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

The human gene *klotho* functions as an aging-suppressor gene. *Klotho*-deficient mice have been found to exhibit premature aging qualities, including shortened lifespan, infertility, skin and muscle atrophy, osteoporosis, and cognitive impairment. Klotho overexpression in cells has been shown to incur resistance to oxidative stress, which is involved in the pathogenesis of various disease conditions and, ultimately, cell death. Despite these observed phenotypes, the mechanisms through which klotho protein acts as an anti-aging factor remain poorly understood. To provide a further characterization of its activities, we have overexpressed the *klotho* gene in the yeast, *Saccharomyces cerevisiae*, using the Invitrogen Gateway System. Our preliminary results suggest that *klotho* enhances yeast cell growth. However, in the process of sequencing our clone, we identified a potential point mutant that would change the amino acid sequence of the klotho protein. We are confirming this finding and correcting it.

Monitoring predation on Oyster Reef Balls® remote set with eastern oysters (*Crassostrea virginica*) in Mount Hope Bay, Bristol, RI

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RI-EPSCoR Summer Undergraduate Research Fellowship

The Eastern Oyster (*Crassostrea virginica*) is an important fishery in the Northeast. Restoration efforts have been underway in order to replenish the natural population which has declined due to overharvesting and disease. Reef Balls® have shown promise in restoration efforts. Oyster Reef Balls® were used for this project because they contain bits of shell on the outside to promote setting and because of their smaller in size (16"x10"). Two study sites, one near the Learning Platform and one away from the platform were chosen to observe predation. Each study site consisted of 7 Oyster Reef Balls ® which were placed in about 3'-4' of water at mean low water. The bottom substrate in the area was generally bare and just contained sand and some macro-algae. The oysters were grown and set onto the Oyster Reef Balls® using the remote setting method. Conditioning of adult oysters started in May and consisted of raising the water temperature and feeding for about 6 weeks. The broodstock were spawned on June 3, 2008 and larvae were collected, inspected, and grown in tanks for about 14 days until they were ready to set. About 800,000 larvae were added to the Reef Ball ® tanks on June 15th and cared for until reaching about 1mm. On July 8th the oysters were counted to obtain an initial stocking density of ~33 oysters/Reef Ball®. The Reef Balls® were deployed into Mount Hope Bay on July 9th. Monitoring consisted of walking out to the sites and using a floating basket to lift up the Reef Balls® one at a time for inspection. Monitoring was done daily for three weeks, July 10th to July 30th. Many predators of the oysters, including crabs [Long Clawed Hermit Crab (*Pagurus longicarpus*), Spider Crab (*Libinia emarginata*), Green Crab (*Carcinus maenas*), Black-Fingered Mud Crab (*Panopeus sp*.), Asian Shore Crab (*Hemigrapus sanguineus*)], fish [Tautog (*Tautoga onitis*), Oyster Toadfish (*Opsanus tau*), Cunner (*Tautogolabrus adspersus*), Tidepool Sculpin (*Oligocottus maculosus*), Winter Flounder (*Pleuronectes americanus*)] and oyster drills (*Urosalphinx cinerea*) were observed in at study sites. The oyster mortality and growth were also monitored.

Identification of Alpha-Toxin in *Staphylococcus aureus*

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Background: Alpha-toxin (hemolysin) production is related to virulence production and has been related to the development of biofilms via quorum sensing. By inhibiting alpha-toxin production with antimicrobial agents that target various sites of protein synthesis, we should therefore be able to effect biofilm production.

Methods: We quantified biofilm formation and alpha toxin production in 24 randomly selected biofilm and alpha toxin producing clinical *S. aureus* strains. We used prototype high-level producers of alpha-toxin and biofilm (ATCC 10832, ATCC 35556, ATCC35984, ATCC 12228) respectively) as controls. Alpha-toxin production was quantified a newly developed, method implementing a 2.0 McFarland and spot plating five ul onto a sheeps blood agar plate. This method was developed to ensure the inoculum size was the same for each sample. The zone of lysis was measured. Biofilm formation was evaluated and quantified using the Biofilm microplate assay previously described by Christenson and Stepanovic. We also identified the MIC of each isolate against clindamycin, gentamicin, linezolid, rifampicin, levofloxacin, oxacilllin, erythromycin, daptomycin and vancomycin. The minimum inhibition concentration is measured with the E-test strips. The results for the controls are given, however the rest of the results with respect to the rest of the 24 clinical isolates is also in progress. Further experimenting with checking biofilm production is in progress.

Results: After identifying 24 clinical isolates, production of alpha hemolysin was highly apparent. The zone of hemolysis was measured quantatively, and characterized with a mean of 5.82mm +/- 1.38 and a range of 4.5 to 10mm. MIC and Biofilm results are as follows:

	ATCC35984 (ug/ml)	**ATCC 10832 (ug/ml)**	**ATCC 35556 (ug/ml)**	**ATCC 12228 (ug/ml)**
LND	0.38	0.75	0.38	0.38
RIF	0.004	0.006	0.006	0.003
CLIN	-	<.016	>.016	<0.016
VAN	0.75	0.5	0.19	0.5
LEVO	0.047	0.094	0.047	0.047
GENT	6	0.19	<.064	<.064
DAP	0.125	0.5	0.023	0.25
ERY	-	0.064	0.047	0.047
OX	0.25	0.064	0.023	0.023
BIOFILM OD_{570}	1.983 +/- 0.31	0.284 +/-0.067	1.33 +/- 0.283	1.443 +/- 0.380

Conclusion: All 25 clinical isolates are alpha toxin producers ranging from 4.5-10mm of hemolysis on blood agar. All 4 control isolates are biofilm producers and are susceptible to the antibiotics tested. We will now move forward with this project to evaluate these isolates in an erythrocyte assay and biofilm inhibition assay.

mTOR activation protects against UVB-induced cell death in skin keratinocytes and dendritic cells

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While UV radiation activates MAP kinase pathway leading to apoptosis, cells fight against widespread cell death through activation of cell survival pathways such as PI3K/AKT pathway. mTOR as a survival factor serves as a signal integrator of several upstream signals, including growth factors, nutrients, energy levels, and stresses. However, the question whether UV activates mTOR pathway remains to be addressed. We used cell culture and Western blot analysis to address above question. We found that UVB induces mTOR activation in human skin keratinocytes and both cultured mouse dendritic cells (XS 106 cell line) and mouse monocyte derived dendritic cells (MoDC). Using specific inhibitors and gene manipulation methods, we observed that UVB induces mTOR activation is dependent on EGFR-mediated AKT activation. TSC2/mTOR, downstream of AKT, is involved in UVB-induced S6K activation. UVB also induces activation of AMPK (AMP-activated protein kinase) and p38 as well as its upstream signal LKB1. We conclude that mTOR activation serves as a novel survival signal against UVB-induced cell death. Our data suggests that LKB1/AMPK/p38 serves as a negative feedback pathway against UVB-induced mTOR activation.

Identification and Characterization of a Putative CUE Domain in the Fanconi Anemia D2 Protein

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RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi Anemia (FA) is a rare autosomal and X-linked recessive disorder characterized by congenital abnormalities, progressive bone marrow failure and pronounced cancer susceptibility. To date, 13 FA genes (*A, B, C, D1/BRCA2, D2, E, F, G, I, J, L, M*, and *N*) have been identified. At the cellular level the hallmark of FA is hypersensitivity to DNA crosslinking agents. In response to DNA damage and during S phase of the cell cycle, the FA core complex mono-ubiquitinates the FANCD2 protein, which signals its activation and localization to chromatin where it interacts with several proteins involved in DNA repair. While the function of FANCD2 mono-ubiquitination has been characterized, the mechanism by which this modification occurs remains largely unknown. Recently, much attention has been given to ubiquitin binding domains (UBDs) that are found in many ubiquitinated proteins and are often required for the ubiquitination of the proteins containing them. These domains may function as an interacting surface between a protein containing a UBD and ubiquitin conjugated to an interacting protein. As FANCD2 is monoubiquitinated and interacts with several ubiquitinated proteins, e.g. PCNA and REV1, we looked for the presence of UBDs within FANCD2. Using a bioinformatics approach we identified a portion of FANCD2 (aa204-234) that shares sequence similarity to a CUE domain, a subclass of UBD. CUE domains contain 3 critical amino acid residues essential for binding to ubiquitin. These consist of a proline separated by approximately 30 amino acids from a di-leucine motif. Using a PCR based site-directed-mutagensis approach we have generated individual FANCD2 CUE domain mutants, containing single and double amino acid substitutions at both the proline and di-leucine motifs respectively. FANCD2 CUE domain mutant retrovirus was generated in a 293GPG retroviral packaging cell line and used to infect patient-derived PD20F FANCD2 null fibroblasts to generate $FANCD2^{P204A}$ and $FANCD2^{LL234AA}$ cell lines. These cell lines are currently being used to characterize the role of this important CUE domain in DNA repair.

Sensitive Electrochemical Immunoassay of a Biomarker α-Fetoprotein Based on Carbon Nanotubes

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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 based on single wall carbon nanotubes (SWNT) for ˜-fetoprotein, a cancer biomarker protein in serum. This novel immunosensor features vertically aligned nanotubes 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 4.0×10^{-10} g mL^{-1} (5.0 pM) corresponding to 50 amol ˜fetoprotein in 10μl of undiluted calf serum. Our results compare favorably with the standard enzyme-linked immunosorbent assays (ELISA). Work is in progress to lower the detection limits using specially designed bioconjugates with multiple enzyme labels for signal amplifications. These easily fabricated SWNT immunosensors show excellent promise for clinical screening of cancer biomarkers and point-of-care diagnosis.

Influence of nekton abundance on egret selection of foraging sites in tidally restricted and unrestricted sections of Galilee Bird Sanctuary.

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Egrets (Ardeidae) are conspicuous and charismatic predators of nekton (small fish and invertebrates), and are useful indicators of environmental health. Little has been published on the influence of the abundance and spatial distribution of nekton on egret habitat selection within salt marshes in New England. We used throw traps to systematically sample the nekton at Galilee Bird Sanctuary. We investigated egret foraging strategies by sampling foraging and random locations at three sub sections with the Bird Sanctuary, two of which were tidally restricted and on that experienced unrestricted tidal-flow. We documented ten species of nekton, with four species more abundant in tidally restricted sites (*F. majalis* (Striped Killifish), *F. heteroclitus* (Mummichog), *Cyprinondon variegates* (Sheepshead minnow), and *Paleomonetes pugio.* (Grass shrimp), and six species more abundant at the unrestricted subsection (*C. crangon* (Sand shrimp), *Carcinus maenas* (Green crab), *Pagulurus longicarpus* (Long-clawed hermit crab), *Myoxocephalus aenaeus* (Grubby Sculpin), *Pseudopleuronectes americanus* (Winter Flounder), and *M. menidia*e(Silversides)). Egrets appeared to select foraging sites where C. crangon and larger Fundulus were more abundant. This information will be useful to develop better management strategies for egrets nesting in Narragansett Bay.

Synthesis and Evaluation of Modified Nucleoside Oligomers Containing 1,2,3-Triazole Internucleoside Linkages as Antisense Agents

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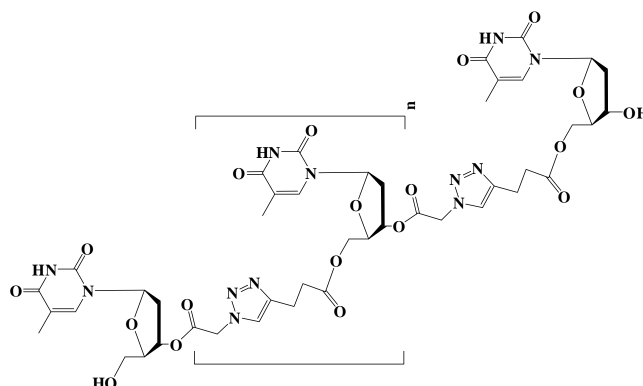
RI-INBRE Summer Undergraduate Research Fellowship Program

Modified oligonucleotides are commonly used in antisense research to silence mRNA of interest. These compounds contain mostly negatively charged mimics of phosphodiester bridges that limit their cellular uptake. The hypothesis underlying this research is that neutral nucleoside oligomers containing 1,2,3-triazole bridges can be used as antisense agents with enhanced cellular uptake and higher binding affinity to the complementary sequences. Herein, we report the synthesis of modified nucleoside oligomers using solution- and solid-phase synthesis. Initially, a dimer of thymidine containing 1,2,3-triazole was synthesized by solution-phase organic synthesis to determine the effectiveness of Click chemistry. 5′-Alkyne- and 3′-azido-substituted thymidine building blocks were first synthesized and then reacted together under the Click reaction conditions to afford a dithymidine derivative linked through 1,2,3-triazole. Alternatively, thymidine was immobilized on Wang resin through 5′-hydroxy group. The 3′-position of thymidine was then substituted with azidoacetate. Subsequent Click reaction with the 5′-alkyne substituted thymidine produced an immobilized dithymidine analog containing 1,2,3-triazole ring. 3′-Azidoacetylation and the Click reaction were repeated several times. Final acidic cleavage from the resin afforded a thymidine oligomer containing 1,2,3-triazole internucleoside linkage that was characterized by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry. The conformation of the compound and the binding affinity of the oligomer toward the corresponding complementary chains of unmodified (deoxyadenosine)$_n$ and (adenosine)$_n$ will be evaluated in the future. This data will provide insight about the effectiveness of the oligomer synthesis and the potential application of these compounds as antisense agents. The long-term goal of this study is to block the expression of c-Src kinase, an enzyme that is overexpressed in breast and colon cancers, at the genome level by designing modified oligomers that silence c-Src mRNA translation for the enzyme.



Use of OffGel Fractionator to Separate Proteins from Rat Kidney Cells Treated with Cadmium

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RI-INBRE Summer Undergraduate Research Fellowship Program

Cadmium is a non-essential element that is extremely toxic to many organ systems and causes cancer. It is found in air, water, food, and cigarettes and accumulates over time in the liver and kidneys; the latter being the main target organ for this metal. Since proteins are essential for cellular function, it was hypothesized that this could be one of the mechanisms by which cadmium may cause nephrotoxicity. Although alteration of cellular protein levels by cadmium has been reported previously, no cause and effect relationships have yet been established. To test our hypothesis, we conducted a preliminary study of the acute effects of cadmium on rat kidney epithelial cell proteins. Before this could be accomplished, it was essential to establish a protocol for the isolation and separation of proteins from the kidney cells. The cells (NRK-52E) were grown to 80% confluence in T75 flasks in the presence of DMEM containing 5% calf serum, treated with 20 μM cadmium chloride for 6 h, and harvested. From another flask untreated control cells were also harvested. To identify contamination by serum proteins, the extract from a flask that contained only the serum-containing medium was also obtained. Proteins were extracted in Protein Extraction Reagent Type 4, pH 10.4 from Sigma's ProteoPrep Universal Extraction Kit and passed through a Hi Trap Blue column to remove most of the structural proteins. The column pass through proteins were concentrated and desalted by YM3 Microcon centrifugal filter device. The concentrated protein samples were separated according to their isoelectric points into twelve fractions using the OffGel Fractionator. Each fraction was then further separated according to molecular weight by SDS gel electrophoresis and the protein bands were silver stained. Preliminary results indicated that the level of expression of a few proteins is indeed altered by cadmium treatment. Further studies are needed to isolate these proteins in sufficient quantity and perform their identification by mass spectrometry

Polymer based multiferroic composite films

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RI-EPSCoR Summer Undergraduate Research Fellowship Program and LSAMP

Multiferroic materials are those that exhibit two or more ferroic properties simultaneously, among them are: ferromagnetism, ferroelectricity, and ferroelasticity. In particular, materials where ferromagnetism and ferroelectricy coexist are termed **magnetoelectric multiferroics**. Even though multiferroics are rare their properties are very desirable. Some of the applications for this type of materials are: multiple-state memory elements, sensors, and microelectromechanical (MEMS) actuators. Homogeneous flexible magnetoelectric multiferroic materials are desirable. To achieve this, the polymer Poly (vinylidene) fluoride (PVDF) was used to provide the ferroelectric component of the material since it can be prepare in a ferroelectric crystalline phase. To obtain the magnetic component in the film, $Co(NO_3)_2 \cdot 6H_2O$ (Cobalt nitrate hexahydrate) was used since cobalt is a magnetic transition metal.

IR spectra of the homogeneous multiferroic composite thin films showed that adding small amounts of cobalt metal salt induced formation of the desired ferroelectric -phase. As Co^{2+} ion in the film was increased the β-phase in the composite PVDF film increased until it reached a maximum at about 2% Co^{2+} (w/w). Peaks indicative of residual solvent, Dimethylformamide (DMF), in the films were identified. DMF was found to coordinate with Co^{2+} complex and to evaporate with film aging. Differential Scanning Calorimetry (DSC) showed a decrease in the polymer's melting point from 160°C to 154°C, indicative of -phase. This melting point decrease is attributed to melting point depression due to $Co(NO_3)_2 \cdot 6H_2O$ impurities in the film. For films containing low Co^{2+} percentages an exotherm was seen at temperatures above 170°C while, for high Co^{2+} ion containing films an endothermic behavior was observed. A second preparation procedure where films were prepared at temperatures close to room temperature showed an improved DSC reproducibility.

Scanning electron microscope (SEM) supported β-phase formation with increase of Co^{2+}ion in film.

A Biosensor for the Measurement of Vitamin D

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

We aim to develop a miniaturized biosensor that accurately and rapidly measures nanomolar quantities of vitamin D metabolite based on electrochemical detection. Vitamin D is an essential prohormone that is obtained through the diet or produced photochemically in the skin, with deficiency causing rickets in children and osteomalacia in adults. Vitamin D is hydroxylated in the liver to become 25(OH)D, which is further hydroxylated in the kidney by the enzyme CYP27B1 to become its active form. The electrochemical approach is based on the hypothesis that the hydroxylation of 25(OH)D can be measured via the catalytic reaction of CYP27B1 immobilized on an electrode. CYP27B1 is a heme-containing monooxygenase that accepts two electrons and reduces one atom of O2 to water, inserting the other oxygen atom onto the substrate. In vitro, electrons can be supplied catalytically, with the catalytic current directly proportional to the amount of 25(OH)D. Other investigators have shown that an enzyme can be integrated with electrodes to form a biosensor, and with the development of microfabrication technology biosensors have become inexpensive, miniaturized, and reproducible.

Integrated Carbon Nanotube-Polyguanine Functionalized Polymeric Nanoparticles for Sensitive Immunodetection of Prostate Specific Antigen (PSA) in serum

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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. We describe herein a sensitive electrochemical immunosensor using polyguanine functionalized polymer nanoparticles (NPs) and carbon nanotube platform for sensitive electrochemical detection of prostate specific antigen (PSA) in serum. The detection of human PSA was achieved using bioconjugates featuring multiple polyguanine labels and secondary antibodies (Ab_2) attached to polymeric nanoparticles coupled to $Ru(bpy)_3^{2+}$ mediated guanine oxidation. The performance of the electrochemical immunosensor was evaluated and experimental parameters including the $Ru(bpy)_3^{2+}$ concentration and PSA incubation times optimized. This approach provided a detection limit of 1.0×10^{-9} g mL^{-1} (30 pM) corresponding 300 amol PSA in 10 l of undiluted calf serum. Our results compares favorably with the standard ELISA assays and are within the 4 ng mL^{-1} clinical diagnostic level for prostate carcinoma. This immunosensor show great promise for a rapid, simple cost effective method for point-of-care diagnostics.

Who’s your daddy?: Utilizing microsatellites to investigate reproductive structure of *Polistes erythrocephalus*

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Short, repeated, DNA sequences of 2 to 8 bases found in all eukaryotic genomes are known as microsatellites. Distance among individuals in a population increases the chances that their microsatellites will vary in number of repeats. Such susceptibility to variation makes these regions the tool of choice as powerful molecular markers in different areas of research. In this case, they are used in a population genetics study. For our project, we employed molecular techniques such as DNA sequencing, PCR, and gel electrophoresis to locate loci with microsatellite regions in a population of tropical paper wasps of the species *Polistes erythrocephalus*. PCR products from these loci will be used to screen this population for allelic variation. We will use genetic variability in these regions to determine kinship among individuals, as well as the reproductive structure of the colony.

Stimulation of Myocardial Glycolysis by Physiological Concentration of Carnitine: Involvement of the MEK 1/2 -ERK Signaling Pathway

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RI-INBRE Summer Undergraduate Research Fellowship Program

Carnitine is a cofactor that promotes oxidation of long-chain fatty acids inside heart and other cells. Low levels of carnitine (50 micromolar) also exist in blood, where it was thought to lack biological activity. However, recent experiments from this laboratory demonstrated that 50 micromolar carnitine produces an insulin-like effect on cardiac metabolism by enhancing glycolysis and glucose oxidation in perfused working rat hearts. The mechanism for this effect is not known. In this study, we investigated the role of the intracellular signal mitogen-activated erk-protein kinase (MEK1/2) in the activation of glycolysis by extracellular carnitine on isolated working rat hearts. We found that the ability of 50 micromolar carnitine to activate glycolysis was blocked by pretreatment with the MEK 1/2 inhibitor PD-98059 (5 micromolar). Western blot analysis revealed a small increase in the ratio of phosphorylated MEK 1/2 to total MEK 1/2 in carnitine-treated hearts when compared to untreated controls. Unexpectedly, carnitine treatment also increased the reactivity of unknown bands in the 90 kDa region in response to phospho-MEK 1/2 antibody exposure. These unidentified bands could be the result of MEK/ERK dimerization, but this has yet to be investigated. The results support the hypothesis that MEK 1/2 mediates the glycolysis-activating effect of carnitine in the heart. However, the signal is weak. Future studies will be directed toward investigating the possible involvement of other intracellular signals known to mediate analogous effects of insulin, including PI3-kinase.

Staphylococcal spp. Biofilm Formation in the Presence of Heparin or Tissue Plasminogen Activator (tPA)

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background. Heparin or tissue plasminogen activator (tPA) is frequently added to catheters to prevent clotting. Heparin enhances *S. aureus* biofilm mass at concentrations of less than or equal to 1000U/ml. It is unknown if increased concentrations of heparin, tPA, or diluents such as bacteriostatic water (0.9% benzyl alcohol) impact biofilm mass. We investigate heparin across a range of concentrations, tPA, and bacteriostatic water in the formation of biofilm.

Methods. Biofilm-producing strains of *S. aureus* (ATCC 35556) and *S. epidermidis* (ATCC 35984), and a biofilm non-forming *S. epidermidis* control (ATCC 12228) were evaluated against heparin (10,000 to 100 U/ml) and tPA (alteplase 1 mg/ml) alone and in combination with bacteriostatic water (benzyl alcohol 0.9%) Quantification of biofilm mass was conducted using the Christensen, colorimetric microtiter plate assay (optical density 570). Minimum inhibitory and bactericidal concentrations (MIC's, MBC's) were determined using the broth microdilution assay. The assays were run in quadruplicate, results averaged, and standard deviations calculated.

Results. MIC's and MBC's for benzyl alcohol were 0.45% for all isolates except *S. epidermidis* ATCC 35984, which had a minimum bactericidal concentration of 1.8%. Heparin concentrations of 5-10,000 U/ml inhibited biofilm mass, </= 2,500 u/ml induced biofilm mass or had no effect on biofilm formation. Benzyl alcohol 0.9%, with or without heparin, showed an inhibitory effect (avg. 97.5% ± 2.4 reduction from growth control) on all isolates except for *S. epidermidis* (38% reduction). Alteplase inhibited biofilm mass (avg. 92% ± 10 reduction from growth control) for all isolates.

Conclusion. Heparin (5-10,000 u/ml) with 0.9% benzyl alcohol was inhibitory to tested isolates of *S. aureus*. However, *S. epidermidis* strains may not be inhibited by benzyl alcohol at these concentrations. Of interest, alteplase has merit to be evaluated as an alternative in preventing staphylococcus biofilm mass in catheter locks.

Damaging the Cell Wall Induces Apoptosis in the Budding Yeast, *Saccharomyces*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Apoptosis or programmed cell death (PCD) is necessary to protect the integrity of an organism or colony by destroying infected, damaged, or unwanted cells. Apoptosis can be induced in *Saccharomyces cerevisiae* cells and occurs when they are exposed to various types of stress, including acetic acid, hydrogen peroxide. The yeast cell wall is essential for survival and is involved in vital tasks such as regulating turgor pressure, providing cell shape, formating buds for cell division. We have discovered that yeast cells exposed to reagents that damage the cell wall, including sodium dodecyl sulfate (SDS), undergo programmed cell death with the hallmarks of apoptosis. We are working to identify the genes involved in this process. Our research should not only lead to a better understanding of the mechanisms behind programmed cell death but may also identify yeast-specific molecular targets for anti-fungal therapies.

GFP as a Biomarker for Signal Peptide Trafficking in *Leishmania*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania tarentolae is a trypanosomatid pathogen that thrives in reptiles residing in subtropical climates. Closely related strains of *Leishmania* can infect humans causing cutaneous and visceral infections. Understanding the biology of the *Leishmania* is vital to produce effective treatments, and for understanding disease prevention. My research was conducted to verify and map the movement of a signal peptide in *Leishmania tarentolae*, using GFP as a biomarker. A signal peptide consists of the first 10 to 20 hydrophobic amino acids in a protein bound for the secretory pathway. When the signal peptide unites with a receptor on the endoplasmic reticulum the entire protein is synthesized and traffics through the cell's the secretory pathway via the ER and Golgi. In the current study, we fused the putative signal peptide of the *LdLIP3* gene from *Leishmania* to GFP using *p*KSNEO, a *Leishmania* expression vector. In addition, a control construct with no signal peptide (GFP alone) was generated. These constructs will be transfected into *Leishmania* promastigotes and the cellular localization of GFP will be detected by means of a fluorescent microscope. We anticipate that if the putative signal peptide functions properly, in the construct with the signal peptide, the GFP will traffic out of the cell and in the control with no signal peptide GFP will remain cytoplasmic.

Nanomaterials-Based Immunoassay for Sensitive Electrochemical Detection of Matrix Metalloproteinase-3 (MMP-3) in Serum

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RI-INBRE Summer Undergraduate Research Fellowship Program

A novel electrochemical immunosensor for the detection of matrix metalloproteinase-3 (MMP-3), a cancer biomarker protein, based on vertically aligned single-wall carbon nanotubes (SWNT) arrays, is presented. The detection of human MMP-3 is based on a sandwich immunoassay in connection with catalytic reactions involving horseradish peroxidase conjugated to a secondary antibody. The single-wall nanotube arrays were characterized using Atomic Force microscopy (AFM) and Resonance Raman spectroscopy. These experiments confirmed bundles of vertically aligned SWNT with average vertical height ~ 20-40 nm. The performance of the electrochemical immunoassay was evaluated and some experimental parameters (e.g. non-specific binding, NSB events, concentrations of both the primary and the secondary antibody etc.) were optimized. This non-amplified approach provided a detection limit of 4.0×10^{-10} g mL^{-1} (5.0 pM), which corresponds to 50 amol of MMP-3 in 10 L calf serum samples. The polymeric beads amplification strategy gave a detection limit of 10.0×10^{-12} g mL^{-1} (125 fM) corresponding to 1250 zepto mol of MMP-3 in 10 L serum sample. This immunosensor based on SWNT arrays offers great promise for a rapid, simple, cost-effective method for clinical screening of cancer biomarkers and point-of-care diagnosis.

Population genetic structure and local adaptation in the acorn barnacle, *Semibalanus balanoides*

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

With an extended planktonic dispersal period, acorn barnacles (*Semibalanus balanoides)* have the ability to cover great distances as nauplii. This life history pattern coupled with the prediction of evolutionary theory, that few effective migrants between populations will prevent local adaptation, suggests that *S. balanoides* will show little genetic structure. Nevertheless, previous research at protein coding molecular markers indicates barnacle populations can become locally adapted, and that the scale of selection stress may vary. This paradoxical result may result from one of two potential processes. First, selective stress on polymorphic loci may be great enough to create population genetic structure despite extensive gene flow. Or second, *S. balanoides* is not as genetically homogenous as previously assumed and barriers to dispersal have preserved local populations. In order to test these hypotheses, barnacles were sampled from 8 sites ranging from southern Rhode Island to the Miramichi estuary in New Brunswick, Canada. Genotyping occurred at 7 putatively neutral molecular markers, the control region of the mitochondrial genome and 6 microsatellite loci. Little local population structure was detected, indicating extensive gene flow among populations with few barriers to dispersal. The effective population size was estimated to be between 10,000 and 100,000 individuals, which when divided by census size leads to an N_e/N ratio that approaches zero. Mitochondrial sequence data provided evidence of three distinct clades that are oriented in a shallow cline. Analysis of microsatellites is ongoing, but preliminary analysis appears to confirm results of the mitochondrial control region, that genetic structure is weak in *S. balanoides* even in populations separated by hundreds of kilometers.

Denitrification and nitrogen fixation organisms in Narragansett Bay sediments

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Nitrogen (N) is important for all organisms to thrive. Two important processes in N budgets include denitrification and nitrogen fixation. Organisms that function in these processes possess biochemical machinery that functions to remove fixed nitrogen by converting NO_3^- and NO_2^- to N_2 gas (denitrification) and fixing N_2 gas into biologically useful NH_4^+ (nitrogen fixation). By tracking genes in these pathways, we can identify organisms in the marine environment that have the potential to fix nitrogen or to denitrify. In this study we are using the *nifH* gene to assess the diversity of nitrogen fixers and the *nirS* and *nirK* genes to assess the diversity of denitrifiers in Narragansett Bay sediments. Our results show that nitrogen fixers and denitrifiers in Narragansett Bay sediments are related to microorganisms in sediments from other estuaries, such as the Chesapeake Bay, implying that similar communities may mediate nitrogen cycling in estuarine ecosystems.

Mercury bioaccumulation and trophic transfer in estuarine fish of Narragansett Bay (Rhode Island, USA)

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RI-INBRE Summer Undergraduate Research Fellowship Program

Coastal marine ecosystems support substantial fisheries, and thus, are the dominant source of mercury to fish-consuming humans. Nevertheless, relative to freshwater environments, very little is known about the fate of Hg in near-shore marine food webs. In this study, we analyzed the total mercury concentration (Hg) of important recreational finfish of Narragansett Bay (Rhode Island, USA). Stable isotope signatures of these target fish and their prey were used to elucidate the effect of feeding history and carbon sources (benthic vs. pelagic pathways) on patterns of Hg burden in fish tissue. In 2006 and 2007, target fish (bluefish, striped bass, tautog, summer flounder, and winter flounder) and their prey (forage fish, squid, decapods, and bivalves) were collected from Narragansett Bay and analyzed for Hg using atomic-absorption spectrophotometry (EPA Method 7473). Stable nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotopes of biota were also measured using continuous-flow isotope ratio mass spectrometry. The Hg of target fish was positively correlated with fish age and $^{15}N/^{14}N$, irrespective of species-type. This verifies Hg is transferred across trophic levels and that all target fish bioaccumulate Hg, albeit at different rates. For example, Hg bioaccumulation rates were lower in benthic/demersal fish relative to pelagic species, and these differences are attributed to the varied feeding ecology of each fish and the Hg content of their prey. Moreover, Hg contamination is more prevalent in pelagic food chains (depleted $^{13}C/^{12}C$) because of this pathway's greater complexity (i.e., more trophic levels), and thus, opportunity for Hg bioaccumulation.

Examining the presence and variation of Podoviruses in the Coastal Waters of Rhode Island

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Cyanophage communities in marine environments are abundant and diverse; containing viruses belonging to three major families, Podoviridae, Siphoviridae and Myoviridae. Recently, cyanophages belonging to the Podoviridae family were isolated from the coastal waters of Rhode Island. This is significant because not many Podoviruses from marine estuaries have been characterized to date. This study examines the presence and diversity of Podoviruses in Narragansett Bay. Using Pulsed Field Gel Electrophoresis, water samples likely to contain Podoviruses were identified. Viruses from these water samples were isolated using plaque purification assays and screened using PCR primers for the Myoviral DNA Polymerase (g43) gene. Viral isolates that did not belong to the Myoviridae family were screened using Podovirus DNA polymerase gene primers. The DNA polymerase genes from podoviruses were analyzed using PCR-RFLP and sequenced. To date, two new podoviruses have been identified: RIP1 and RIP2. To determine host specificity, host range tests were performed by plating each Podovirus with five strains of *Synechococcus.* The two Podoviruses are highly specific and only infect the strain that they were isolated on. The two new podoviruses were also tested for host-derived photosystem II D1 and D2 reaction center (psbA and psbD) genes. Both podoviruses contain a psbA gene, but not a psbD gene. This suggests the exchange of genes among viruses or between virus and its host. Viral isolates from Narragansett Bay are continuing to be analyzed to identify additional Podoviruses. Although Myoviruses were thought to be the main viral family in estuarine environments, Podoviruses may also be a key component of estuarine viral communities.

1-N-substituded Cyclised Payrazoline Analogues of Thiosemicarbazones as a potential antimebic drug

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RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica is a protozoan parasite that infects more than 50 million people world wide and causes 100,000 human deaths annually. *E. histolytica* lacks mitochondria and therefore uses fermentative pathway to generate energy anaerobically to survive inside the human intestine through a bifunctional alcohol and aldehyde dehydrogenase enzyme, *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2). EhADH2 is a potential target for antiamebic drug due to its low homology to the human alcohol dehydrogenase, and its crucial role in the survival of the parasite. This study focuses on synthesizing1-N-substituded cyclised pyrazoline analogues of thiosemicarbazones, and monitoring their effect against purified EhADH2 enzymatic activities and inhibition of amebic growth. The synthesis of 3-phenyl-2-pyrazoline-1-(N,N-diethyl) thiocarboxamide was achieved by refluxing Acetophenone Manic base and N'diethyl-thiosemicarbazide. Structural confirmation was done by IR and 1H NMR. EhADH2 activities are measured indirectly based on the conversion of cofactor NADH into NAD at 340 nm. Monitoring the inhibition of this compound against EhADH2 has been problematic because the chemical has a strong absorption at 340 nm. Future research will concentrate on finding an alternative ways to monitor EhADH2 activities either by measuring the end products (ethanol), or another cofactor (Thioester bond between coenzyme A and acetic acid). Since EhADH2 is an essential enzyme in *E. histolytica* that differ greatly from the human alcohol dehydrogenase, the goal of this research is finding an inhibitor for EhADH2 as an alternative way to treat amoebiasis.

Genetic Characterization of Acetic Acid-Induced Cell Death in *Saccharomyces cerevisiae*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Apoptosis or programmed cell death (PCD) is necessary to protect the integrity of an organism or colony by destroying infected, damaged, or unwanted cells. Apoptosis is inducible in *Saccharomyces cerevisiae* cells and occurs when they are exposed to various types of stress including acetic acid, a natural byproduct of yeast metabolism. We have generated single and double mutant yeast cells using PCR-directed gene disruption techniques to knockout the following genes involved in apoptosis: *AIF1, NUC1, BIR1, UTH1*, and *YCA1.* We are now characterizing these mutant strains to determine their phenotypes after being exposed to acetic acid to identify the molecular pathway involved.

Mercury exposure for Rhode Island residents consuming local fishery resources

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant affecting human health, and exposure occurs mainly through dietary uptake of contaminated fish. To minimize Hg exposure, public health officials issue consumption advisories to inform citizens of the possible health risks associated with eating fish. While consumption advisories have been developed on a site-specific basis for fish inhabiting freshwater systems, advisories regarding the consumption of saltwater species lack geographic specificity. In this study, measurements of Hg in fish collected from Narragansett Bay (RI, USA) were incorporated into exposure assessment models. Results from these spatially-explicit models, in turn, will support the development of effective consumption advisories for fish-eating residents of RI. Estimates of human exposure to Hg due to local fish consumption were calculated from exposure assessment models modified from the U.S. Environmental Protection Agency (U.S. EPA). Daily exposure to Hg was estimated for children (0-14 years), women of childbearing age (15-44 years), and the general adult population (18+ years). To evaluate the efficacy of the RI-specific exposure assessment model, results were compared to: (1) national estimates of human exposure to Hg, and (2) the reference dose (RfD) established by the U.S. EPA. Estimates of Hg exposure for RI residents, in many instances, do not reflect nationally aggregated data. Moreover, selectively consuming bluefish, striped bass, and tautog (>1 meal per week) result in Hg intake exceeding the RfD, whereas eating black sea bass, summer flounder, and winter flounder are not expected to cause deleterious effects on human health. The cumulative result of the modeling exercise is the improved assessment of human dietary exposure to Hg at the local scale.

Engineering dually fluorescent quantum dot liposomes for advanced imaging and diagnostics

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RI-INBRE Summer Undergraduate Research Fellowship Program

Lipid bilayer vesicles called liposomes are being investigated for their potential use as imaging agents for diagnostic applications. Fluorescent liposomes have been engineered using semi-conducting, photostable nanoparticles known as quantum dots (QDs). Since QDs are composed of toxic metals, they are incorporated within liposomes containing common cellular lipids to reduce their *in vivo* toxicity. To advance the imaging of carcinoma cells from singular to dual fluorescence, liposomes with two QDs of different emission wavelengths were synthesized. Hydrophobic QDs were embedded within the lipid bilayer and hydrophilic QDs were encapsulated in the inner aqueous core. The self-assembly of liposomes was achieved by reverse phase evaporation. Fluorescence and confocal microscopy indicated the formation of dually fluorescent quantum dot liposomes (L-QDs). The L-QDs were tested on a human hepatoma cell line, HuH-7, to determine the extent and efficacy of cellular uptake as well as intracellular fluorescence. Fluorescence and confocal microscopy also confirmed the uptake of cationic, dually fluorescent L-QDs in HuH-7 cells within 24 hours. Once inside the cells, however, the L-QDs did not appear to break down and release the encapsulated QDs. Further exploration of lipid composition is necessary to produce dually fluorescent L-QDs that are easily taken up by carcinoma cells and subsequently broken down for advanced intracellular imaging and diagnostics.

Development of a practical, non-invasive method for determining CYP3A4 activity by measurement of urinary cortisol and 6β-hydroxycortisol levels by HPLC-UV

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RI-INBRE Summer Undergraduate Research Fellowship Program

Cytochrome P450 (CYP) is a family of enzymes that are responsible for the metabolism of many endogenous and exogenous compounds. The CYP 3A4 subfamily, found primarily in the liver, is the most clinically significant CYP family (as it metabolizes a broader range of xenobiotics than any other CYP type). Measuring an individual's CYP3A4 activity can theoretically predict the pharmacokinetic and/or pharmacodynamic response of CYP3A4 substrates. CYP3A4 is the major isoform responsible for production of 6β-hydroxycortisol from endogenous cortisol. Hence, the ratio of 6β-hydroxycortisol (6βOHF) to cortisol (F), measured using HPLC-UV in urine, is a known method for non-invasive determination of CYP3A4 activity. The existing methods typically have a chromatographic runtime of ~40 minutes, thus are time-consuming for the measurement of a large number of clinical samples. The objective of this study was to develop a faster and more straightforward assay for the measurement of 6βOHF/F in urine. Trials of mobile phase composition between different column types produced a much shorter runtime than previously developed methods. A Luna® Phenyl-Hexyl 5μm column produced an 11 minute runtime. Oasis® SPE cartridges were used for urinary extraction. The extraction technique requires further adjustments to increase analyte recovery. Recovery complications were most likely attributed to the difference in polarity between the two compounds. For future studies, upon an improved extraction method, this non-invasive method will be used to determine whether CYP3A4-related dose adjustments are required for special classes of patients.

Molecular and Behavioral Characterization of Mice with a Mutation in *Wnt1*

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RI-INBRE Summer Undergraduate Research Fellowship Program

The dopamine (DA) neurons of the midbrain (Mb) are involved in the regulation of important behavioral processes including addiction, attention, motivation, and reward. Furthermore, their aberrant function or loss has been implicated in a number of neurological diseases. The present study has used fluorescent immunotcytochemistry in combination with Volocity Morphometric Software in order to develop a semi-quantitative method of representing the 3-D spatial distribution of the MbDA neuron sub-populations. We are currently quantitatively assessing DA neurons distribution of wild types. Specifically, tyrosine hydroxylase positive Mb neurons were analyzed for co-localization with g-protein inward rectifying potassium channel 2 (GIRK2), calbindin, or calretenin. We are further applying our analytical methods on a novel mutant mouse model with extensive neurological defects of the midbrain and cerebellum. *Wnt1*$^{sw/sw}$ (Swaying) mutants exhibit substantial loss of MbDA neurons and model salient features of Parkinson's disease and schizophrenia. The spatial distribution map coupled with extensive behavioral analysis and systematic stereology of the aforementioned MbDA sub-populations is being done in order to determine the relationship between the observed phenotype and the specific MbDA neuron loss. Determining the molecular identity of the lost MbDA neurons coupled with the behavioral characterization of the *Wnt1*$^{sw/sw}$ mutant phenotype will provide further insight in understanding embryonic brain development and may shed light on possible medical therapies in regards to human disease.

The characterization of the genetic basis of chondroitin AC lyase in *Flavobacterium columnare*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Flavobacterium columnare is the causative agent of Columnaris disease, a disease affecting fresh water fish. Chondroitin AC lyase activity has been proposed as a virulence factor of *F. columnare* along with biofilm formation, protease activity, and adherence factors. The rate of chondroitin AC lyase activity was studied in two clinical isolates of *F. columnare*, C#2 and 94-081. These wild type strains were analyzed for variations in the rate of chondroitin sulfate A and C degradation. Strain C#2 degraded both chondroitin A and C more rapidly than the second strain, 94-081. The *cslA* gene has been previously shown to degrade chondroitin A when expressed in *Escherichia coli.* The contribution of this gene in *F. columnare* to chondroitinase activity was examined by the creation of *cslA* mutants in both wild type strains. Both *cslA* mutant strains, Fc48 and FcMR03, exhibited reduced degradation of chondroitin A and C. The residual chondroitin lyase activity in the mutant suggested that an additional gene is responsible for the remaining activity. A putative glycosaminoglycan lyase gene was identified from the partially sequenced genome of *F. columnare*. The DNA sequence was used to generate a mutation in C#2 by single crossover homologous recombination. This mutant strain, FcMR01, showed no reduction in chondroitin A or C lyase activity when compared to the wild type. Our results show that *cslA* is responsible for 95% and 72% of chondroitin A and C degradation, respectively. In addition, a mutation in a putative glycosaminoglycan lyase gene, did not affect chondroitin AC lyase activity. The creation of these *cslA* mutant strains will allow us to determine whether chondroitin AC lyase is a virulence factor.

The identification of SNPs in a segment of a matrix metalloproteinase gene of the eastern oyster, *Crassostrea virginica*

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RI-EPSCoR Summer Undergraduate Research Fellowship

Previous studies of matrix metalloproteinases (MMPs) in mammals have shown that they are responsible for a varied set of tasks, including cell migration, activation and regulation of immunity and inflammation, and tissue repair. We believe that MMPs may play similar roles in the eastern oyster *Crassostrea virginica*. There are currently three major diseases, known as dermo (caused by the protozoan parasite *Perkinsus marinus*), MSX (caused by the protozoan parasite *Haplosporidium nelsoni*) and Juvenile Oyster Disease (JOD, caused by the bacteria *Roseovarius crassostreae*) which affect the eastern oyster. Our laboratory has isolated and characterized the genes coding for two metalloproteinases in oysters, *Cv1mmp* and *Cv2mmp.* Patterns of tissue expression suggest that *Cv1mmp* is involved in hemocyte (the immune cells of oysters) migration, digestion, immune defenses, and shell formation. The scope of this project is to identify genetic variation, in the form of single nucleotide polymorphisms (SNPs), in a fragment of the *Cv1mmp* gene. Four populations of oysters have been selected for this study: Rutgers NEH, developed at Rutgers University for resistance to dermo and MSX; Green Hill Pond, a Rhode Island native strain which has been exposed to high levels of MSX and dermo in the past and has been believed to be naturally selected for disease resistance; Canada, a strain from the cooler waters of Canada which is naive to both MSX and Dermo; and samples from the wild stock of Rhode Island with moderate to low exposure to dermo and MSX. A fragment of the *Cv1mmp* has been sequenced for 3 – 5 individuals from each population, and several SNPs have been identified. Sequence information will be used to design a protocol to screen large numbers of individuals from relevant oyster populations. Ultimately the goal is to try to correlate genetic variation in the *Cv1mmp* gene with levels of disease resistance in oysters.

Extracts from marine actinomycetes as novel anti-amebic agents

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RI-INBRE Summer Undergraduate Research Fellowship Program

The parasitic protozoan, *Entamoeba histolytica*, causes 50 million cases of diarrhea and 100,000 deaths a year. There are few options for treatment and all comprise multiple side-effects. The primary treatment for amebiasis is the drug metronidazole; and, its mutagenic, carcinogenic, and neurological complications encourage the search for new amebic therapies. Secondary metabolites produced by marine actinomycetes represent a chemically rich and completely untapped resource for anti-protozoan drug discovery. In preliminary in vitro studies, echinomycin A and tirandamycin inhibited trophozoite growth. At increased concentrations, both chemical principles decreased cell growth. *E. histolytica* trophozoite growth was inhibited using echinomycin A and tirandamycin concentrations of 25µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL. Echinomycin A and tirandamycin's mechanism of action, efficiency against E. histolytica clinical isolates and toxicity, need to be elucidated. The scope of this study is to discover novel marine secondary metabolites with inhibitory effects on trophozoite growth. Identified compounds will be tested on *E. histolytica* clinical isolates as new therapies against amebiasis and other protozoan diseases.

Other strategies for discovering chemical principles are currently underway in our laboratory. Iron chelators have anti-malarial and anti-mycotic activities suggestive to have inhibitory effects on the iron thriving *E. histolytica*. Preclinical studies testing iron scavengers and chemically synthesized pyrazoline analogues of thiosemicarbazones inhibiting *E. histolytica* trophozoite growth could provide alternative amebiasis chemotherapeutics.

In Silico Search for Novel Estrogen Receptor Ligands

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RI-INBRE Summer Undergraduate Research Fellowship Program

The estrogen receptors (ERs) α and β are intracellular proteins responsible for controlling transcription of genes necessary for human development and reproduction. ER activity is normally modulated by the endogenous hormone 17β-estradiol which binds to the ERs resulting in recruitment of coregulatory complexes. Irregularities in ER activity can lead to a number of conditions including breast, ovarian, colon, prostate, and endometrial cancers. Selective estrogen receptor modulators (SERMs) are compounds which can bind to ERs and modulate ER activity in specific combinations of cell types and are used to treat ER-related disorders. The search for novel SERMs is necessary to develop better treatments for these disorders. In order to discover new estrogen receptor ligands, libraries of compounds were assembled on the computer based around the several molecular scaffolds including the common SERM substructures triphenylethylene. Searches were performed on the ZINC database in order to identify available compounds with these substructures as well as available precursors which could yield compounds with our desired form. The resulting dataset of compounds was screened virtually against x-ray crystallographic structures of ERα using the automated docking program eHiTS. The best performing compounds are being obtained in order to screen against ZR-75-1 breast cancer cells to determine if they indeed have estrogenic activity.

Pyrethroid effects on mouse spermatozoa motility and capacitation

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RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroid insecticides have a wide spectrum of use and are effective in controlling a variety of insect pests both on agricultural commodities and in the prevention of insect-mediated human and animal disease transmission. The increased spread of the aforementioned diseases will likely result in increased efforts to control medically important insects and, as a consequence, increased exposure situations for individuals. Thus, it is critical that these insecticides are evaluated for potential adverse effects on reproduction. The purpose of this research was to develop a motility and capacitation assay to observe the effects of pyrethroids on reproductive characteristics of mouse spermatozoa. Spermatozoa were isolated from the mouse caudal epididymis and incubated at 37°C in the presence or absence of deltamethrin or DMSO (solvent control). For the motility assay, spermatozoa were aliquoted to a single chamber and motility readings were evaluated using the CEROS Computer-Assisted Sperm Analysis system (CASA). In the capacitation assay, chlortetracycline stained spermatozoa was assessed by visual examination of under a fluorescent microscope. Percent motile and percent progressively motile spermatozoa were increased in the presence of BSA compared to the absence. Deltamethrin, however, had no effect on the motility characteristics compared to DMSO control. BSA capacitates sperm significantly after 20 minutes 1.4 fold compared to BWW control. Deltamethrin increased the number of capacitated spermatozoa by 39% in the presence of BSA 1.5 fold compared to a DMSO control. DDT had no effect on capacitation. Our data suggest that deltamethrin increases the percent capacitation of mouse spermatozoa *in vitro* also suggesting pyrethroid insecticides may be potential reproductive toxicants.

An analysis of eukaryotic ribosomal protein L32 and ribosomal RNA in ribosome biogenesis and cell cycle control

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RI-EPSCoR Summer Undergraduate Research Fellowship

Ribosomes are macromolecular RNA-protein complexes found in all cells that catalyze the conversion of mRNA-encoded information into proteins. Rapid advancements have been made in understanding the roles of rRNA and ribosomal proteins in ribosome function using the prokaryotic system. The invariant nature of regions of rRNA, and the presence of many ribosomal protein homologues in all kingdoms suggests a conservation of basic functions throughout evolution. However, there are considerable differences in the biogenesis and structure of ribosomal subunits between the prokaryotic and eukaryotic kingdoms, and an analysis of these differences is critical to understanding the eukaryotic ribosome.

The LSU in yeast contains ribosomal proteins that are absent in eubacteria. Homology modeling using the ribosome crystal structure of *H. marismortui* revealed that Rpl32p, which lacks a eubacterial homologue, potentially contacts two rRNA elements that are perfectly conserved in eukaryotes, but degenerate in prokaryotes. We propose that the absence of a eubacterial homologue, as well as its implicated interactions with these conserved regions, suggest a specialized function for Rpl32p. We have genetically depleted Rpl32p from yeast cells to understand its contribution to ribosome biogenesis. The data confirm that *RPL32* is an essential gene. Additionally, *RPL32* depletion compromises cell cycle progression, further supporting the suggested synchronization of ribosome biogenesis and DNA replication in eukaryotes. The characterization of Rpl32p should help elucidate eukaryotic-specific functions of ribosomal protein-rRNA interactions in ribosome biogenesis and cell cycle control.

Transition Metal Oxide Catalysts for Gas Sensors

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RI-EPSCoR Summer Undergraduate Fellowship Program & LSAMP

This research project is a multi-faceted project whereby research groups from URI, Georgia Tech and Sensor Tech, Savanna, GA are working together to develop novel combinations of transition metal oxide catalysts for the detection of specific gas molecules of interest to the US Army and Department of Homeland Security. Several sensor templates are being used for the detection of gas molecules including one based on ceramic microheaters and another based on MEMs microheaters. These sensor platforms utilize thin film (sputtered) coatings of transition metal oxides that are deposited over the heater elements to affect the electrical output relative to a reference sensor that has no catalytic coating. Using specific target gases such as ammonia, the sensor response was evaluated for each catalyst combination and the most responsive catalysts were further characterized. Changes in the electrical power were used to monitor the response of the catalyst combinations in terms of sensitivity and specificity of gases.

Characterization of Sulforaphane-Resistant Mutants in the Yeast, *Saccharomyces*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Sulforaphane (SFN) is a member of the class of antioxidants known as isothiocyanates, found in broccoli and other cruciferous vegetables. Reports from several laboratories have shown that SFN has both anticancer and antimicrobial activity. However, the mechanism by which SFN acts in living cells remains elusive at this time. Recent studies that have investigated the mechanism of SFN's chemotherapeutic effect have suggested that SFN works by causing cell cycle arrest and/or apoptosis, but the mechanism of cell death is not fully understood. To elucidate the mechanism of action of SFN, we have initiated studies of its effects on the budding yeast, *Saccharomyces cerevisiae*. We have determined that at a concentration of 160 µg/ml, SFN significantly retards the growth of wild type *S. cerevisiae*. We have also identified mutants that are show resistance to SFN. These SFN resistant mutants also show a greater sensitivity to rapamyacin were slow growing as compared to the wild type strain. Complementation analysis shows that the mutants are recessive. We are now attempting to clone the genes involved. This will aid our efforts to identify the genetic mechanism in which SFN influences apoptosis in yeast cells, a pathway that could be conserved in human beings.

Inducible expression of RGS9 in mouse embryonic stem cell-derived neurons

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RI-INBRE Summer Undergraduate Research Fellowship Program

Problem: Regulator of G protein Signaling 9-2 (RGS9-2) is a member of the RGS family of Gα GTPase accelerating proteins that is specifically expressed in the striatum, a brain region that is important in controlling movement. Emerging evidence suggests that RGS9 can protect against the development of a major toxicity of chronic antipsychotic drug treatment called tardive dyskinesia, which results in irreversible and involuntary hyperkinetic movements. Our hypothesis is that RGS9-2 suppresses TD through a neuro-protective action on striatal neurons. Since primary cultured striatal neurons do not express RGS9 we plan to test the action of apoptotic agents on mouse embryonic stem cell-(mESC) derived neurons engineered to inducibly express transgenic RGS9-2 constructs. We plan to produce neurons expressing wild-type (wt) RGS9-2 as well as mutant forms of RGS9-2, lacking RGS activity or with deletions in the previously defined DEP and GGL domains present in the RGS9-2 protein. Neurons engineered in this manner will in addition help us to further characterize the cellular functions of RGS9-2 in neurons. In parallel, we investigated the effect on protein stability of five non-synonymous mutations in the RGS9 gene identified in an ongoing study of schizophrenic patients.

Methods: We constructed a Cre-recombinase dependent targeting vector for the genomic integration and inducible expression of wt RGS9 and RGS9 mutants. PCR based strategies were used to engineer cDNAs for RGS9 N364H, and RGS9 DEP, and GGL domain deletion constructs. RGS9 N364H is a point mutation of RGS9 proposed to disrupt its catalytic activity. To determine whether this mutation disrupts RGS9 catalytic activity, RGS9 wt and RGS9 N364H were expressed in *Xenopus* oocytes and their activities were compared in electrophysiology recordings. In separate experiments for evaluating the effects on protein stability of the point mutations discovered in schizophrenic patients, RGS9 constructs incorporating the mutations were transiently expressed in HEK293 and CHO cells. After inhibiting protein synthesis, we measured the rate of RGS9 protein degradation using western analysis.

Results: The vectors required for supporting inducible expression of RGS9 and RGS9 mutants were successfully engineered. In addition, we constructed cDNAs for RGS9 lacking its DEP domain, GGL domain, and point mutation in the RGS domain. We also found that the degradation rate of the RGS9 point mutants from schizophrenic patients was similar to the wild-type.

Future directions: Future studies include employing the DNA constructs developed in this project for expression of RGS9 in mESC-derived neurons and determining if the mutations in RGS9 identified in schizophrenic patients alter other cellular functions of RGS9.

BAX-induced Cell Death in the Yeast, *Saccharomyces*, 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. The budding yeast, *Saccharomyces cerevisiae*, is a simple eukaryote that does not naturally have Bax. However, when Bax is placed in yeast cells, they undergo a series of events that mirror programmed cell death in mammalian cells. We are investigating the role of calcium in BAX-induced cell death in yeast by overexpressing mammalian BAX in yeast calcium mutants. We have discovered that mutants lacking genes important for calcium regulation including *CNB1*, *CCH1*, and *PMC1* are relatively resistant to BAX-induced toxicity and that BAX-induced toxicity rises as extracellular concentrations of calcium rises. We are working to decipher the molecular mechanism involved in this phenomenon.

Characterization of six new marine viruses using multiple structural and host derived genes

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RI-INBRE Summer Undergraduate Research Fellowship Program

Marine viruses are one of the most abundant and genetically diverse entities in coastal waters. Viral communities play a crucial role in the marine food web by limiting the growth of bacterial populations. In Rhode Island, the Myoviridae virus family is particularly dominant in the coastal waters. Myoviruses are genetically diverse and gene exchange among viral isolates can occur. Previous studies have used g20 (a viral structural gene) as a molecular marker for genetic diversity. This study examines the genetic diversity and gene evolution of seven core-structural and host-derived genes (g20, g23, g43, psbA, psbD, phoH, and cobS) in six new Myoviral isolates from Narragansett Bay. PCR products from each gene were sequenced. These gene sequences were compared to determine their divergence from one another and were used to reconstruct their evolutionary histories. The phylogenetic gene trees were not congruent, suggesting that gene exchange may have occurred. Levels of divergence also differed among the genes, suggesting that some genes are more highly conserved than others or have more recently been acquired by the viruses. By examining the evolutionary history of seven different genes, a better understanding of viral genome evolution will be gained.

Caloric restriction alters transporter expression in the liver

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RI-INBRE Summer Undergraduate Research Fellowship Program

Transporters aid in the absorption and excretion of endogenous and exogenous chemicals. The transporters we are interested in are involved in detoxification and countering oxidative stress. Caloric restriction (CR), which requires a reduction in calories without malnourishment, is known to extend lifespan and reduce the occurrence of multiple diseases. Optimal results are seen with a restriction of 25-60% below ad libitum. The purpose of this study was to determine whether CR changes the transporter expression in the liver, which could affect chemical absorption and excretion. Lean and obese male mice were fed ad libitum or placed on a 40% reduced calorie diet for approximately thirteen weeks. In order to see if CR alters transporter RNA levels in the liver, we isolated RNA from the livers of these mice and performed the Branched DNA Signal Amplification assay to detect the expression levels of various transporters. In lean mice, CR decreased Oatp1, Mrp3, Mrp4, and Mrp5. In obese mice, CR increased Mrp2 and decreased Mrp1 and Mrp5. CR did not change Oatp4, BCRP, Mrp1, Mrp2, and Mrp6 expression levels in lean mice. In obese mice, Oatp1, Oatp4, BCRP, Mrp3, Mrp4, and Mrp6 expression levels also remained unchanged. The data demonstrate that CR alters transporter expression. This suggests that CR may alter chemical disposition in humans.

Exposure-related Alterations to miRNA Expression: Mechanism and Consequences During Intrauterine Development

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Problem: Heritable control of gene function without modification to the DNA sequence is known as epigenetic regulation. This mechanism is important to normal molecular functioning in cells. When epigenetic regulation is altered, certain pathologies can result. While able to maintain integrity through serial cell divisions and generations, epigenetic control is also unique it is capacity to undergo modification by the environment.

Methods: The placenta was studied as a model organ in which epigenetic alterations could be environmentally induced. To examine the role of DNA methylation in the human placenta, a specific microRNA and environmental toxin were analyzed. Cell culturing systems were also central to the study.

Preliminary Results: A plasmid was created to over-express the mircoRNA being examined so that its response to toxin could be more readily observed once treated. This plasmid was then transfected into the placenta cell line.

Energy from Biomass: A microbial fuel cell

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RI-EPSCoR Summer Undergraduate Research Fellowship

Waste streams containing biomass can be very useful. Because the stream has energy value, we would like to recover it. This project can help to reduce our dependence on imported fuel oil. We have demonstrated the production of electricity from simple biomass. Applications are sewage sludge, biodiesel production and trash.

Exploring morphological differences of bloom forming green algae in Greenwich Bay

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Macroalgal blooms are an increasingly frequent phenomenon worldwide resulting in a number of negative ecologic and economic impacts. In Greenwich Bay, Rhode Island, the increased frequency of macroalgal blooms has resulted in the increased occurrence of anoxia. Despite their prevalence, the identification of some bloom forming species remains problematic, complicating the research of macroalgal blooms. This is particularly true of species in the green algal genus *Ulva*, which is the most prevalent bloom-forming genus found in Greenwich Bay. Due to a vast overlap in morphological characteristics, a close examination of morphology is needed. Our research addressed this need through a survey of Ulvoid species located both in Greenwich Bay and along the outer coast of Rhode Island. We anticipated a similar species composition in Greenwich Bay and on the outer coast, though we expected the morphologies of these species to differ depending on their location. Green algal samples were collected in February, April, and June 2008 from eight different sites. All samples were subjected to both macro and microscopic morphological examination and then pressed to form a voucher specimen. The surveys demonstrated that bloom forming species were more prevalent in Greenwich Bay while a more diverse species assemblage was found at sites located outside of Greenwich Bay.

SIRT1 confers protection against UVB and H_2O_2-induced cell death in skin keratinocytes

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RI-INBRE Summer Undergraduate Research Fellowship Program

SIRT1 is a member of a highly conserved gene family encoding NAD^+-dependent deacetylases, originally found to deacetylate histones leading to increased DNA stability and prolonged survival in yeast and mammals. SIRT1 functions as a deacetylase for numerous protein targets involved in many cellular pathways, including cellular stress responses and apoptosis. However, the role of SIRT1 in UV signaling pathways remains unknown. We used cell culture and Western blot analysis to address above question. We found that SIRT1 is expressed in human skin keratinocytes. Both UV and H_2O_2, the major inducers of skin cell damage, down-regulate SIRT1. ROS-mediated JNK activation is involved in SIRT1 down-regulation. SIRT activator resveratrol protects against UV-and H_2O_2-induced cell death, while SIRT inhibitors sirtinol and nicotinamide enhance cell death. Activation of SIRT1 negatively regulates p53 acetylation, since nicotinamide and sirtinol as well as SIRT1 siRNA enhances UV- and H_2O_2-induced p53 acetylation while SIRT1 activator resveratrol inhibits it. SIRT1 activation is also involved in UV-induced AMPK and downstream ACC and PFK phosphorylation. Our data provide new insights into understanding of the molecular mechanisms of UV-induced skin aging, suggesting that SIRT1 activators such as resveratrol could serve as a new anti-skin aging agent.

Extensive variability of the shark Aryl Hydrocarbon Receptor 2 (AHR2) in a domain containing multiple tandem octapeptide repeats

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RI-INBRE Summer Undergraduate Research Fellowship Program

The aryl hydrocarbon receptor (AHR) is an intracellular protein regulating genes that encode biotransformation enzymes. Upon ligand activation, conformational changes of AHR promote its translocation into the nucleus and binding to promoter elements to upregulate some cytochromes P450 and other genes involved in cell physiology. *Squalus acanthias* (spiny dogfish) is the earliest diverging vertebrate investigated for AHR function. We have previously discovered 3 distinct genes encoding AHR in sharks that are highly conserved in their bHLH and PAS domains among vertebrates, but diverge in the amino acid sequence in the carboxyl-terminal half of the protein. Shark AHR2 is unique because it contains an **o**cta**p**eptide repeat **m**otif (OPM) domain in the carboxyl-terminal region not present in other vertebrate AHR2 orthologues. To investigate the structure and function of the OPM domain, we isolated RNA from multiple tissues of three *S. acanthias* individuals, synthesized cDNA, and used primers designed to amplify the entire OPM domain. Our sequencing results from these PCR products reveal at least 6 isoforms that differ in the number of octapeptide repeats (27-36 repeats); these differences occur at two specific sites in the AHR. Together these results and our investigations of genomic structure using OPM DNA probes with the *S. acanthias* BAC library demonstrate an impressive variability in the OPM region whose function is yet undiscovered. While it is unclear whether these AHR2 isoforms represent different genes, alternative splicing of a single AHR2 gene, or another mechanism promoting genetic variability, our studies will clarify the role for AHRs in responses to environmental chemicals at cellular and genetic levels in cartilaginous fishes and provide additional information about the endogenous function of the AHR signaling pathway in vertebrates.

Invasive and native epiphytes in Narragansett Bay and the influence of the marine snail, *Lacuna vincta*

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RI-EPSCoR Summer Undergraduate Research Fellowship Program

Macroalgal epiphyte-herbivore interactions are an important part of marine community dynamics, but have received little attention in the scientific literature. This relationship provides an excellent model for studying herbivore-producer dynamics in rocky intertidal zones. We investigated two related questions in our study: does the presence of epiphytes affect the recruitment of the marine snail, Lacuna vincta, and vice versa. Lacuna is an herbivorous species of snail known to prey on macroalgal epiphytes. Using nylon rope as a macroalgal mimic, we installed some mimics onto the rocks where the Lacuna could recruit freely. These mimics were weeded for epiphyte species and density, with a focus on the invasive species *Neosiphonia harveyi* and the native *Ceramium virgatum*. We placed other mimics in covered buckets so that Lacuna recruitment was restricted but the epiphytes could still recruit on the mimic inside. We placed a controlled density of Lacuna inside each bucket. We found a significant difference in Lacuna numbers where the epiphyte density was altered, but not among the bucket treatments. The Lacuna do not appear to influence epiphyte recruitment.

Eugenia jambolana L. (Jamun) berries: Anthocyanin isolation and antiproliferative effects on human breast cancer cells

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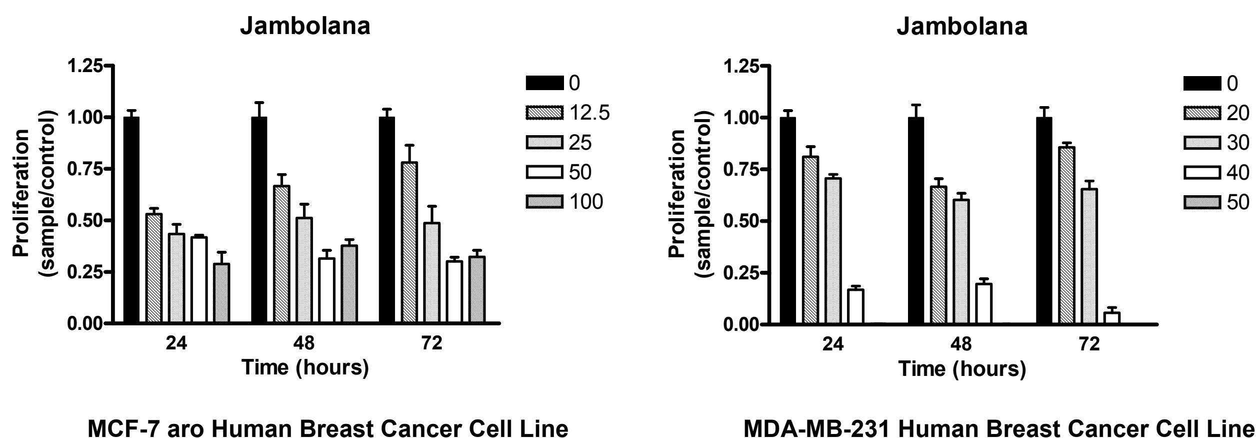
RI-INBRE Summer Undergraduate Research Fellowship Program

Anthocyanins are a colorful class of biologically active compounds responsible for the rich reds, deep blue, and purple hues seen in some flowers and plant foods, most notably berries. Anthocyanin-rich berries have been shown to inhibit the growth of human cancer cell lines *in vitro*. The berries of *Eugenia Jambolana* L., commonly known as Jamun or Jambolana, have been used for centuries by ancient cultures for a variety of medicinal purposes. An anthocyanin-rich Jamun fruit extract (AJE) was prepared using Amberlite resin (XAD-16) column chromatography and evaluated for anthocyanin content by high performance liquid chromatography (HPLC). The AJE was found to contain three major (delphinidin, petunidin and malvidin diglucosides) and two minor (peonidin and cyanidin diglucosides) anthocyanins. AJE was tested for its ability to inhibit human breast cancer cell lines (MDA-MB-231, MCF-7aro) *in vitro* using an ATP cell viability assay. AJE inhibited both breast cancer cell lines at concentrations ranging from 100-12.5 µg/mL with greater activity observed against the triple negative MDA-MB-231 cells. Further *in vivo* studies are required to evaluate the anti-cancer potential of Jamun anthocyanins.



MCF-7 aro Human Breast Cancer Cell Line

MDA-MB-231 Human Breast Cancer Cell Line

Three novel alternative splice forms of Ufd2a are conserved in zebrafish

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Ufd2a is a ubiquitylation enzyme that is involved in protein degradation. It is known to be critical to cell division and may also participate in apoptosis signaling in vertebrates. Ufd2a is most widely expressed in a form which includes exons 1-6 and 8-27 (referred to here as Ufd2a I). However, data has shown that cardiac and skeletal muscle cells of adult rodents and humans express alternatively spliced isoforms, Ufd2a II and III (which include exons 7 and exons 7 and 7a, respectively). In addition, Ufd2a I, the ubiquitous form, has been found to be expressed in murine myoblasts. However, upon differentiation, myotubes began to express Ufd2a II followed by Ufd2a III. Moreover, fully differentiated myofibers expressed Ufd2a III exclusively. Interestingly, when Ufd2a was knocked out in mice, embryos died *in utero* due to massive cardiomyocyte apoptosis. Since zebrafish are an ideal *in vivo* model organism for the study of skeletal and cardiac muscle development, we have begun to examine the expression pattern of the three Ufd2a isoforms in developing embryos and adult tissues. RT-PCR of whole 7 dpf embryos demonstrated that the alternatively spliced exons 7 and 7a are conserved in zebrafish. In addition, expression of Ufd2aI was detected early in development, whereas Ufd2aII was found at 10 hpf, followed by Ufd2aIII (starting at ~24 hpf). Also, Ufd2aIII expression was detected in adult zebrafish heart tissue by this method. Finally, preliminary ISH results suggest that the localization of expression of the three isoforms differs.

Elucidating the role of Ufd2a alternative isoforms in muscle cell differentiation

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Ufd2a is an E3/E4 enzyme that catalyzes the addition of polyubiquitin chains to proteins. Ufd2a has three alternative splice forms, which differ in their inclusion of two tissue specific exons. Ufd2aI, the short form excludes exons 7 and 7a, Ufd2aII includes exon 7 and Ufd2aIII includes both exons 7 and 7a. Expression of the three splice forms is cell type specific; Ufd2aI is detected in most proliferating cells, Ufd2aII solely in differentiating myoblasts and Ufd2aIII in differentiating myotubes and exclusively in mature cardiac muscle fibers. To test whether Ufd2a affects differentiation in C2C12 myoblast cells, morpholino olignonucleotides (MOs) were designed to block expression of each of the three Ufd2a isoforms. Western blot analysis of differentiating cells treated with MOs targeting exons 7 and 7a showed expression of Ufd2aII and III to be significantly reduced, while gross morphological observation suggested reduced cell fusion and differentiation occurred in these cells as compared to cells given a control MO. Currently, we are developing methods to validate the specificity of these effects.

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Arylimines Synthesis and Modification by Phase-Transfer Catalysis to Prepare Estrogen Receptor Modulators

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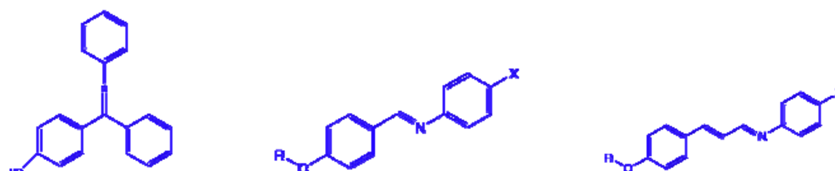
One-step microscale synthesis to prepare a library of arylimines that are structural analogs of GSK4716, and Tamoxifen, potent selective estrogen receptor modulator (SERM), is underway. After characterization by MP, HPLC, LC-MS, IR and GC-MS, these compounds are being screened for estrogen-receptor activity. Modification of the compounds where X = OH can be done in a subsequent step using a Williamson ether synthesis in a phase-transfer reaction with aliquat and potassium carbonate.



Target Compounds; Variables; R and X