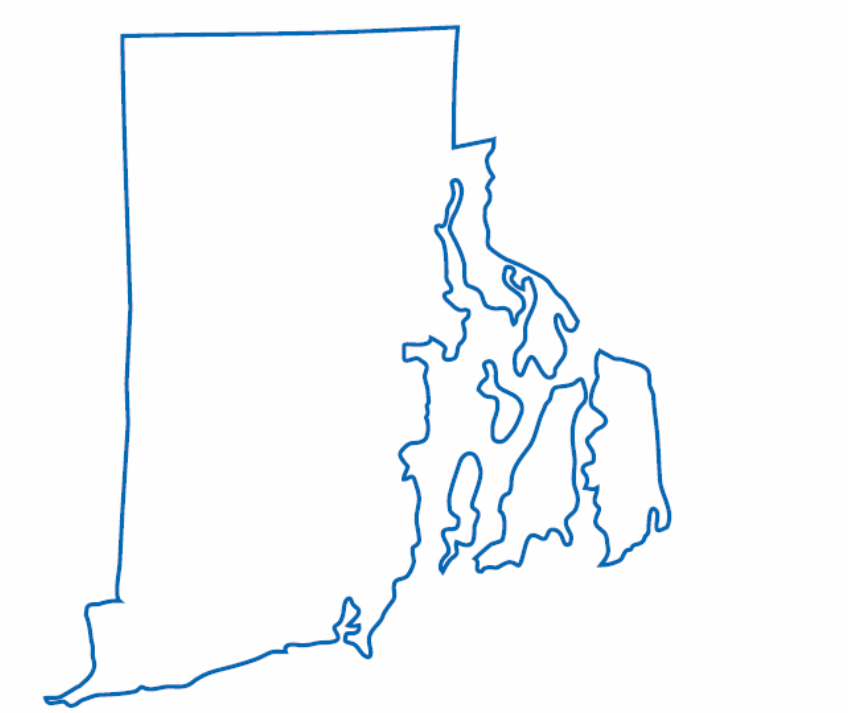
2009 Rhode Island Summer Undergraduate Research Fellowship Conference



Monday, August 3, 2009
8:00 AM

The Ryan Center, University of Rhode Island

Supported by

Rhode Island INBRE

RI EPSCoR
Experimental Program to Stimulate Competitive Research

RI-INBRE & RI EPSCoR

SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

MONDAY, AUGUST 3, 2009

THE RYAN CENTER CONCOURSE

UNIVERSITY OF RHODE ISLAND

KINGSTON, RI

8:00 – 8:30 AM	***CONTINENTAL BREAKFAST & GROUP A POSTER SET-UP***
8:30 AM	***WELCOMING REMARKS***
	ELIZABETH ROBERTS, LIEUTENANT GOVERNOR, STATE OF RHODE ISLAND
	DR. ZAHIR SHAIKH, RI-INBRE PROGRAM DIRECTOR
	DR. ANDREW STAROSCIK, RI EPSCOR ACADEMY DIRECTOR
8:45 – 10:15 AM	***SUMMER UNDERGRADUATE RESEARCH FELLOWS POSTER SESSION***
	GROUP A
10:15 AM	***INTERMISSION & GROUP B POSTER SET-UP***
10:30 – 12:00 PM	***SUMMER UNDERGRADUATE RESEARCH FELLOWS POSTER SESSION***
	GROUP B
12:00 PM	LUNCH

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.*

University of Rhode Island

Poster #	Summer Fellow	Mentor
4	Alexandera Barbosa	Carol Thornber, Ph.D.
12	Benjamin Carroll	Keykavous Parang, Ph.D.
14	Jillian Chase	Abraham Kovoor, Ph.D.
18	Patricia Coutts	Stanley Barnett, Ph.D.
21	Siobhan Curran	Roberta King, Ph.D.
26	Russell Dunn	Tatiana Rynearson, Ph.D.
29	Jocelyn Friel	David Rowley, Ph.D.
31	Marian Gaviola	Daniel Udwary, Ph.D.
34	Neil Greene	Paul Cohen, Ph.D.
39	Wusung Kim	Bongsup Cho, Ph.D.
41	Alline Lelis	Geoff Bothun, Ph.D.
46	Kathryn Marks	Brenton DeBoef, Ph.D.
51	Jemima Monchery	Zahir Shaikh, Ph.D
52	Jennifer Moniz	Clinton Chichester, Ph.D.
60	Afeez Olalekan	Stanley Barnett, Ph.D.
64	Saima Qamar	Matthew Stoner, Ph.D.
70	Simon Sarkisian	Kerry LaPlante, Ph.D.
72	Joanna Scimeca	Bongsup Cho, Ph.D.
75	Mae Shen	Niall Howlett, Ph.D.
78	Amanda St. Germain	Angela Slitt, Ph.D.
85	Daniel Woolridge	Navindra Seeram, Ph.D.

Brown University

Poster #	Summer Fellow	Mentor
19	Erin Cummings	Barbara Stonestreet, M.D.
50	Jamieson Mellor	Daniel Weinreich, Ph.D.
57	Kao Chin Ngeow	William Fairbrother, Ph.D.
73	Justin Shaya	Wayne Bowen, Ph.D.
79	Benjamin Stein	Wolfgang Peti, Ph.D.

Providence College

Poster #	Summer Fellow	Mentor
9, 48, 56	Gabriella Brum	Yinsheng Wan, Ph.D
15	Christopher Chin	Nicanor Austriaco, Ph.D.
25	William Dundon	Joseph DeGiorgis, Ph.D.
40	David Laprade	Nicanor Austriaco, Ph.D.
9, 48, 56	Michael McCauley	Yinsheng Wan, Ph.D.
63	Adam Miller	Christopher Bloom, Ph.D.
54	Kevin Murphy	Nicanor Austriaco, Ph.D.
9, 48, 56	Ari Nalbandian	Yinsheng Wan, Ph.D.
59	Michael O'hara	Elisabeth Arévalo, Ph.D.
63	David Pucino	Christopher Bloom, Ph.D.
68	Colin Samoriski	Joseph DeGiorgis, Ph.D.
68	Jean-Pierre Sarkis	Joseph DeGiorgis, Ph.D.
63	Jesse Sheinhite	Christopher Bloom, Ph.D.
83	Nicholas Vincent	Charles Toth, Ph.D.

Rhode Island College

Poster #	Summer Fellow	Mentor
1	Kenneth Almeida	John Williams Jr., Ph.D.
6	Krystal Bergeron	Karen Almeida, Ph.D.
44	Rosalie Berrios-Candelaria	Thomas Malloy, Ph.D.
8, 49	Lily Brown	Karen Almeida, Ph.D.
10, 37	Liam Burke	Deborah Britt, Ph.D.
17	Brenda Cordeiro	Sarah Spinette, Ph.D.
20, 66	Elizabeth Cuoto	John Williams Jr., Ph.D.
23, 84	Jennifer Desjarlais	Robin Montvilo, Ph.D.
36	Jacob Hicks	John Williams Jr., Ph.D.
37, 38	Shira Hirshberg	Deborah Britt, Ph.D.
27	Daniel Fisher	John Williams Jr., Ph.D.
37	Crystal King	Deborah Britt, Ph.D.
44	Lorin Kinney	Thomas Malloy, Ph.D.
8, 49	Kirsten Mello	Karen Almeida, Ph.D.
44	Peter Murphy	Thomas Malloy, Ph.D.
65	Daniel Reeves	Rebeka Merson, Ph.D.
20, 66	Tomaz Rosadzinski	John Williams Jr., Ph.D.
23, 84	Erica Russo	Robin Montvilo, Ph.D.
77	Jose Solares	Sarah Spinette, Ph.D.
23, 84	Shana Warot	Robin Montvilo, Ph.D.

Roger Williams University

Poster #	Summer Fellow	Mentor
2	Nichole Ares	David Taylor, Ph.D.
3, 61	Keith Austin	Dan Von Riesen, Ph.D.
22	Diana Denio	Lonnie Guralnick, Ph.D.
30	Elizabeth Futoma	David Taylor, Ph.D.
32	Carissa Gervasi	David Taylor, Ph.D.
33	Cait Gosselin	Marcia Marston, Ph.D.
43	Jennifer Linehan	David Taylor, Ph.D.
3, 61	Barbara Mann	Avelina Espinosa, Ph.D.
47	Todd Massari	David Taylor, Ph.D.
3, 61	Monichan Phay	Avelina Espinosa, Ph.D.
62	Angela Possinger	Loren Byrne, Ph.D.
3, 61	Laura-Ashley Przondo	Dan Von Riesen, Ph.D.
71	Jasmine Schonwald	Marcia Marston, Ph.D.
76	Lisseth Silva	Avelina Espinosa, Ph.D.
33, 80	Samantha Taylor	Marcia Marston, Ph.D.
3, 61	Robert Yaeger	Dan Von Riesen, Ph.D.
	High School Teacher	
53	Christopher Munzert	Marcia Marston, Ph.D.
53	John Wemple	Marcia Marston, Ph.D.

Salve Regina University

Poster #	Summer Fellow	Mentor
5	Jessie Barowski	Alison Shakarian, Ph.D.
7, 67	Amanda Borges	Sándor Kádár, Ph.D
11	Mark Byrne	Alison Shakarian, Ph.D.
16	Amy Coffey	Bernard Munge, Ph.D.
16	Jaimee Doucette	Bernard Munge, Ph.D.
24	Richard Dowd	Bernard Munge, Ph.D.
28, 69	David Fraulino	Alison Shakarian, Ph.D.
35	Carin Heaney	Sheila Quinn, Ph.D.
42	Mandy Letourneau	Sándor Kádár, Ph.D
45	Justin Mare	Alison Shakarian, Ph.D.
55, 82	Edwin Mutanguha	Steven Symington, Ph.D.
58	Jessica Odobasic	Sándor Kádár, Ph.D
35	Alexandra Pereira	Sheila Quinn, Ph.D.
7, 42, 67	Deanna Salter	Steven Symington, Ph.D.
35	Samantha Sandland	Sheila Quinn, Ph.D.
69	Carlos Santos	Alison Shakarian, Ph.D.
74	Alison Shea	Steven Symington, Ph.D.
24	Michael Sullivan	Bernard Munge, Ph.D.
55, 82	Zachary Valentine	Steven Symington, Ph.D.
	High School Student	
81	Mary Tobin	Alison Shakarian, Ph.D. & Steven Symington, Ph.D.
13	Nathan Charette	Alison Shakarian, Ph.D. & Steven Symington, Ph.D.

Trialyphosphonium conjugates with 1-naphthol and flouresceine tested for fluoresce and FRET

Almeida, K., Alexander, A., and Williams Jr., J. C.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Triaryl(X)phosphonium salts cross lipid membranes and can carry other covalently bound groups (X) with them that would not diffuse easily alone. Attaching a known bioactive moiety as “X” might allow selective accumulation in target tissues. The salts themselves are generally cytotoxic to bacteria, trypanosomes, schistosomes, malignant and normal cells in culture, Lemna minor, and mice. As such they are in use as drugs in treatment of sleeping sickness in humans and have been shown to kill schistosome parasites in mice. Phosphonium salts are used as additives to paints used on drilling rigs in the North Sea to suppress growth of marine organisms. They inhibit acetylcholinesterase and some oxidases and bind to genomic DNA *in vitro*. Imaging these compounds *in vitr*o and *in vivo* using a fluorescent maker is the goal in this work. This would help elucidate the mechanism(s) of toxicity for these compounds. Para-(triphenylphoshonium)toluic acid bromide was conjugated with 1-naphthol and flouresceine using thionyl chloride to form the acid chloride then reaction with the hydroxyl group on each compound in a one tube synthesis. The fluorescence spectra were observed. Preliminary results indicate that the naphthol conjugate does not show any fluorescence different from a variety of other Triphenyl(X)phosphonium salts but that the flouresceine conjugate does and in fact exhibits FRET.

Mercury accumulation in brain and muscle tissues of bluefish (*Pomatomus saltatrix*) and tautog (*Tautoga onitis*)

Ares, N.L. and Taylor, D.L.

Department of Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant that negatively affects human health, and exposure occurs mainly through the consumption of finfish. Consequently, previous research has been dedicated to measuring Hg levels in muscle filets of edible fish, including the bluefish (*Pomatomus saltatrix*) and tautog (*Tautoga onitis*). While Hg contamination in muscle tissue of these species is well documented, there is little information on Hg concentrations in other tissues. The brain is a tissue of particular concern because Hg is a neurotoxin. The objectives of this investigation were to: (1) examine Hg bioaccumulation in brain and muscle tissue of bluefish and tautog, and (2) evaluate the relationship between Hg levels in the two tissue types. From June to August 2007-2009, target fish were collected from Narragansett Bay (RI, USA) using otter trawls and rod & reel. Length (cm) was recorded for each fish, after which total Hg was measured in excised muscle and brain tissue using combustion atomic-absorption spectroscopy (ppm dry wt). For bluefish and tautog, Hg concentrations of muscle and brain tissue were positively correlated with fish length (Blue: R^2=0.110, n=7; Taut: R^2=0.256, n=17), indicating that the Hg bioaccumulates in both tissues. There was also a positive correlation between muscle and brain tissue Hg concentrations for both target fish (Blue: R^2=0.868, n=7; Taut: R^2=0.468, n=17). Among these relationships, tautog experienced elevated brain Hg concentrations relative to bluefish. Interspecies differences in Hg contamination are attributed to tautog being older, and thus, having a protracted period in which they accumulate Hg. Future research will include the analysis of target fish livers, as well as the possible role of selenium in mitigating the toxic effects of Hg.

Synergistic approach to find alternative drugs against amebiasis: 3, 5-phenyl-1-(Phenyl carboxamide)-2-pyrazoline series

Austin, K.[2], Przondo, L.[2], Yaeger, R.[2], Mann, B.[1], Phay, M.[1], Von Riesen, D.[2] and Espinosa, A.[1]

[1]Department of Biology, Roger Williams University, Bristol, RI
[2]Department of Chemistry, Roger Williams University, Bristol, RI

RI-INBR Summer Undergraduate Research Fellowship Program

Entamoeba histolytica is a parasitic protozoan which infects 50 million people worldwide causing 100,000 mortalities annually. The primary treatment of amebiasis utilizes metronidazole which is known to have unwanted side effects and cause complications. Lacking mitochondria and residing within the anaerobic environment of the intestinal lumen causes the organism to utilize a fermentative pathway for metabolism. The terminal fermentation step is catalyzed by the bifunctional enzyme *E. histolytica* alcohol dehydrogenase 2 (EhADH2) which converts acetyl-CoA to acetaldehyde and acetaldehyde to ethanol, regenerating NAD^+ from NADH. It has been found that EhADH2 is required for both growth and survival of the parasite. The crucial pathway EhADH2 completes and low homology to human dehydrogenase makes the enzyme an ideal chemotherapeutic target. The lack of sufficient treatment options has increased the search for new antiamebic compounds and chemotherapeutic targets. Nine compounds of the 3, 5-phenyl-1-(Phenyl carboxamide)-2-pyrazoline series have been synthesized and tested for antiamebic activity and inhibition of EhADH2. None of the compounds exhibited antiamebic or EhADH2 inhibitory properties during screening. However, when compared to 3-phenyl-1-(phenyl carboxamide)-2-pyrazolines, which display both antiamebic and EhADH2 inhibition activities, information can be gathered on both successful inhibitor design and enzyme structure. The synthesis of a new series is in progress.

Partially funded by the 2008-2011 Merck/AAAS URSP grants

Macroalgal blooms: The rate of tissue decomposition and its relevance to the estuarine food web

Barbosa, A. and Thornber, C.

Department of Biological Sciences, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship

Macroalgal blooms are present in estuaries around the world. The goals of this project were to determine the rate of algal bloom tissue decomposition and to determine if algal nutrients were recycled into the estuarine food web. The bloom-forming species *Gracilaria vermiculophylla*, tubular branched *Ulva* spp., *Ulva rigida*, and *Ulva compressa* were collected in the field, weighed, and placed into mesh bags. Bags were placed in the shallow subtidal at the University of Rhode Island Bay Campus in Narragansett, Rhode Island, and at Oakland Beach, Warwick, Rhode Island. We found significant variation in decomposition rate among species. Tubular branched *Ulva* was found to decay the fastest after a 2 day period, followed by *Gracilaria* and *Ulva compressa*. However, *Ulva rigida* did not decay, but instead grew, over a 5 day period. Algae decayed significantly faster at Oakland Beach, an estuary site, than at Narragansett, a rocky site. The organic content of algae significantly decreased during the experiment, indicating the algae was decaying. Our data indicated that algal decay is quite rapid in this system, and can be an effective means of nutrient recycling in Narragansett Bay.

Protein purification and calculation of specific activity of an HA-tagged secretory lipase from the human pathogen *Leishmania donovani*

Barowski, J. and Shakarian, A.M.

Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Lipase is an enzyme that catalyzes the hydrolysis of ester bonds and breaks down lipids into usable forms of energy. In many organisms, including parasites, lipase aids in nutrient acquisition and is known increase the virulence of some pathogens. There is little known about the role of lipase in the human parasitic protozoan, *Leishmania.* Thus, investigating the biochemical aspects of lipase from *L. donovani* should aid in revealing the role of lipase in the biology of these organisms and possibly provide insight to the virulence of *Leishmania*. Previously, an episomal expression vector bearing the *LdLIP3* putative secretory lipase gene was constructed with an HA epitope tag (*pKSNEO::LdLIP3::HA*) and subsequently transfected into *L. donovani* promastigotes. Control cells were transfected with the expression plasmid, *pKSNEO*. In the current study, the HA-tagged LdLIP3 protein was purified from culture supernatants of mid-log phase promastigotes by anti-HA affinity matrix column chromatography. Column elution fractions were tested for lipase activity at 37^0C and 42^0C at pH6.0 and 7.0 using 4-methylumbiliferyl stearate as substrate. In addition, BCA assays were performed to determine the amount of protein present in each sample and to calculate the specific activity (nmol/min/mg) of the purified enzyme. Results revealed that there was an optimal 675% fold increase in specific activity of purified protein from *pKSNEO:LdLIP3::HA* transfectants verses unpurified samples at 42^0C, pH 6.0. As expected, control pKSNEO transfectants showed no enzyme activity. In addition, Western Blot analysis verified that the purified HA-tagged lipase was ~33 kDa and was only present in culture supernatants from cells transfected with the lipase gene. Taken together, this data shows that the *LdLIP3* gene of *L. donovani* codes for a protein with lipase activity. Further studies will be carried out to determine the optimal pH, temperature, and substrate specificity of the expressed lipase.

Far western analysis of the bloom syndrome protein and Rad51

Bergeron, K.L., Murphy, E.L., and Almeida, K.H.

Physical Science Department, Rhode Island College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Bloom syndrome is a rare recessive disorder, resulting from a mutation in the Bloom Syndrome protein (BLM). BLM is a DNA helicase that is involved in the overall stability of the genome but, the exact role of BLM in the genome is unknown. BLM is involved in homologous recombination, the DNA repair pathway for double strand breaks. When BLM is mutated or absent, an increase of homologous recombination occurs. Therefore, a diagnostic symptom of patients with Bloom Syndrome is an increased in sister chromatid exchange, a marker of homologous recombination. Most importantly patients that suffer from Bloom Syndrome are predisposed to cancer, presumably due to genomic instability. This research consisted of the production and purification of fragments of BLM. Literature reports that Rad51 interacts with BLM on the N-terminus and the C-terminus of BLM. Rad51 is a homologous recombinant pathway protein that is essential for survival. Elaborating on previous literature, this study of protein-protein interactions between BLM and Rad51 refines the partnering domains via Far Western analysis. Results confirmed that the N-terminus and C-terminus of BLM interacts with Rad51. More specifically, the last 114 amino acids of the N-terminus (BLM 100-214) and the first 50 amino acids of the C-terminus (BLM 1317-1367) are responsible for the interaction with Rad51. The majority of the interaction on the N-terminus is between amino acids 150-214 (55%), with BLM 100-150 contributing 22% to the interaction. Similarly, amino acids 1317-1367 are responsible for 24% of the interaction. Further investigation of the exact amino acid sequence of the protein binding domain of BLM to Rad51 will be conducted via a competitive peptide assay. The last 64 amino acids of the N-terminus (150-214) were entered into a BLAST search, revealing that thirteen of these amino acids were similar to the Intein homing endonuclease, PI-Tko II. This suggests that BLM 150-214 may be the site of a secondary DNA binding domain. To better understand the role of BLM in the genome, future experiments will consist of studying BLM 1-214 with DNA.

Modeling catecholamine secretion using a comprehensive mechanism for intracellular calcium oscillations

Borges, A., Salter, D., Kádár, S., and Symington, S.

Department of Biology & Biomedical Science, Salve Regina University, Newport, RI

RI-EPSCoR Summer Undergraduate Research Fellowship Program

We propose a comprehensive two-cell mechanism describing Ca^{2+} dynamics in the cell and a possible mechanism for information transmission between cells. The model was constructed from the model by Cuthbertson and Chay which accounts for the cascading G-protein mechanism and from the model by Borghans, Dupont, and Goldbeter which places emphasis on the Ca^{2+} -induced Ca^{2+} release (CICR). The model was refined by accounting for the biphasic Ca^{2+} -dependence of the IP_3 receptor on the endoplasmic reticulum. A two-cell configuration was considered to establish a realistic scenario: the first cell is stimulated by a commonly accepted manner with an artificial piece of information which is converted to a neurotransmitter-carried signal (dopamine, DA) by the first cell for the second cell. The model is also refined to account for the neurotransmitter-dependent down-regulation of the voltage-gated calcium channel. The feasibility of the model is being studied experimentally. Two key dynamical parameters, the cytosolic Ca^{2+} concentration and the DA concentration in the synaptic cleft are targeted through studying the effect of externally added DA and potassium ions which triggers Ca^{2+} influx into the cell. The cytosolic Ca^{2+} concentration is being monitored with Ca^{2+} -green and we are currently working on developing an amperometric method to monitor DA concentration.

Overlapping bloom syndrome protein ORF expression: Amino acids 150-700 and 642-1290

Brown, L.W., Mello, K., Capuano, M., and Almeida, K.H.

Physical Sciences Department, Rhode Island College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Bloom syndrome (BLM) protein is a 160 kD protein thought to stabilize the genome through helicase activity. BLM is involved at the replication fork as well as the DNA pathway, homologous recombination, (HR). This research describes the investigation of protein-protein interactions between BLM and other HR proteins. To further investigate BLM protein interactions the full length protein was segmented into smaller overlapping sections. The BLM amino acids 150-700 and 642-1290 were PCR amplified to incorporate a C-terminus FLAG epitope tag then inserted into pENTR (Invitrogen) vector for *E.coli*. Individual colonies were verified by restriction enzyme digestion and confirmed by DNA sequencing. *E.coli* expression plasmids containing the fragment of interest fused to an N-terminal 6x His epitope tag were generated, induced, then verified via Western blot analysis. BLM $_{150\text{-}700}$ and BLM $_{642\text{-}1290}$ proteins have expected weights of 63.8 kD and 74.6 kD respectively. Both protein inductions via IPTG addition produced only degraded product on Western blots against the FLAG and His epitope tags, showing degradation from both ends. An alternative protein induction media (Genscript) produced a small amount of the expected ~75 kD protein for both fragments. Affinity column purification under denaturing conditions resulted in the expected BLM $_{642\text{-}1290}$ product but contained degradation of BLM $_{150\text{-}700}$. Currently, other avenues of expression are being investigated, including a change in *E.coli* background and Insect *in vitro* transcription/translation protocols.

Effect of hypertonic stress on appotosis and cell survival

Brum, G., Nalbandian, A., Fredette, R., McCauley, M., and Wan, Y.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Hypertonic conditions cause cells to lose water into the environment, to shrivel and eventually die. Overactive exercise or sports result in excreting excess amount of sweats with high concentration of salts, creating a hypertonic condition that may damage skin cells, if chronically, eventually leading to skin premature aging. The cell signaling pathways involved in hypertonic stress-induced skin cell damage remain elusive. To understand how skin cells respond to hypertonic environments, we cultured human skin keratinocytes in a medium with increasing amount of NaCl ranging from 150mM to 200 mM. Western blot analysis data show that AMPK is activated with the increasing concentration of NaCl in the medium 30 minute post treatment, while AKT is deactivated. Cell migration test data show that NaCl treatment decreases cell migration and wound healing. Interestingly, we found that AQP3 is down-regulated in response to NaCl treatment. Those results have been also confirmed with another hypertonic solution of sorbitol. Taken together, we conclude that hypertonic stress induces apoptotic signal AMPK activation and cell survival signal inactivation in skin cells that may lead to premature skin aging.

Tamoxifen mimics for screening as potential SERM's are synthesized by phase-transfer Wittig reactions.

Burke, L. and Williams Jr., J.C.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship
RI EPSCoR Summer Undergraduate Research Fellowship (2008)
RI Science and Technology Advisory Council

Tamoxifen is a selective estrogen receptor modulator (SERM) and is an important drug in the treatment of breast cancer, but it has a variety of side effects. It starves cells of estrogen in certain tissues and enhances estrogenic activity in other tissues. The latter effect has been shown to cause uterine and endometrial cancers. The goal of this work was to develop and improve a total synthesis scheme with five or fewer steps to make a variety of tamoxifen analogs without using either exotic or particularly toxic compounds that would allow significant variation of the molecular structure in the last step. We have prepared triarylethenes in up to 50% yield with one aryl group having a para carboxylic acid substituent using aliquat as a phase-transfer catalyst and sodium hydroxide in dichloromethane. Several ester and amide derivatives have been prepared from this via formation of the acyl chloride with thionyl chloride followed by reaction an alcohol or amine. Scale-up of the triarylethene reaction is underway. Screening against the estrogen receptor using a luciferase reporter assay is underway (Matt Stoner, URI College of Pharmacy) and has identified six compounds showing some activity, either estrogenic or anti-estrogenic. Computational screening using the eHitsLightening® docking program has identified several compounds with scores the same order of magnitude as tamoxifen that are accessible by this synthetic pathway.

A comparison of *LdLIP3* transcripts copy numbers in promastigotes and amastigotes of the human pathogen *Leishmania donovani* using one-step RT-qPCR SYBR green analysis

Byrne, M. and Shakarian, A.M.

Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania are parasitic protozoa that have a digenetic lifecycle appearing as flagellated promastigotes in the sandfly vector and as non-flagellated intracellular amastigotes in the reticuloendothelial macrophages of the human host. *Ldlip3* is a gene in *Leishmania* which codes for a secretory lipase. It is hypothesized that lipases play a major role in the biology of the pathogenic parasite, *Leishmania spp*. In the current study, RT-qPCR was used to further investigate the role lipase plays throughout the life cycle of these important human pathogens by examining the relative amount of transcript expressed from the *LdLIP3* gene by *L. donovani* promastigotes and amastigotes. For these studies, total RNA isolated from each life cycle stage, gene specific primers and SYBR Green were used in Real-Time one-step RT-PCR assays. In addition, standard curves were constructed using the cloned *Ldlip3* gene of known concentration/copy number. These standard curves were subsequently used to determine the number of *LdLIP3* transcripts in the promastigote and amastigote total RNA samples. The relative number of copies of *LdLIP3* specific transcript in *Leishmania donovani* promastigotes and amastigotes were normalized by comparison of the *LdLIP3* transcript levels to those determined in parallel studies for the housekeeping gene, β-tubulin. Preliminary results indicate that both forms of the parasite express the *LdLIP3* gene at the level of transcription which is consistent with previous lipase activity data indicating both amastigotes and promastigotes secrete this enzyme during their growth *in vitro*.

Design of lipophillic bifunctional masked nucleoside monophosphates as anti-HIV agents

Carroll, B., Ahmadibeni, Y., and Parang, K.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

HIV reverse transcriptase is a critical enzyme involved in converting RNA into DNA during the life cycle of the virus. The biological activity of antiviral nucleoside reverse transcriptase inhibitors is dependent upon many factors. The nucleosides used in reverse transcriptase inhibition are the polar molecules that make their cellular uptake a potential challenge. Another major factor is the requirement for the nucleosides to be phosphorylated by nucleoside kinases before becoming biologically active drugs. The issues of development of viral resistance, cellular uptake of nucleosides and negatively-charged active nucleoside monophosphates, and first rate limiting phosphorylation step are potentially diminishing the efficacy of reverse transcriptase inhibitors. Therefore, there are major interests to develop compounds that can effectively deliver different nucleosides intracellulary. The purpose of this project was to develop a multifunctional antiviral prodrug that can deliver different masked nucleoside monophosphates into cells. The hypothesis of the project was that a lipophillic bidendate scaffold can be used to mask nucleoside monophosphates in order to improve their cellular uptake, bypass the first rate limiting phosphorylation step and release different nucleoside monophosphates intracellulary. A lipophillic bisalicyl alcohol derivative was synthesized as a scaffold from salicyl alcohol in a Friedel Crafts reaction. The scaffold was used in reactions with phosphorylating reagents, conjugation with two of three nucleosides (lamivudine, emtricitabine, alovudine), and oxidation respectively. Three combinations of nucleosides (lamivudine and emtricitabine, lamivudine and alovudine, alovudine and emtricitabine) were used in the synthesis of the masked dinulceoside monophosphate bi*cyclo*Saligeny derivatives. Symmetrical bi*cyclo*Saligeny derivatives, single lipophillic *cyclo*Saligenyl derivatives, and single *cyclo*Saligenyl derivatives are synthesized as control compounds. The chemical structures for all synthesized structures were confirmed by mass spectrometry, ^{1}HNMR, ^{13}CNMR, and ^{31}PNMR. The cellular uptake and other biological activities will be determined against cell-free, cell-associated, and multi-drug resistant virus.

Identification, cloning and sequencing of the β-tubulin gene from the lizard parasite *Leishmania tarentolae*

Charette, N.[1], Symington, S.B.[2], and Shakarian, A.M.[2]

[1]Tiverton High School, Tiverton, RI

[2]Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

Salve Regina University High School Outreach Program

In the current study, the gene for β-tubulin was identified, cloned and sequenced from *Leishmania tarentolae*, a lizard parasite used as a model system for human disease. For these experiments, *L. tarentolae* promastigote cells were grown in M119+ media and harvested by centrifugation. The genomic DNA (gDNA) was isolated and used as template in PCR amplifications using primers based on β-tubulin genes from other *Leishmania* species. Agarose gel electrophoresis of the PCR reactions showed an expected ~1300 bp amplified product which was then ligated into the pTOPO plasmid vector and transformed into *E. coli*. Transformed colonies were selected for on ampicillin agar plates. Colony PCR and agarose gel electrophoresis was used to identify those colonies that contained plasmids with β-tubulin inserts. Sequence analysis of the purified pTOPO::β-tubulin plasmid revealed a 1329 bp β-tubulin gene which codes for a protein of 443 amino acids. Taken together, this data constitutes the first report of the identification, cloning and sequencing of a β-tubulin gene in *Leishmania tarentolae*.

Developing bioluminescence resonance energy transfer assays for monitoring interactions between the D2 dopamine receptor and RGS9-2 and arrestin

Chase, J.[1], Celver, J.[1, 2], Sharma, M.1, and Kovoor, A.[1, 2]

[1]Dept. of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI
[2]Kovogen, LLC

RI-INBRE Summer Undergraduate Research Fellowship Program

Introduction: Drug-induced dyskinesias (DID) are unexplained and irreversible movement disorders which result from the pharmacotherapy of Parkinson's disease and schizophrenia. These side effects are thought to result from the chronic actions of the respective drugs at striatal D2-like dopamine receptors (D2R). Regulators of G-protein signaling (RGS) proteins accelerate GTPase activity of the G□, a G protein subunit. RGS9-2 is specifically expressed in the striatum and regulates D2R responsiveness. Similarly, arrestin is able to bind to agonist activated and phosphorylated G-protein coupled receptors and can regulate D2R activity and cellular localization. Understanding the underlying mechanisms responsible for regulating D2R activity and localization may help to elucidate mechanisms that lead to aberrant D2R signaling associated DID. We are developing Bioluminescence Resonance Energy Transfer (BRET) assays to study the interactions of the D2 dopamine receptor with RGS9 and arrestin in real-time in living cells.

Design: Our objective was to construct yellow fluorescence protein (YFP) and Renilla luciferase (LUC) fusion proteins for expression of appropriate donor and acceptor pairs such that interactions with D2R and RGS9 or arrestin can be monitored using BRET in living cells. These constructs include D2R-YFP, RGS9-2-LUC, and arrestin-LUC. Upon addition of the luciferase substrate to cells expressing the fusion proteins, close proximity of the RGS9-LUC or arrestin-LUC with D2R-YFP should enable the light produced by the luciferase fusion proteins to excite D2R-YFP. This excitement is expected to produce an emission peak for D2R-YFP that is dependent on an interaction between D2R and either RGS9 or arrestin.

Results: We have successfully constructed the appropriate constructs for expression of the YFP and luciferase fusion proteins described above. We will report on preliminary BRET assay results conducted between D2R and RGS9 or arrestin. Successful application of the BRET technology with D2R, RGS9 and arrestin will provide useful tools in understanding mechanisms by which D2R activity and cellular localization are regulated by RGS9 and arrestin; regulation that could underlie aberrant D2R signaling associated with DID.

Caspofungin induces Aif1 dependent apoptosis in the budding yeast, *Saccharomyces cerevisiae*

Chin, C. and Austriaco, N.

Department of Biology, Providence College, Providence RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Caspofungin is the first drug of the new echinocandins class of drugs. It works by inhibiting the enzyme β(1,3)-D-glucanase, disturbing the cell wall of the target cells. Caspofungin was already known to be effective against *Candida* and *Aspergillus* fungal infections. We have shown that culturing the budding yeast *Saccharomyces cerevisiae* in media containing caspofungin induces the generation of reactive oxygen species, fragmentation of the mitochondria and degradation of the genomic DNA. These are hallmarks of apoptosis. We then cultured a *Saccharomyces cerevisiae* mutant lacking the *AIF1* (or apoptosis inducing factor 1), a gene necessary for caspase-independent apoptosis and found decreased levels of reactive oxygen species, of mitochondrial fragmentation and lower levels cell death suggesting that caspofungin induces apoptotic yeast cell death through an *AIF1*-dependent pathway. This gives insight into the manner by which caspofungin kills, as well as identifying a possible way for resistance to be acquired.

Sensitive electrochemical immunosensor for interleukin 8 (IL-8), cancer biomarker protein based on gold nanoparticle platform

Coffey, A., Doucette, J., and Munge, B.S.

Department of Chemistry, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Sensitive electrochemical immunosensor based on glutathione gold protected nanoparticles (GSH-AuNP) was developed for the detection of cancer biomarker interleukin-8 (IL-8) in calf serum. The electrochemical detection of IL-8 is based on a sandwich immunoassay in conjunction with catalytic reactions involving horseradish peroxidase conjugated to a secondary antibody. The formation of GSH-AuNP was characterized using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and atomic force microscopy (AFM). The UV-Vis of the particles gives a characteristic Plasmon band at 514 nm, indicating an even size distribution. The FT-IR shows peaks which are consistent with the disappearance of the sulfur hydrogen bond (2500 cm^{-1}) and the presence of carboxyl groups (3000-3500 cm^{-1}, and 1717 cm^{-1}). The AFM show particles of approximately 5 nm in size. Non-specific binding (NSB) events were minimized by optimizing the primary and secondary antibodies concentrations and use of BSA and Tween-20. The immunosensor gave a detection limit of 1 pg mL^{-1}, which is 10-fold lower than the standard ELISA method and that of IL-6 at 10 pg mL^{-1} in a 10 μL new born calf serum sample reported in our lab recently. These GSH-AuNP based immuonsensors show great promise for the fabrication of ultrasensitive biosensor microarrays for point-of-care cancer diagnosis.

Expression and purification of human Ufd2a isoforms in *Saccharomyces cerevisiae*

Cordeiro, B. and Spinette, S.

Biology Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Ufd2a is an ubiquitylation protein that labels substrates for proteolysis by acting as an ubiquitin ligase and polyubiquitin chain extension enzyme. Previous studies have identified three isoforms of Ufd2a in mammals that result from alternative splicing: *Ufd2aI*, *Ufd2aII*, and *Ufd2aIII*. The isoform expression changes during muscle injury and regeneration in mice, indicating that they may have different functions in this tissue type. Its muscle-specific substrates of ubiquitylation have not been identified, indicating the various isoforms may have different substrates, and the ubiquitylated proteins in muscle differentiation are possible candidates. The *Ufd2aI* and *Ufd2aIII* isoforms, as well as a catalytically inactive mutant, *Ufd2aIII*∆*Ubox* were cloned into yeast expression vectors, and transformed into *Saccharomyces cerevisiea.* Ufd2a expression was induced with a Gal1 promoter and the proteins were extracted at various time points after induction. Preliminary results have indicated that yeast cells were successfully transformed with the expression vectors containing the Ufd2a isoforms. Western blots revealed that the cells are expressing *Ufd2aI*. Additional clones will be induced in order to express *Ufd2aIII* and *Ufd2aIII*∆*Ubox* in yeast cells. The recombinant proteins will then be purified using a nickel affinity column. Future studies will utilize known substrates in order to assess their relative ubiquitylation activity. These proteins will serve as valuable tools for the testing of candidate molecules as substrates of Ufd2a activity.

Microbial fuel cell for energy and chemical by-products

Coutts, P.L., Hanselman, S.L., and Barnett, S.M.

Department of Chemical Engineering, College of Engineering, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

There is a growing need to convert the glycerol waste stream from the production of biodiesel into other valuable products. This work uses a microbial fuel cell to generate electricity and produce useful green chemicals from the waste. A microbial fuel cell (MFC) uses coupled oxidation-reduction reactions to generate electricity through the transfer of electrons. A new cell was designed and constructed to provide the option of continuous or batch operation. The *Pseudomonas sp* microorganism in our MFC oxidizes the glycerol, and other components of the waste stream from the production of biodiesel. For this study, it reduces potassium ferricyanide, an electron acceptor, to produce a maximum potential drop of 0.594 volts and a power density of 4.19 mW/m^2. Chemicals, such as 1,2 propanediol have been produced from these reactions.

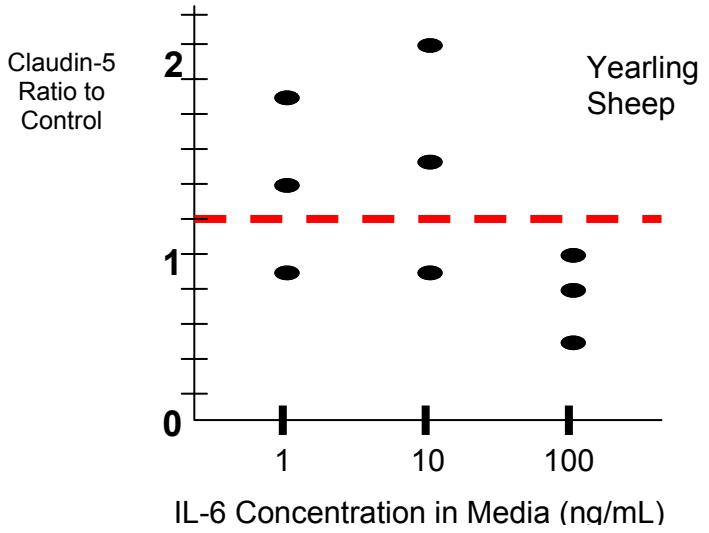
The effects of pro-inflammatory cytokines on ovine brain endothelial cells

Cummings, E.E., Cohen, S.S., and Stonestreet, B.S.

Department of Pediatrics, Alpert Medical School, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Hypoxic-ischemic brain injury, marked by a reduction in oxygen and blood supply to brain regions, in the perinatal period is closely linked to the pathogenesis of many neurodevelopmental impairments. Pro-inflammatory cell signaling molecules called cytokines are believed to exacerbate the brain injury because circulating products of systemic inflammation could damage the endothelium of the blood-brain barrier (BBB) and potentially lead to brain damage. Regulation of BBB permeability is crucial to preserving brain homeostasis and protecting the brain from unwanted substances in the mature and immature brain. Although there has been much speculation that cytokines cross the BBB and damage the brain, data is not available to support this contention. The major site of the BBB is the endothelial tight junction, and information on the effects of specific cytokines on the tight junction is lacking. We hypothesize that pro-inflammatory cytokines injure the BBB by reducing the expression of tight junction proteins in the BBB of mature and young brains. We examined the effects of the cytokine Interleukin 6 (IL-6) on the expression of tight junction proteins in the endothelium of microvessels derived from adult and yearling sheep brains. Ovine brains were obtained and the microvessel endothelial cells isolated through dissection, homogenization, and filtration. Microvessels were placed into culture and then incubated with IL-6 at doses of 0, 1, 10 and 100 ng/mL for 24 hours. The microvessels were harvested and preserved for protein analysis by Western immunoblotting for claudins and occludin. Preliminary results (Figure).



Our preliminary results suggest that IL-6 could potentially reduce the relative amount of claudin-5 in the tight junction of yearling sheep.

Arylimines are synthesized then modified by the Williamson ether synthesis with phase-transfer catalysis to make a library of GSK-4716 analogs as potential SERMs and antioxidants

Cuoto, E., Rosadzinski, T., and Williams Jr., J.C.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Undergraduate Research Fellowship Program
RI Science and Technology Advisory Council

A library of potential selective estrogen receptor modulators (SERMs) and antioxidants structurally similar to GSK-4716, a powerful SERM, were synthesized and are being screened for activity against the estrogen receptor *in vitro* (Matt Stoner, URI College of Pharmacy) and in the MTS assay for cytotoxicity (Rebeka Merson, RIC, Biology). Thousands of structure variations are being screened computationally using the eHitsLightening® docking program. An essentially quantitative Schiff reaction of aldehydes or ketones with arylamines provides one library of GSK-4716 analogs. Those with a phenolic hydroxy group were modified by the Williamson ether synthesis using aliquat phase-transfer catalysis to make another library. Modification of the extraction process using dichloromethane yielded solids from otherwise intractable oils formed in some of the aliquat reations. The overall yields for the second set of products is 60-80%. Oxygen charge-transfer complexes are apparently formed by some of these compounds. Physical chemical experiments to verify this phenomenon and use it to predict antioxidant activity will be performed.

Production and purification of tick sulfotransferase for crystallization studies

Curran, S.[1] and King, R.S.[2]

[1]Department of Biology, Providence College, Providence, RI
[2]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Sulfotransferases are enzymes that alter the activity of a substrate by facilitating the transfer of a sulfate group ($-SO_3^-$) from the universal donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to an oxygen or nitrogen acceptor. The main vector for Lyme Disease, *Ixodes scapularis*, is known to produce 2 different sulfotransferases in its salivary gland; one during the fed state and one during the fasted state, referred to as *Ixosc* Sult 1 and Sult 2 respectively. Their specific role in feeding and salivation is currently under investigation as is the similarity between *Ixosc* Sult1 and Human Dopamine Sulfotransferase (SULT1A3) which sulfonates monoamines in the brain and has been implicated in diseases such as schizophrenia and Parkinson's. In order to further understand these *I. scapularis* enzymes, theoretical structures based on homology modeling were generated prior to this study. The aim of this study was to elucidate the actual crystal structures of both sulfotransferases. Towards this end, *E. coli* were transfected with plasmids coding for the proteins, cultured in a nutrient broth and induced to produce *Ixosc* Sult 1 and Sult 2. These proteins were then purified using an affinity column, concentrated using centrifugal filtration, and finally screened against possible crystallization reagents. While the production and purification procedure of *Ixosc* Sult 1 and Sult 2 were optimized and the screens established, the protein solutions have yet to yield crystals for X-ray crystallographic analysis.

Portulaca grandiflora: CAM and C_4 photosynthetic pathways in cotyledons

Denio, D. and Guralnick, L.

Department of Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Portulaca grandiflora belongs to a unique genus within the Portulacaceae by virtue that the species utilizes both the C_4 and Crassulacean acid metabolism (CAM) photosynthetic pathways in mature leaf tissues. The leaves of *P. grandiflora* have a Kranz anatomy typical of C_4 plants and an inner succulent tissue containing the CAM pathway. It has previously been observed that both pathways operate simultaneously in their respective tissues and that increased CAM activity can be induced by water stress in mature leaves. Our goal of this project is determine if both pathways develop simultaneously and are functional in cotyledons of *P. grandiflora*. We are also studying whether CAM can be induced by water stress in cotyledons. We investigated the enzyme activity PEP carboxylase and NADP- Malic Enzyme (the decarboxylase) of cotyledons, 10-14 days old, under well watered and drought conditions to understand the activity of the C_4 and CAM pathway. If the younger leaf tissues have CAM photosynthesis it should show elevated nighttime enzyme activity and increased diurnal acid fluctuations. After 3-6 days of water stress, the enzyme levels in cotyledons were higher by about 25 percent at evening and midnight times, then the well watered plants, indicating that CAM photosynthesis has developed in cotyledons of the *Portulaca grandiflora.* Titratable acidity measurements taken at sunrise and sunset under well water conditions and drought conditions indicate that the CAM pathway develops at the same time as the C_4 pathway, matching our enzyme activity results. Thus, it appears that both pathways develop simultaneously in the cotyledons. Further work will be needed to understand the genetic control of these pathways and this may shed light on the evolution of C_4 and CAM photosynthesis in the Portulacaceae.

Timeline for change: An approach to change in the field of chemical dependency and addiction

Desjarlais, J.L., Russo, E., Warot, S.S., and Montvilo, R.K.

Department of Psychology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Throughout the country, most especially in RI, we are faced with growing numbers of individuals who are encountering problems with addiction. While there are many institutions dedicated to assisting these individuals with their recovery, there are still many individuals who fall through the cracks in the system, and do not receive the attention or treatment that they need. Part of this problem may be due to significant gaps within different aspects of our system. Problems can be encountered with accessing the system, with the actual service providers, within the courts and judicial system, in the health care system or in the legislative system. There is still disagreement in the field as to what Recovery means, and to recognition of the fact that Addiction is a chronic disorder which requires long-term follow-up. Learning about the problem(s) is an important way to start on the path to finding a solution(s). By learning about what services are available and what services are lacking, and how available services are being utilized (accessed or not accessed) we may be able to better assist in the positive change that needs to occur in the Chemical Dependency field. By going to the state house, schools, conferences (throughout RI and neighboring states), task force meetings, focus groups, and meeting with professionals in the field, as well as doing literary reviews, we have been learning about the framework of the continuum of care within this field. Utilizing these means (from May to August of this year), we have recognized that there are significant gaps within the system, and many obstacles to its use by individuals in need. It has become clear that although there are a multitude of resources available in the Chemical Dependency field there is a lack of unity among the components of the system. The existence of many different programs within the state approaching the problem(s) from multiple different perspectives seems only to enhance the gaps to treatment and recovery. Recognition of the fact that there remain many obstacles that may hinder an individuals' diagnosis, treatment, or recovery, as well as their access to any part of the system, may be the first step in overcoming these obstacles. There needs to be more cohesiveness among the many avenues dedicated to assisting individuals involved with substance abuse and addiction. There also needs to be greater consideration given to special populations within the field of Addictions, such as: women, people with developmental disabilities or physical disabilities, the elderly, Lesbian/Gay/Bisexual/Transgender/Questioning, or veterans. By looking at special needs across groups, we will be better able to identify any obstacles specific to each population, as well as those which are common across the spectrum, affecting people in each group. By identifying these obstacles we may help to bridge some of the gaps which continue to be a barrier to recovery. Fostering collaboration across agencies within the field of Chemical Dependency/Addiction, should also serve to bridge the gaps, leading to a better system of Prevention, Treatment, and Recovery within RI.

Ultrasensitive immunosensor for interleukin 6 (IL-6), cancer biomarker protein using gold nanoparticle decorated electrodes and magnetic particles amplification

Dowd, R.S., Sullivan, M., and Munge, B.S.

Department of Chemistry, Salve Regina University, Newport RI

RI-INBRE Summer Undergraduate Research Fellowship Program

A glutathione protected gold nanoparticle (GSH-AuNP) platform was used to develop a sandwich electrochemical immunosensors for the detection of interleukin 6 (IL-6), cancer biomarker in calf serum. The immunosensor is greatly amplified by using 1 μm magnetic beads coated with horseradish peroxidase (HRP) and conjugated to the secondary antibody. The amperometric detection is based on peroxidase catalytic reduction of H_2O_2. As quality control, the GSH-AuNPs were characterized using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and atomic force microscopy (AFM). The UV-Vis of the particles gives a characteristic Plasmon band at 514 nm, indicating an even size distribution. The FT-IR shows peaks which are consistent with the disappearance of the sulfur hydrogen bond (2500 cm^{-1}) and the presence of carboxyl groups (3000-3500 cm^{-1}, and 1717 cm^{-1}). The AFM show particles of approximately 5 nm in size. Using the optimized concentrations of the primary and secondary antibody from the non-amplified IL-6 immunosensor we achieved a remarkably low detection limit of 1 fg mL^{-1}. This represents a 10,000-fold decrease in the DL over the non-amplified system and the industry standard ELISA for IL-6. These GSH-AuNP based immuonsensors show great promise for the fabrication of ultrasensitive biosensor microarrays for point-of-care cancer diagnosis.

Movement of pigment granules in the squid photoreceptor: a model for understanding motor regulation

Dundon, W.S., and DeGiorgis, J.A.

Biology Department, Laboratory of Neurobiology, Providence College, Providence, RI
Marine Biological Laboratory, Woods Hole, MA

RI-INBRE Summer Undergraduate Research Fellowship Program
NIH Summer Internship Program

The squid eye lacks an iris as a means of controlling the quantity of light that enters the eye and stimulates the photoreceptors of the retina. Instead, these organisms possess pigment granules that move from the base of the photoreceptors to their distal tips and act as molecular sunglasses to shade the light sensitive machinery of the cells. Here, we demonstrate the dynamic movement of pigment granules in response to light and dark adaptation. The movement of granules from the base to the tip of the photoreceptor requires a 20-minute time interval. Movement in the opposite direction requires a longer 30-minute time interval suggesting that different motors mediate bidirectional movement. Pigment granules isolated from the photoreceptor by sucrose density gradient fractionation bind to exogenous microtubules in a reconstituted binding assay. Pigment granules are also found in contact with microtubules in vivo by electron microcopy. These data suggest that granule movement occurs along microtubules and are therefore powered by microtubule-based motors. Antibodies raised against kinesins and dyneins identify these motors in the squid retina by Western blot. An immunocytochemistry project is underway to determine whether these motors are directly associated with motile granules.

The effect of competition on growth rates of *Ditylum brightwellii*

Dunn, R., and Rynearson, T.

Graduate School of Oceanography, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Diatoms are unicellular photosynthetic algae that are ecologically important in the world's ocean. Diatoms have a profound effect on the environment; they produce twenty percent of the oxygen we breathe while absorbing carbon emissions and countering the green house effect. Many diatoms have no method of movement, and drift passively through the ocean by the tides and currents. It has been reasoned that through this drifting lifestyle and the limited geographical barriers of the ocean, diatoms are spread throughout the ocean. Although global dispersal implies that individuals within a single species collected from different regions of the ocean will not display genetic and physiological differentiation, patterns of genetic and physiological diversity have been observed on local spatial scales, as seen in the diatom *Ditylum brightwellii*. Previous research indicates that genetically distinct *D. brightwellii,* characterized by the different ITS regions in their RNA, show distinct physiological traits, such as variable cell diameter. These specific genetic differences affect growth rates and the creation of algal blooms. By understanding how to classify and separate different samples, genetically different isolates were prepared to test the theory that under stable environmental conditions the individual with the fastest growth rate in a population is expected to become numerically dominant in a short period. Isolates from the water near Marlborough, New Zealand were placed in competition with isolates from Martha's Vineyard. A mixed culture of both isolates was made, as well as two pure samples, which were all grown in a 14°C, 12 hour light-dark cycle incubator. Daily cell counts and florescence tests were done to observe growth rates. Through two separate tests, the experiments showed slightly different growth rates between a pure sample and a mixed sample. The Martha's Vineyard sample grew slower in competition, while the Marlborough sample grew quicker. The Martha's Vineyard sample in both cases, however, grew to have almost three times as many cells. This experiment suggests that genetically different isolates can coexist, and only affect each others growth rate slightly. However, manipulating starting cell numbers or changing the various experimental parameters are some of the vast ways of doing competition experiments that were not tested, and could show different results.

Investigation into the binding stoichiometry of arylphosphonium salt/DNA complexes using AMBER, AutoDock, and the continuous variation method

Fisher, D.B. and Williams Jr., J.C.

Department of Physical Sciences, Rhode Island College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Arylphosphonium salts (APS) are both lipophilic and cationic. They are soluble in both water and oil based solvents. This amphiphillic character allows APS to be absorbed into cells or cellular compartments. Because carcinoma cells have a larger charge gradient than normal cells, APS have been shown to preferentially accumulate in cancer cells. This phenomenon has the potential for exploitation in tumor imaging or chemotherapy. Previous studies indicate that some APS compounds form complexes with DNA, and that this association is structure dependant. The binding stoichiometry of the arylphosphonium salts p-xylenebis(trisphenylphosphonium bromide) and o-xylenebis-(trisphenylphosphonium bromide) in complex with DNA has been investigated using computational and experimental methods. Crystal structures of DNA oligomers d(CGCAAATTTGCG)2 and d(CGCGAATTCGCG)2 were obtained from the protein database. PDB files were generated for the arylphosphonium salts using Hyperchem. These ligand files were structurally optimized using the AMBER99 force field. Blind, rigid docking calculations were performed using AutoDock in order to establish the ten lowest free energy conformations of the DNA/Ligand complex. The method of continuous variation was used to probe the binding stoichiometry. Equimolar solutions of salmon testes DNA and p-xylenebis(trisphenylphosphonium bromide) were prepared in a phosphate buffer of pH 7.0. Ten solutions were prepared such that the summed concentration of DNA and ligand was held constant. Fluorescence spectra and UV/Vis absorption spectra were taken for each solution. A job plot was generated with the photometric data. The procedure was repeated for three different concentrations. The intersection between two least squares regression lines generated from the data was used to estimate the binding stoichiometry. The results indicate that both the para and the ortho APS isomers studied bind to DNA with similar binding free energy changes and can occupy multiple binding sites.

Identification, cloning and sequence analyses of *LtLIP3*, a gene encoding the secretory lipase from *Leishmania tarentolae*

Fraulino, D.A. and Shakarian, A.M.

Department of Biology and Biomedical Science, Salve Regina University Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania is a trypanosomatid protozoan parasite that causes Leishmaniasis, a disease that is spread by the bite of an infected sandfly. The parasite has a unique ability to survive in mammalian host macrophages and it is hypothesized their resistance and survival results in part from a secretory lipase capable of hydrolyzing lipids into simple fatty acids. In *Leishmania* this secretory lipase is encoded by the *LIP3* gene. However, to date no such gene has been identified in the *L. tarentolae* species. Thus, in the current study, the *LtLIP3* gene was identified, cloned and sequenced from *Leishmania tarentolae* by PCR amplification using primers based on homologous genes from other species. Nucleotide sequence analysis revealed a 927 bp ORF coding for a deduced protein of 308 aa. BLASTP searches through the online National Center for Biotechnology Information (NCBI) and Clustal W Alignment searches through EMBL online databases were performed to express evolutionary relationships with other known lipases. Results of these analyses showed high homology with known lipase from other species of *Leishmania* such as a 90% aa similarity with a lipase from *Leishmania infantum,* and an 89% similarity with a lipase from *Leishmania major.* In addition, Conserved Domain Database searches showed that the *L. tarentolae* deduced aa sequence contained an aa triad (Ser-Asp-His) consistent with the catalytic site of serine class 3 lipases. Taken together, this data constitutes the first report of the identification, cloning and sequencing of a secretory lipase gene in *Leishmania tarentolae*. Further characterization of this gene and protein will lead to a better understanding of the role of secretory lipases within the biology of the important group of human pathogens.

Biotransformation of biologically active aromatic molecules by marine bacteria possessing Type III PKS genes

Friel, J.E., Sheffer, R., Worthen, D.R., and Rowley, D.C.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Biotransformation is an alteration made by an organism to a chemical compound. This process can be useful in the development of new drugs and for the detoxification of environmental contaminants. We hypothesize that microorganisms possessing biosynthetic pathways related to secondary metabolite production are good candidates for biotransformation reactions. In this study, a panel comprising 124 marine bacteria was screened using PCR for the presence of Type III polyketide synthase (PKS) pathways. Type III PKS gene clusters generally produce small aromatic molecules and frequently contain accessory genes that modify these products. Type III PKS genes were detected in 24 of the environmental isolates, and these bacteria were then tested for their ability to modify five target molecules. The plant-derived aromatic compounds resveratrol and quercetin were selected due to their diverse structures and medically relevant biological properties. Bisphenol A, a common plasticizer and suspected hormonal modulator and environmental contaminant, was also examined. The other targets were oxydianiline and diaminodiphenyl sulfide, compounds which have demonstrated strong in vivo activity as anticonvulsant agents.[1]

The bacterial strains were grown overnight in 10 mL marine broth and then treated with 1 mg of the appropriate target molecule. The cultures were incubated at 24° C with shaking for five days. The mixtures were extracted with ethyl acetate and then examined by thin layer chromatography (TLC) in order to detect possible products of biotransformation reactions. No new products were observed for resveratrol, quercetin, and Bisphenol A. However, oxydianiline and diaminodiphenyl sulfide were extensively metabolized by most of the bacterial strains. Based upon these results, 1 L scale reactions were carried out on these molecules in order to prepare sufficient biotransformation products for chemical characterization and biological evaluations. One-liter cultures of strain C-12, a marine *Bacillus pumilus*, was treated with 400 mg of diaminodiphenyl sulfide and incubated with shaking for five days. The products were extracted with ethyl acetate and purified by silica gel column chromatography. An identical experiment was conducted with oxydianiline and strain RI06-95, also a marine Bacillus pumilus. Analysis of the biotransformation products by NMR and mass spectrometry is underway.

[1]Worthen, D.R. *et al*., In vivo evaluation of diaminodiphenyls:anticonvulsant agents with minimal acute neurotoxicity, *Bioorg Med Chem Lett*, 2009, *in press*.

Maternal transfer of mercury to gonad tissue and its effect on the fecundity of the Atlantic silverside, *Menidia menidia*

Futoma, E.K. and Taylor, D.L.

Department of Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic contaminant that is prevalent in estuarine environments. Dietary intake is the main route of Hg exposure in estuarine fish, after which it potentially bioaccumulates in their muscle and gonad tissue. This Hg exposure may cause population-level effects if females transfer Hg to their offspring or experience reduced fecundity. The objectives of this study were twofold: (1) examine the maternal transfer of Hg to the gonad tissue of a ubiquitous estuarine fish, the Atlantic silverside, *Menidia menidia*, and (2) evaluate the effect of Hg exposure on silverside egg production. In June-July of 2008 and 2009, silversides were collected from six sites in Narragansett Bay (RI, USA) that differed in environmental Hg contamination (3 pristine and 3 polluted sites). Total lengths (cm) and weights of excised muscle and gonad tissues (g wet wt) were measured for each silverside. All tissue samples were then freeze dried for 48 hr, homogenized, and analyzed for total Hg content using combustion atomic absorption spectrometry (ppm dry wt). Average gonad and muscle tissue Hg concentrations were significantly higher in fish from polluted sites (n=21; muscle=0.858 ppm; gonad=0.156 ppm) than those from pristine sites (n=22; muscle=0.527 ppm; gonad=0.089 ppm). Moreover, Hg concentrations in muscle and gonad tissue were positively correlated for fish collected from both areas (R^2=0.785 and R^2=0.494 for pristine and polluted, respectively). Although a positive correlation also exists between fish length and gonad mass for individuals from pristine and polluted areas, unexpectedly, fish from polluted sites had a higher gonad mass (mean=0.985±0.126 g) than those from pristine sites (mean=0.795±0.071 g). Future work will investigate the rates of gonad growth in pristine and polluted locations and examine the effect of Hg exposure on egg number and size.

Improved genome assembly and heterologous gene expression in Bacillus pumilus strain RI06-95

Gaviola, M.L., Killian, C.G., and Udwary, D.W.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Narrow River of Rhode Island is home to a new strain of *Bacillus pumilus* (designated RI06-95), one that creates the molecule amicoumacin which shows probiotic activity towards shrimp and oysters, and antibiotic activity towards *Helicobacter pylori*. By assembling its genome, we can better understand the biosynthetic clusters that produce this molecule, as well as pave the way for the production of new drugs at the genetic level. Using bioinformatics, we have succeeded in an improved partial assembly of its genome as well as the identification of different biosynthetic clusters through comparison with additional strains of *B. pumilus* and current knowledge of polyketide synthase protein structures. We are now developing synthetic biology methods to predict the chemistry of gene cluster products which we can alter to create new molecules with drug activity more desirable than those originally made by the bacteria.

Abundance, growth, and diet of juvenile summer flounder (*Paralichthys dentatus*) and winter flounder (*Pseudopleuronectes americanus*) In the Seekonk River, RI and the Taunton River, MA.

Gervasi, C.L., and Taylor D.L.

Department of Marine Biology, Roger Williams University, Bristol, RI

RI-EPSCoR Summer Undergraduate Research Fellowship

Summer flounder, *Paralichthys dentatus*, and winter flounder, *Pseudopleuronectes americanus* utilize estuaries as nursery habitat during their early life history stages. In southern New England estuaries, however, little is known regarding the spatiotemporal overlap and potential biotic interactions between the flounder species. The purpose of this research was to assess the abundance, growth, and dietary habits of juvenile summer and winter flounder to determine if predator-prey and/or competitive relationships exist. From May to August 2009, flounder in the Seekonk River (5 sites) and Taunton River (6 sites) were sampled biweekly using beach seines. Captured flounder were enumerated, measured for total length (mm), and a sub-sample was preserved for subsequent stomach content analysis. Summer flounder abundance (mean = 0.54 fish/m^2) decreased significantly over time, but the abundance of Winter flounder (mean = 0.30 fish/m^2) remained relatively constant during the sampling period. Summer flounder grew significantly faster than winter flounder (growth rates = 0.98 and 0.49 mm/day, respectively), which may be attributed to differences in dietary habits. Decapods and fish were an important component of the summer flounder diet (44% and 5% by volume, respectively), while amphipods and nematodes were favored by winter flounder (35% and 25% by volume, respectively). These data suggest that competition for food resources is minimal between species. Among the identifiable fish prey in summer flounder stomachs, however, there was evidence of predation on winter flounder, albeit to a limited extent. In order to better understand flounder interspecies interactions, future work will examine the abiotic factors affecting abundance and growth of the two species, as well as observe prey availability.

Diversity of *Synechococcus* psbA and psbD genes in Narragansett Bay

Gosselin, C., Taylor, S., Marston, M.

Department of Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Cyanobacteria, such as *Synechococcus*, are important primary producers in the marine food web. *Synechococcus spp*. are commonly found in temperate latitudes and coastal regions. Marine viruses, specifically cyanophages, infect *Synechococcus* and may control their populations. Marine viruses contain a broad range of both viral and host derived genes, such as the photosynthetic genes psbA and psbD, believed to increase phage fitness upon infection. Cyanophages acquired these host genes at some point in their evolutionary histories and there may be continued exchange of these genes between hosts and phages. Previous studies have thoroughly examined the extent of diversity of the psbA and psbD genes in Narragansett Bay cyanophages; however, very little is known about the diversity of these genes in their cyanobacterial hosts. This study examines the diversity of *Synechococcus* in Narragansett Bay using ITS (an internal transcribed spacer region) as well as the photosystem II psbA and psbD genes. Seawater was collected from several locations in the Bay. For each sample, approximately 1 liter of seawater was filtered (5 uM) to remove larger unwanted organisms. All remaining cells were collected on a 0.22 or 0.45 uM filter and DNA was isolated. PCR was used to amplify ITS, psbA, and psbD sequences. PCR products were cloned and subsequently sequenced. Analysis of ITS sequences revealed sequences that closely resembled previously known *Synechococcus* ITS sequences, while other sequences fell within a distinct clade of other uncharacterized cyanobacteria. While over 16 psbA and psbD sequences were obtained from the clone libraries, none of these sequences fell within known *Synechococcus* clades, rather these sequences resemble sequences from other cyanobacterial genera or in some cases, diatoms. Based on the information obtained thus far *Synechococcus* sp. are present in Narragansett Bay; however, methods for acquiring *Synechococcus* psbA and psbD sequences must be refined.

Neither *E.* coli Nissle 1917 nor *E. coli* MG1655 sequester L-arabinose from the other when colonizing the mouse intestine

Greene, N.G., Leatham, M.P., and Cohen, P.S.

Department of Cell and Molecular Biology, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

When colonizing the streptomycin-treated mouse intestine, different commensal strains of *E. coli* appear to metabolize different sugars. However, it has been shown that both *E. coli* Nissle 1917 and *E. coli* MG1655 metabolize L-arabinose *in vivo*, illustrated by approximately a three-log difference between each wild-type strain and its respective mutant. The present study investigated whether it was possible for either commensal to appropriate L-arabinose from the other when one strain was pre-colonized for ten days. Neither *E. coli* Nissle 1917 nor *E. coli* MG1655 was able to sequester L-arabinose away from the pre-colonized strain. In each case, there was approximately a four-log disparity between the wild-type and respective mutant strains. These results suggest that when co-colonizing the mouse intestine, *E. coli* Nissle 1917 and *E. coli* MG1655 are equally capable of using L-arabinose for growth. Alternatively, it is possible that, if each strain grows independently in separate intestinal niches, these two strains are not in direct competition for L-arabinose as a carbon source.

A multiple-baseline study of joint attention as a pivotal behavior for children with autism

Heaney, C.[1], Pereira, A. [1], Sandland, S. [1], Chait, A.[2], and Quinn, S. [1]

[1]Department of Psychology, Salve Regina University, Newport, RI
[2]Pathways Strategic Teaching Center, Warwick, RI

RI-INRE Summer Undergraduate Research Fellowship Program

Pivotal behaviors are defined as skills that, when mastered, result in the more rapid acquisition of other skills. Joint attention (JA) is theorized to be a pivotal behavior related to other social skill and language development. This poster documents the preliminary results of a study that examined the relationship between the development of JA and collateral social behaviors in 3 children with autism. If, with the development of JA, the children's other social behaviors began to show similar acceleration patterns that could be interpreted as evidence of the pivotal behavior status of JA.

A JA activity that required the experimental participants to respond to 1 of 3 choices on the basis of adult gaze was designed and a task analysis of this activity was developed. The task analysis specified behaviors that ranged from merely turning toward the experimenter when the child's name was called (Step 1) to being able to correctly interpret the experimenter's eye gaze by choosing 1 of the 3 objects (Step 5). All the children in this study were determined to be on Step 3 of the task analysis. Placement on Step 3 indicated the child had not mastered the ability to interpret another's facial orientation.

Once a baseline level was determined and a stable baseline trend on that step was achieved, the child began the acquisition phase. Discrete trail teaching (DTT) with mastery interspersed trials (MI) was administered to teach the JA skill appropriate for that child.

An archival examination of the child's treatment plan was also conducted to determine if other behaviors showed an acquisition trend that could be related to the child's JA program.

The results indicated that while JA behavior increased during the acquisition phase, the results for other, theoretically related behaviors were less consistent.

Triazoles are synthesized by “click chemistry” to make tamoxifen and GSK-4716 analogs for estrogen receptor screening.

Hicks, J., and Williams Jr., J. C.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program
RI Science and Technology Advisory Council

Tamoxifen and GSK-4716 are potent selective estrogen receptor modulators (SERMs). Tamoxifen, a major chemotherapy agent in breast cancer, inhibits estrogen binding to estrogen receptors in breast tissue. However, this is frequently accompanied by an increase in estrogenic activity in other tissues, sometimes resulting in deleterious effects. The goal of this work is to find SERMs with anti-cancer efficacy and fewer side effects using a reaction sequence of five or fewer steps that can generate a library with a large number of structure variations for *in vitro* screening against the estrogen receptor. One compound, out of fewer than ten screened of this class (Matt Stoner, URI College of Pharmacy), was identified in the initial set prepared for screening as having estrogenic activity. Several others accessible by this route have been identified by *in silico* screening with the eHitsLightening® docking program. Triaryl-1,2,3-triazoles were prepared by the electrocylic reaction of diphenylacetylenes and an aryl azide catalyzed by pentamethyhlcyclopentadienlybis(triphenylphospine) ruthenium(II) chloride. The diarylacetylenes were prepared from *trans*-stilbene by bromination/debromination. Modification of the aryls on the *meso*-dibromo stilbene was also done by textbook organic reactions to make nitro and amino substituted dibromostilbenes which were then debrominated with KOH in TEG. The final step is the “click” chemistry 2-3 electrocyclic reaction to make the triazoles.

Elucidating the role of Bcp1 protein in DNA repair using *Saccharomyces cerevisiae* as a model organism

Burke, L., Hirshberg, S., King, C. and Britt, D.

Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Studies indicate that the human protein BCCIP promotes cell cycle arrest and participates in homologous recombination repair (HRR) in response to DNA damage, but the function of the protein remains to be determined. BCCIP is associated with the tumor suppressor BRCA2 and has a homolog, Bcp1, in the budding yeast *Saccharomyces cerevisiae*; therefore, investigation of Bcp1 holds the potential to clarify our understanding of the role of this important gene in mammalian systems. *S. cerevisiae* was chosen as a model organism due to its characteristics as a single-celled organism whose DNA damage repair and cell cycle regulation are well-characterized and highly conserved between eukaryotes. The goal of this project is to examine whether or not Bcp1 is a necessary component of the DNA damage response in *S. cerevisiae*. This study utilizes two strains of *S. cerevisiae*, a wild type with a functional *BCP1* gene and a temperature-sensitive mutant where this essential protein is deactivated at 38°C. The phenotypes of both strains were confirmed by counting colonies after incubation at permissive and non-permissive temperatures. To quantify the ability of mutant yeast to recover from various types of DNA damage, cell viability counts were conducted after treatment with DNA damaging agents hydroxyurea, bleomycin and cisplatin at permissive and non-permissive temperatures. Preliminary data suggest that viability of the temperature sensitive mutant is reduced at the non-permissive temperature, and that viability of both strains is reduced by drug treatment, but the mutant does not appear to be more sensitive than the wild type.

Triarylphosphonium salts with polymerizable functional groups are bacteriacidal

Hirshberg, S., Williams Jr., J. C., and Britt, D.

Physical Sciences Department and Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Arylphosponium salts are toxic to bacteria, mammalian cells (both normal and malignant), whole animals (shistosomes, trypanosomes, mice) and plants (Lemna minor). They complex *in vitro* with genomic DNA and inhibit acetylcholinesterase and some oxidases. The goal of this work was to find compounds that are bacteriacidal and have functionality allowing polymerization or co-polymerization for formulation of plastic tubing and containers for hospital use. Immobilization of the toxins by covalent bonding into the plastic will avoid their leaching into the fluids in contact with the inner surface of tubing and containers. This will avoid toxicity to the patient while resisting colonization of resistant stains. We have identified candidates for polymerization studies and for grafting to polyvinyl alcohol.

Thermodynamic studies of arylamine-dG adduct at the replication fork: Role in mutagenesis

Kim, W., Ganesan, V., and Cho, B.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Arylamines are important class of chemical carcinogens which forms covalent DNA adducts by metabolic activation. Arylamines and its derivatives exhibit strong sequence selectivity in the mutational assays. In this study, we carried out the thermodynamic analysis of N-deacetylated form of fluorinated aminofluorene DNA adduct (FAF-dG) at the replication fork by using UV thermal denaturation, differential scanning calorimetry and circular dichroism spectroscopy. Thermodynamic studies on primer extension from n-1 to n+6 using match and mismatch base opposite oligonucleotide containing DNA lesion (16-mer) was performed to mimic the translesion synthesis by a DNA polymerase at the replication fork. The present thermodynamic data will provide an insight in understanding the polymerase-DNA interaction in vivo and its possible cause for mutagenesis. The results obtained from various techniques will be discussed in detail at the poster session.

Filamentation makes yeast cells more resistant to programmed cell death in *Saccharomyces cerevisiae* and *Candida albicans*

Laprade, D., Cao, Y., and Austriaco, N.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Apoptosis, or programmed cell death, is a controlled 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*. Much as the human body consists of several cell types (from muscle cells to epithelial cells) yeast also occur in several, distinct cell types. Among these are the haploid **a** and **α** cells, haploid invasive cells, the diploid **a/α** cells, and diploid pseudo-hyphal 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 from the ∑1278b strain background that is still capable of dimorphic shift, the ability to become filamentous. Thus, by inducing invasive growth under controlled conditions, we have been able to investigate the responses of different cell types to ethanol-induced apoptosis. Filamentation, in both haploid and diploid cells, showed a marked resistance to apoptotic death compared to regular yeast cells when placed in 22% ethanol. Finally, to determine whether or not this phenomenon is exclusive to *S. cerevisiae*, we are testing filamentous cells and regular yeast-form cells of the human pathogen *C. albicans* for resistance to the drug amphotericin B – an anti-fungal medication commonly used to treat patients with yeast infections. If the hyphal cells are resistant, our data would suggest that one reason why *C. albicans* infections are so notoriously hard to kill in patients suffering from severe immuno-deficiency is that the hyphae required to produce said infections are, themselves, more resistant to normal cell suicide than its non-invasive form.

Design of multifunctional liposome/nanoparticle assemblies as targeted “cancer bombs”

Lelis, A.[3], Bothun, G.[1], and Stoner, M.[2]

[1]Department of Chemical Engineering, College of Engineering, University of Rhode Island, Kingston, RI
[2]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI
[3]Biology Department, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

A stable multifunctional targeted liposome system was designed with the purpose of selectively delivering chemotherapeutic drugs to cancer cells. This system contained a folic acid lipid derivative in the lipid bilayer of the liposome that allowed efficient uptake by cancer cells, which overexpress surface folate receptors. The chemotherapeutic drug doxorubicin (DOX) and five nanometer (5nm), negatively charged superparamagnetic iron oxide (SPIO) nanoparticles were encapsulated within the liposome aqueous core and bound to the lipid bilayer, respectively, using a thin film hydration method. Initial screening using fluorescence microscopy showed that the liposomes were stable in phosphate buffered saline (PBS) and attached to the surface of Huh7, HEK293T/17, COS-1, and HeLa cancer cells within an hour. HeLa cells showed greater attachment than the other cells lines after an hour and complete uptake after three hours. To demonstrate external control over drug release, HeLa cells incubated with liposomes, both with and without SPIO nanoparticles, were subjected to *in situ* inductive radio frequency (RF) heating. Our hypothesis was that the alternating electrical current of RF would heat the nanoparticles, which in turn would release DOX on demand by disrupting the liposome bilayer. The heat itself may also damage internal cell structure, providing combined drug delivery and hyperthermia. Preliminary results showed that cell death resulted after one and four days treatments with liposomes carrying DOX and SPIO nanoparticles, and DOX alone. A modest increase in cell death was observed with RF heating, which suggests that liposome disruption aided DOX release. Additional experiments, including control samples and optimized RF heating profiles have to be conducted to determine the efficacy of this treatment. This preliminary study shows that the multifunctional liposome concept may provide efficient and selective drug delivery for cancer patients, especially for those who have reached the stage of metastasis.

Development of an amperometric detection method to monitor dopamine concentrations

Letourneau, M. [1], Salter, D. [2], Kádár, S. [1], and Symington, S.B. [2]

[1]Department of Chemistry, Salve Regina University, Newport, RI
[2]Department of Biology, Salve Regina University, Newport, RI

[2]RI-INBRE Summer Undergraduate Research Fellowship Program
[1]RI EPSCoR Summer Undergraduate Research Fellowship Program

Our goal is to develop an amperometric detection method using carbon electrodes that will be sensitive enough to monitor the release of dopamine from a single cell. We used a combination of cyclic voltammetry and amperometry to measure the oxidation of dopamine and to assess the functionality and sensitivity of a carbon electrode in the presence and absence of a catalyst. Our electrodes were prepared with a sol-gel solution and tyrosinase (an enzyme that catalyzes the oxidation of dopamine). Our preliminary results indicate that the carbon electrode prepared with tyrosinase increased the oxidation of dopamine by 2.5-fold compared to an electrode prepared without the enzyme. Furthermore, the tyrosinase-coated electrode increased dopamine oxidation by 1.5-fold compared to an uncoated carbon electrode. Therefore, tyrosinase successfully amplified the signal as detected through cyclic voltammetry and amperometry. Current research efforts focus on developing a functional miniaturized system capable of detecting smaller amounts of dopamine release from a single cell.

Estuarine invertebrates and forge finfish as bio-indicators of environmental mercury levels in the Narragansett Bay (Rhode Island, USA)

Linehan, J.C. and Taylor, D.L.

Department of Biology and Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Estuarine environments are susceptible to anthropogenic perturbations, including the deposition and mobilization of a variety of contaminants in the sediment. Environmental mercury (Hg) contamination is of particular concern because Hg bioaccumulates in aquatic food webs and exposure has deleterious effects on biota, including humans. The potential health risks associated with Hg exposure justifies the development of monitoring programs that link environmental and biological Hg contamination. In this study, we assessed the utility of estuarine invertebrates and forage finfish as bio-indicators of environmental Hg pollution. Specifically, surface sediments (0-2 cm; 54 sites) and biota (finfish, macrocrustaceans, bivalves, polychaetes, and zooplankton; 83 sites) were collected from the Narragansett Bay (Rhode Island), and analyzed for total Hg concentration using atomic absorption spectroscopy. Spatial relationships between sediment and biota Hg concentrations were then statistically compared using Geographic Information System and least-squares linear regression models. There was a significant positive correlation between sediment Hg levels and the Hg content of bivalves (R^2 =0.389; $p < 0.0006$) and zooplankton (R^2 = 0.2613; $p < 0.005$). Conversely, polychaete, macrocrustacean, and finfish Hg body burdens were not significantly related to environmental Hg levels (R^2 = 0.091, 0.322, 0.012; p =0.2746, 0.1463, 0.5236). Preliminary results therefore indicate that the effectiveness of estuarine biota as bio-indicators of environmental Hg contamination is taxon-specific, and is likely influenced by feeding ecology, longevity, and site fidelity.

Differentiation of out-group members: Relative intergroup status and familiarity

Berrios-Candelaria, R., Nurmikko, T., Lewis, B., Murphy, P., Kinney, L., and Malloy, T.E.

Psychology Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

That humans form social groups is axiomatic. Group formation increases security, permits task allocation, offers a social identity, provides mating opportunities, and consequently increases the likelihood of survival. The group one is a member of is the in-group; groups that one is not a member of are out-groups. A reliable finding in social psychology is that individuals fail to differentiate the unique traits of out-group members while they do differentiate the traits of in-group members. This is termed the out-group homogeneity bias; a perceiver concludes “they’re all the same” when judging the characteristics of the out-group. We designed an experiment to estimate the effects of *familiarity with the out-group* and the *relative status of the in-group and out-group* on the differentiation of out-group members. A significant main effect due to relative group status was observed ($F(29,29) = 3.76$, $p < .01$); perceivers from a low status group differentiated the traits of high status out-group members to a greater extent than did high status perceivers when judging low status out-group members. A significant main effect on was found but was inconsistent with past findings ($F(29,29) = 2.15$, $p < .05$). There was less differentiation of out-group members in a high familiarity condition compared to a low familiarity condition. The ***“They’re all the Same”*** phenomenon is rooted, in part, in differential group status. Members of a high status group focus on the features that define category membership (e.g. a dark skin tone) and ignore unique features that permit differentiation of category members

Generating plasmid constructs for the stable integration and expression of a secretory lipase in *Leishmania*

Mare, J. and Shakarian, A.M.

Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania is a genus of protozoan parasite that is indigenous to sub-tropical areas in Asia, Africa, South and Central America and some parts of southern Europe. We hypothesize that a secretory lipase produced by *Leishmania* contributes to its ability to thrive inside of host cells. To further investigate this hypothesis and to characterize the leishmanial secretory lipase, an expression plasmid is currently being constructed using the pFX4.1 system. The pFX4.1 plasmid constructs bearing the lipase gene, *LdLIP3* will be integrated into the ribosomal locus of *Leishmania tarentolae* to maximize protein expression.

A multi step cloning process is being utilized to generate the expression plasmid construct for integration into the *L. tarentolae* genome. For example, the secretory lipase gene of *Leishmania donovani*, *LdLIP3*, was amplified by PCR and analyzed by agarose gel electrophoresis. Result showed an amplified product of the expected size, ~ 960 bp, the size of the gene with the addition of an HA epitope tag and the restriction sites *Not* I and *Nco* I at the 5' and 3' ends, respectively. This PCR product was cloned using the TOPO vector (pTopo:LdLIP3::HA[*Not* I and *Nco* I]). Secondly, to generate the final construct, *Not* I and *Nco* I restriction endonucleases were used to isolate and gel purify the LdLIP3 insert from *pTopo:LdLIP3::HA*[*Not* I and *Nco* I]. In ongoing studies, this purified LdLIP3 insert will be ligated with the Pfx4.1 vector. The *pFx4.1::LdLIP3::HA* construct will be verified by sequence analysis and subsequently integrated into the *L. tarentolae* ribosomal locus by transfection and homolgous recombination via nucleotide sequences in the expression vector. Transfectants are selected with increasing concentrations of the drug hygromycin. Once established, this system should produce large amounts of the secretory lipase for further biochemical analysis.

Synthesis of cyclodextrin cocaine derived MR contrast agents for dopamine transporter imaging

Marks, K., Lincourt, G., and Naumiec, G.

Organic Chemistry Department, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Magnetic Resonance Imaging (MRI) is an extremely important tool used to help diagnose medical conditions in such fields as neurology, oncology, and orthopedics. The development of paramagnetic contrast agents that enhance relaxivity at high magnetic fields has the potential to greatly improve successful medical imaging and diagnosis. Part of this development includes designing contrast agents that specifically target certain receptors and transporters. In this case, a contrast agent targeting dopamine transporters (DAT) in the brain is being synthesized. The agent consists of a modified cyclodextrin cage with seven arms. Six of these arms were linked via “click” reactions to paramagnetic Gadolinium-DOTA ligands, and one arm was linked to a cocaine analog. Gadolinium is used because of its high magnetic moment and symmetric electron ground state, while cocaine analogues compete with dopamine in binding DAT with high affinity. Since DAT concentrations can be used to indicate such conditions as Parkinson’s disease, depression, and propensity towards drug addiction, the use of this contrast agent for MRI could greatly improve the detection of such neurological conditions.

Restored oyster reefs and their impact on the abundance and diversity of local fauna

Massari, T.L.[1,2], Taylor, D.[1], Chintala, M.[2], Ayvazian, S.[2], and Leavitt, D.[1]

[1] Department of Marine Biology, Roger Williams University, Bristol, RI
[2] Atlantic Ecology Division, U.S. Environmental Protection Agency, Narragansett, RI

U.S. EPA 2008 GRO Fellowship

The Eastern Oyster (*Crassotrea virginica*) is an ecologically important species that provides multiple ecosystem services, including the potential to increase complex habitat for resident fauna. To this end, the objective of this study was to determine if the presence of restored oyster reefs in Narragansett Bay (RI, USA) increased the abundance and diversity of local macro-invertebrates and finfish. In June and July 2009, six baited traps were deployed biweekly one hour ± of high tide at three oyster reef (OR) and three non-oyster reef (NOR) sites in the Bay. An initial assessment was conducted at the OR sites to determine the density and size-class frequency of the oysters (mean density = 44 ± 18.5 oysters/m^2, mean size = 43.9 ± 16.5 mm shell height). There was no significant difference in the abundance of fish and macro-invertebrates between the OR (2.5 fish/trap; 4.2 invert/trap) and NOR (1.5 fish/trap; 4.6 invert/trap) sites. The diversity of finfish at OR (0.7 spp./trap) sites was significantly greater than that of the NOR (0.3 spp./trap) sites, but there was no difference in the diversity of invertebrates (OR=1.2 spp./trap; NOR=1.1 spp./trap). The catch composition at the OR sites was similar to that of the NOR sites, with Mummichogs and Striped Killifish the most common fish and Green Crabs, Mud Snails and Grass Shrimp the most common invertebrates. These data suggest that oyster reefs impact the diversity of finfish species that occupy a habitat, although they do not necessarily increase the overall abundance of finfish and invertebrates. Further work for this study will examine the effect of oysters on sediment nitrogen and infaunal invertebrates.

Optimization of stripping and reprobing for Li-Cor western blot analysis

McCauley, M., Brum, G., Nalbandian, A., Fredette, R., Lu, S., Cao, C., and Wan, Y.S.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Li-Cor Odyssey Infrared Imaging System is pioneering the way for standard Western blot analysis by using direct infrared fluorescence detection. Compared to once popular chemiluminescence and visible fluorescence techniques, Odyssey's infrared detection system provides accurate quantification, multiplex detection, high sensitivity, and clear data. It also provides a safer and cleaner method to detect proteins compared to traditional darkroom autoradiography where toxic developing and fixation reagents are used. However, in our initial experiments we found that one hurdle with the Odyssey imaging system is that its standard stripping protocol fails to remove all of the antibodies from each membrane. We decided to further investigate this issue and ascertain the optimal stripping procedure. In order to completely strip each membrane, some elements of the standard protocol had to be altered. We chose to manipulate the amount of time the membranes were exposed to the Newblot Stripping Buffer, the temperature at which the membranes were soaked in the buffer solution, and the concentration of the stripping buffer. We conclude that the optimal stripping procedure involves soaking the membranes in a 1:5 diluted buffer solution at 50°C for 30 minutes. Stripping antibodies from a membrane is critical because it allows one to reuse the same membrane and test for presence of different proteins. Our data suggest that these modifications to the original protocol offer a more effective stripping procedure.

Overlapping bloom syndrome protein ORF expression: Amino acids 1217-1417

Mello, K., Brown, L.W., Capuano, M., and Almeida, K.H.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bloom Syndrome is a rare autosomal recessive chromosomal disorder caused by a mutation in the BLM gene. The BLM gene is thought to be responsible for stabilizing the genome. Characteristics of this disorder are a high frequency of breaks and rearrangements in chromosome of an affected individual. These disruptions in the chromosomes cause individuals to develop a wide array of cancers. There is no cure for Bloom Syndrome. Studying the BLM gene and it's interaction with other proteins involved in homologous recombination, like BRCA1, helps to better understand the role of genomic stability in cancer progression. Overlapping sections of the BLM protein were generated in order to express the complete open reading frame of BLM, 1417 amino acids total. These experiments focus on the section of BLM between amino acids 1217 and 1417. PCR primers were designed to amplify the appropriate cDNA sequence. In addition a FLAG epitope tag was engineered into the C-terminus. The correct DNA segment was inserted into the pENTR vector (Invitrogen). Restriction enzyme analysis verified and DNA sequencing confirmed the correct insertion. The pENTR $BLM_{1217\text{-}1417}$ vector was recombined into a destination vector containing an N-terminal 6x His epitope tag and was expressed in C41 *E. coli*. Protein expression was induced with IPTG and verified by western blot analysis against the FLAG and 6x His epitope tags. The expected band at 25 kD was seen for both blots. In the future, interactions between BLM and BRCA1, NBS1, and H2AX will be studied.

Antibiotic resistance: Designing high-throughput methods for mutating beta-lactamase

Mellor, J.P., Tierney, C.M., Knies, J.L., and Weinreich, D.M.

Department of Ecology and Evolutionary Biology, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Through biological evolution many bacteria have developed plasmid genes that code for beta-lactamase, an enzyme that deactivates Ampicillin and a majority of beta-lactam drugs. The emergence of these defiant microbes has caused concern towards the treatment of diseases that may be unaffected by antibiotics. Beginning with vector pBR322, which carries a beta-lactamase gene, we performed site-directed mutagenesis to design and construct a plasmid that isolates the beta-lactamase gene sequence with novel restriction enzyme recognition sites. Specifically an AgeI site was introduced seven nucleotides upstream of the mature beta-lactamase protein, and an NcoI site was introduced eight nucleotides downstream from the stop codon of the gene. In the future this specificity will allow experimental disruption of just the mature protein through error-prone polymerase chain reaction (epPCR), a process that modifies selected DNA into a variety of mutated alleles. The formation of this specialized plasmid, epTEM322, offers a convenient high-throughput method for disorganizing the resistant gene so that the structure and function between the original and mutated enzymes can be studied.

The estrogenic effects of divalent metals on human breast cancer

Monchery, J.[2], Scully, K.[1], and Shaikh, Z. [1]

[1]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI
[2]Biology Department, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Estradiol is a hormone responsible for mammary development and cell growth and also upregulates breast cancer proliferation. SKBR-3 is a human breast cancer cell line which lacks classical estrogen receptors and expresses GPR30, a 7-transmembrane G protein-coupled membrane estrogen receptor. In response to estrogen, GPR30 initiates cellular signaling and cell growth. Previous studies have identified several divalent metals as xenoestrogens; compounds with estrogenic effects. The aim of this study was to examine the effects of divalent metals on breast cancer proliferation through GPR30. Cell proliferation was measured using the MTT assay. Results provided evidence that breast cancer cells expressing only GPR30 show can increased cell growth when treated with cadmium and some of the other divalent metals at concentrations that do not produce acute toxicity. This data suggests that exposure to non-essential metals at non-toxic concentrations may be one of the causative factors in breast cancer proliferation.

Urinary biomarkers in arthritis induced rats

Moniz, J., Ahmed, A., Jean-Gilles, D., and Chichester, C.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Rheumatoid Arthritis (RA) is the most common form of inflammatory arthritis affecting approximately 1% of the world's population. Urinary proteomics is a rapidly growing field that studies the quantitative changes in protein levels in the urine and holding the promise of discovery of potential biomarkers. A protein biomarker is an indicator which can be objectively measured is specifically reflecting the process of the disease. This study used urinary proteomics to analyze for potential protein biomarkers in arthritis. Arthritis was induced in male rats by injecting methylated bovine serum albumin in the right and left femorotibial joints. Urine samples were than collected after the third injection from arthritic and non-arthritic rats in twenty-four hour increments. The samples were concentrated with the use of dialysis and lyophylization. The urine samples peptides and proteins were initially analyzed using FPLC-gel filtration with the Superose 12, (32 x 3cm) column. The sample was also analyzed by ion exchange by using the HiTrap Q HP, 5ml column which produced better separation of the sample. The samples were collected based on major peaks and then concentrated by using the YM-3 centrifugal filtration device. Further processing of the samples was achieved by RP-HPLC using a Prosorb C18, 4.6 x 250mm RP-HPLC column with a 30% gradient of acentonitrile over 108 minutes. The concentrated urine samples by the YM-3 filtration was also analyzed by PAGE gel electrophoresis. There appears to be significant differences in the protein patterns between control and arthritis test urine samples. With these results it is anticipated that it may be possible to identify a urine biomarker that can be used for the early detection of RA.

Characterization of coastal cyanophages that infect *Synechoccocus* cyanobacteria WH 8101 and WH 8012 from upper and lower Narragansett Bay

Munzert, C.M.[1], Wemple, J.S.[2], and Marston, M.F.[3]

[1]Mt. Hope High School, Bristol, RI
[2]William B. Cooley HST High School, Providence, RI
[3]Department of Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Teacher Research Fellowship

The Narragansett Bay's productive cyanobacteria population is limited in part by the local distribution of cyanophages whose life cycle results in the lysis of its bacterial hosts. The purpose of this investigation is to characterize viral populations, isolated from Riverside (upper bay) and Jamestown (lower bay) water samples, which are capable of infecting *Synechococcus* WH 8101 and WH 8012. The Most Probable Number Assay was used to determine host-specific viral abundance (virus/mL seawater) in Riverside and Jamestown (and RWU) samples. Jamestown samples contained 372 virus/mL seawater for WH 8101 and 608 virus/mL seawater for WH 8012. Riverside samples contained 87 virus/mL for WH 8101 and 104 virus/mL for WH 8012. For both cell types (WH 8101 and WH 8012) Jamestown samples contained a greater number of virus/mL seawater. PCR products for the DNA polymerase gene (g43) were digested using Mse1 and Mbo1 resulting in two sets of Community Profiles for each location and each host cell-type. Community Profile banding patterns suggest there are numerous viral types infecting WH 8101 and WH 8012 and that these viral types differ by cell type and also by location. Viruses were isolated by plaque purification using host cells WH 8101 and WH 8012. PCR g43 products from viral isolates were digested with Mbo1 and compared with the community profile to identify specific viral types within the community profiles. For each unique viral type, sequences for the host-derived photosystem II psbA and DNA Polymerase g43 genes were obtained. Sequence comparisons with all known viruses through a Genbank BLAST search can reveal new sequences and can be used to examine evolutionary relationships among viruses. More complete characterization of viral populations that infect specific coastal bacterial hosts will provide key information regarding host-phage interactions, host specificity, and location specific variations.

BAX-induced cell death in the budding yeast, *Saccharomyces*, involves calcium

Murphy, K.R., Sullivan, J.J., and Austriaco, N.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bax is a proapoptotic member of the Bcl-2 family of proteins. Upon activation, Bax binds to the outer mitochondrial membrane, which ultimately induces programmed cell death in mammalian cells. Naturally *Saccharomyces cerevisiae* does not contain BAX; however, when Bax is transformed into the yeast it functions as it does in mammalian cells. We are investigating the role of calcium in Bax-induced cell death in yeast by overexpressing mammalian BAX in our yeast calcium mutants. We have discovered that mutants lacking genes important for calcium regulation including *CCH1, CNB1*, CRZ1/TCN1, *PMC1, PMR1 and VCX1* are all relatively resistant to BAX-induced toxicity. We have observed an increase in BAX-induced toxicity as extracellular concentrations of calcium rise. We are currently working to definitively show that calcium levels change intracellularly by constructing the first yeast cameleon. Previously, it has been difficult to detect intracellular calcium level changes due to the laborious processes of aequorin testing. This protein complex was taken from jellyfish and is effective in determining calcium concentrations that are in the magnitude of micromolar. Instead of conducting this laborious testing, we can engineer and localize our cameleon to show us intracellular cytoplasmic, endoplasmic reticular and mitochondrial calcium levels. This construct contains two fluorophores which show normal calcium levels (cyan fluorescence) and elevated calcium levels (yellow fluorescence). This in hopes will validate our preliminary calcium data and prove that as intracellular calcium levels rise, so does BAX-induced toxicity leading to apoptosis or programmed cell death.

Structure activity relationships of pyrethroid insecticides on the human t-type voltage-sensitive calcium channel.

Mutanguha, E.M., Valentine, Z., and Symington, S.B.

Department of Biology and Biomedical Science, Salve Regina University Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The purpose of this research was to determine the effects of pyrethroid insecticides on the peak current characteristics of a human t-type voltage-sensitive calcium channel ($Ca_v3.2$). Human $Ca_v3.2$ cDNA was transcribed into cRNA and injected into defollicated *Xenopus* oocytes. Human $Ca_V3.2$ currents were electrophysiologically characterized using the two-electrode voltage clamp technique with Ba^{2+} as a charge carrier. Dose response curves were generated and relative indices of potency (EC_{50}) and efficacy (β_{max}) were obtained. Pyrethroid effects on the peak current were determined following perfusion with various concentrations of deltamethrin, λ-cyhalothrin, bifenthrin, fenvalerate, esfenvalerate, fenpropathrin, permethrin, tefluthrin, bioallethrin, and β-cyfluthrin. Overall, pyrethroids as a class did not modify $Ca_v3.2$ in a consistent manner. Pyrethroids that possess an α-cyano moiety were more potent and efficacious than the ones that lack this moiety. Moreover, the presence of a halogenated site of unsaturation in the acid portion of the molecule appears to also play a role in the mechanism of action of these compounds on this channel.

Effect of hypotonic osmotic stress on cultured human skin keratinocytes

Nalbandian, A., Fredette, R., Brum, G., McCauley, M., and Wan, Y.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

While it is known that a cell in a hypotonic environment swells from an influx of water due to an osmotic imbalance, the pathways activated inside the cell are not as clearly observable and less well studied. To investigate whether apoptotic and cell survival signaling pathways are activated, cultured human skin keratinocytes (HaCaT cell line) were exposed to increasing levels of hypotonicity from 0% dilution up to 50%. Western blotting analysis was used after 30 minutes of incubation. The results show that AMPK is activated when the dilution is increased, and the activity is peaked at 40% dilution and then dropped at 50% dilution. Similarly, AKT is activated and the activity is peaked at 40% and 50% dilutions, suggesting that at this level of hypotonicity these pathways are working hard to ensure cell survival. The results also demonstrate that p38 and JNK are activated as the dilution is increased, and the activity is peaked at 40% dilution. Interestingly, the data from cell migration assay show that cell migration is increased gradually as dilution is increased, but dropped significantly at 50% dilution. Collectively, our data suggest that hypotonic stress affect both apoptotic and survival pathways and to certain degree enhance cell migration.

Optimization of bacterial transformation for construction of plasmid libraries

Ngeow, K.C. and Fairbrother, W.G.

Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Bacterial transformation plays a key role in molecular genetics, particularly in the construction of plasmid libraries. Although bacterial transformation is a well-studied phenomenon, most studies have been made with single variants of supercoiled, closed plasmids. The preparation of a plasmid library is considerably more complex because it involves the product of a ligation reaction between a plasmid vector and our inserts of interest (a pool of oligonucleotides). Transformation efficiencies are generally lower when using such ligation products because of factors such as poor ligation efficiency and chemical interference. In order to generate a representative library of clones from a small sample of DNA, transformation efficiency would have to be increased. Hence, there is a need to optimize the various factors involved in transformation for our specific ligation mix. As high transformation efficiencies were required, the standard calcium chloride heat shock method proved to be insufficient for our purposes. Our protocol is based loosely upon the one developed by Inoue et al in 1990 to induce ultra-competency in *E. coli* cells, with our own modifications after taking into account differences in bacterial strains, insert DNA, reagents and equipment. Using the HB101 strain of *E. coli*, we were able to attain maximum transformation efficiencies on the order of 10^8 cfu/µg, which is similar to that obtained in the original protocol. Our cells also retained their competency upon long-term storage in -80°C, with no significant decrease in transformation efficiency over a 30 day period. By varying factors such as the length of heat shock and recovery period, we were able to attain up to 21% higher transformation efficiencies than if we had followed the original protocol. In addition, we were also able to attain similar transformation efficiencies using cheaper and more readily available supplies, thus minimizing costs, which is an important consideration when constructing plasmid libraries on a large scale. By performing our transformation protocol on the ligation mix, we were able to obtain a respectable 3000-4000 colonies on a single plate.

Noise-facilitated Ca^{2+} oscillations through the mechanism of stochastic resonance

Odobasic, J. and Kádár, S.

Chemistry Department, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Intracellular calcium (Ca^{2+}) is one of the most important secondary messengers in the cytosol of living cells, and it plays a significant role in controlling many cellular functions and processes. Due to the complexity of the cell, there is always inherent noise (fluctuations in concentrations and temperature) present in biological systems. Natural noise is proposed to promote signal propagations in biological processes through the mechanism called stochastic resonance, in which an otherwise undetectable periodic signal becomes viable due to the presence of noise in a system. The proposed model to account for the noise-mediated signal transmission (a form of oscillation of calcium concentration) is a combination of the Cuthbertson and Chay model that accounts for G-protein signal transduction and the Borghans, Dupont, and Goldbeter model which emphasizes calcium-induced calcium release. The numerical integration was performed with MATLAB and the analysis was based on the Signal to Noise ratio (SNR) obtained from the Fast Fourier Spectrum (FFT) of the concentration-time series. According to our findings, increasing the noise level provides increasing support for signal transmission up to an optimum level after which further increase of the noise level corrupts the signal. Experimentally, the dynamics of the oscillation of calcium concentration is studied in PC12 cells. Collected experimental data indeed shows the presence of noise.

The uphill climb to determining levels of relatedness in *Polistes erythrocephalus* using microsatellites

O'Hara, M. and Arévalo, E.

Department of Biology, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

When considering the evolution of sociality in insects, Hamilton's theory of inclusive fitness predicts that the existence of a non-reproductive caste would be favored by natural selection. These individuals sacrifice their chance for direct reproduction, while ensuring the passage of their genes via helping reproductive relatives. Bees, ants, and wasps (Hymenoptera), are models for studying the evolution of sociality because of their haplodiploid system that produces high levels of relatedness up to 0.75. In these groups, the range of sociality spans from species considered to be eusocial with predetermined sterile castes, to more simple systems considered primitively eusocial in which all individuals of the colony could potentially reproduce. Paper wasps of the genus, *Polistes*, are an example of this low end of the spectrum. *Polistes* are widely used by researchers because of their worldwide distribution, small nest sizes, high abundance, and ease to collect. For the present study, in order to assess the reproductive structure within a colony of *Polistes* we used highly variable molecular markers, microsatellites. Forty colonies of *Polistes erythrocephalus* from Costa Rica were collected in 2006 for this study. A total of forty-one microsatellite loci designed for this species were used to screen these colonies. We observed lower levels of heterozygosity across these loci; only 10% showed heterozygosities greater than 0.50. Similar studies on other species of *Polistes* (*P. annularis*, *P. bellicosus*, and *P. dominulus*) have significantly higher heterozygosities ranging from 37% to 84% greater than 0.50. This discrepancy in genetic variability might be attributed to differences in the colony structure of temperate (these three species are from temperate regions) versus tropical species (*P. erythrocephalus*). To determine if such levels are a reflection of the structure of these populations or if there was a problem with the markers used, we are testing the *P. erythrocephalus* loci on two other species of *Polistes*, *P. dominulus* and *P. carnifex*. In addition, we are using the microsatellites designed for those temperate species with *P. erythrocephalus* samples. The low levels of heterozygosity found so far will require screening our samples through a larger number of loci in order to have the necessary power to discriminate between highly related individuals of the colony.

Electroporation device

Olalekan, A.[2], Ferguson, A.[1], and Barnett, S.M.[1]

[1]Department of Chemical Engineering, University of Rhode Island, Kingston, RI
[2]Department of Electrical, Computer and Biomedical Engineering, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Oil trapped in micro-algal cells can be used for biodiesel production. An electroporation device is designed and constructed to disrupt cell walls when a high electric field is applied to it. The device features an orifice whose length and width 200 times shorter and 30 times narrower than the length and width of the microchannel the algae flow through. On each end of the channel is pair of external electrode (platinum wire) is attached to the dc voltage supply. With a high voltage applied, the device generates a high electric field strength at the orifice to break apart the cell and low electric field strength across the remainder of the microchannel to generate an electroosmatic flow.

Synergistic approach to find alternative drugs against amebiasis: 3-phenyl-1-(phenyl carbamoyl and thiocarbamoyl) -2-pyrazolines

Phay, M.[1], Austin, K.[2], Przondo, L.[2], Mann, B.[1], Yaeger, R.[2], Von Riesen, D.[2], and Espinosa, A.[1]

[1]Department of Biology, Roger Williams University, Bristol, RI
[2]Department of Chemistry, Roger Williams University, Bristol, RI

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Entamoeba histolytica causes 50 million infections and 100,000 deaths annually. Deficient in mitochondria and cytochrome oxidative system, these parasites use a fermentative pathway for energy metabolism by means of a bi-functional enzyme, *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2). Due to the enzymes' crucial role in *E. histolytica's* survival and its dissimilar characteristics to human alcohol and aldehyde dehydrogenases, EhADH2 is an excellent target to treat amebic infection. Series of pyrazoline analogues were synthesized and tested against EhADH2 activity because of their broad use in medicine as anesthetics and antibiotics. All pyrazolines compounds tested showed inhibitory effects on *E. histolytica* trophozoite growth and EhADH2 enzymatic activities. The efficiency of each compound on growth and activities varied depending on the side halogens: chloride affect amebic growth and biochemical activities more than bromide. Furthermore, thiocarbamoyls are better inhibitors than carbamoyls. These compounds are being modified to improve their solubility and efficiency. Cyclic pyrazolines are thought to act as alcohol analogs but more work is needed to elucidate the mechanism of action on EhADH2.

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Effect of buckwheat (*Fagopyrum esculentum*) treatments on available soil phosphorus and oxalate^{2-} concentrations in a field experiment

Possinger, A.R. and Byrne, L.B.

Department of Biology and Marine Biology, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Phosphorus (P) is an important element of successful plant growth, and many low-input farming systems are being developed to increase P availability in soil through use of cover crops, including buckwheat (*Fagopyrum esculentum*). It is proposed that buckwheat alters the available P concentration and consequently its own P uptake by means of root exudates, which remain understudied. The exudation of oxalate^{2-} organic anions has been observed in buckwheat, and this compound has also been identified as having a role in increasing P availability in other plant species. In this study, field buckwheat plots with added P and with existing P condition treatments were created to test the effect of the culture of buckwheat on the presence of available P and oxalate^{2-} in the bulk soil, rhizosphere, and root tissues. Available P and oxalate^{2-} concentrations will be measured using Bray P Extraction methods and gas chromatography-mass spectrometry (GC-MS), respectively. Additionally, fall crops will be grown in each buckwheat treatment plot to compare the efficacy of buckwheat in increasing available P for the next crop rotation. The findings of this study are intended to develop greater understanding of the bioprocesses involved in the buckwheat - P relationship and consequently develop more informed means of using buckwheat in sustainable agricultural systems.

The role of physiological arousal and environmental stressors in an animal model of non-suicidal self-injury

Pucino, D.M., Sheinhite, J.S., Miller, A.M.P., Wante, K.M., and Bloom, C.M.

Department of Psychology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Non-suicidal self-injury (NSSI) is estimated to affect between 4 - 21% of the population, leading to both personal distress and occasionally serious health risks. Current theory implicates a combination of nervous system singularities and environmental stressors in the development and maintenance of this disorder. An animal model was designed to replicate these conditions using 200mg/kg of the stimulant drug pemoline and an avoidance conditioning paradigm. Sixteen rats were randomly assigned to the four conditions of a 2 (Pemoline/Vehicle) X 2 (Discriminated Reinforcement/ Discriminated Avoidance) factorial design. Analyses were conducted across conditions for both degree of injury and operant performance.

Screening compounds for ligand activity with constitutive androstane receptor (CAR) and pregnane X receptor (PXR)

Qamar, S., Dring, A., Hamel, L., and Stoner, M.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) are closely related proteins, share some ligands and target overlapping sets of genes that influence all phases of drug metabolism. CAR is less well-understood than PXR; at least 15 CAR splice variants have been found to date and its ligand binding domain (LBD) is smaller than that of PXR. Studies have shown that the few known ligands of CAR have different types of structure associated with agonist and inverse agonist (antagonist) effects. To investigate putative structure-activity relationships, sixty-one commercially-available compounds, encompassing 6 different core chemical structures, were selected for a cell-based chemical ligand activity screen in human hepatoma cell line HuH7. Expression plasmids for PXR and 2 CAR variants found in human liver--one with constitutive activity (CAR1) and one with inducible activity (CAR3)--were co-transfected with Luciferase reporter plasmids under the control of consensus regulatory elements for CAR or PXR. Luciferase activity was measured and used to define ligand binding, agonist, antagonist and inverse agonist activity. Twenty-eight compounds were ligands of CAR1, CAR3 and PXR, 13 were ligands of CAR only and 5 were PXR ligands only. One compound was toxic to cells and 14 did not modulate receptor activities. Results were analyzed at different stringency levels to discover high, medium and low confidence groups of ligands, split into agonist and inverse agonist subsets and related to chemical structures. Compounds with demonstrated unique activity on any one receptor are potentially valuable diagnostic tools for future dissection of the *in vivo* molecular targets of the related nuclear receptors PXR and CAR.

Projects on gene divergence of aryl hydrocarbon receptors (AHR) in sharks

Merson, R., Albanese, A., Hall, J., Hersey, S., Reeves, D., and Schmidt, M.

Biology Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Aryl hydrocarbon receptor (AHR) protein binds and is activated by structurally diverse chemicals including environmental contaminants and putative endogenous compounds. AHR protein moves into the nucleus to regulate genes encoding biotransformation enzymes and numerous other genes involved diverse cell processes, but multiple molecular targets of the AHR make it difficult to establish mechanisms by which AHR agonists disrupt cell physiology.The overall objective of our research is to distinguish the role of aryl hydrocarbon receptors (AHRs) in xenobiotic-induced toxicity from their endogenous role(s) in by studying structural and functional diversity among the multiple early vertebrate AHRs and separately testing their function. We traveled to the Mount Desert Island Biological Laboratory (MDIBL) to use the spiny dogfish shark *Squalus acanthias* embryo-derived (SAE) cell line and the *Squalus acanthias* BAC library resources to 1) perform functional studies of receptor proteins, and 2) use comparative genomics and targeted mapping of AHR genes. Our presentation gives a broad overview of the projects underway in the laboratory and the work we accomplished at MDIBL.

Arylimines are synthesized then modified by the Williamson ether synthesis with phase-transfer catalysis to make a library of GSK-4716 analogs as potential SERMs and antioxidants

Cuoto, E., Rosadzinski, T., and Williams Jr., J.C.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE Undergraduate Research Fellowship Program
RI Science and Technology Advisory Council

A library of potential selective estrogen receptor modulators (SERMs) and antioxidants structurally similar to GSK-4716, a powerful SERM, were synthesized and are being screened for activity against the estrogen receptor *in vitro* (Matt Stoner, URI College of Pharmacy) and in the MTS assay for cytotoxicity (Rebeka Merson, RIC, Biology). Thousands of structure variations are being screened computationally using the eHitsLightening® docking program. An essentially quantitative Schiff reaction of aldehydes or ketones with arylamines provides one library of GSK-4716 analogs. Those with a phenolic hydroxy group were modified by the Williamson ether synthesis using aliquat phase-transfer catalysis to make another library. Modification of the extraction process using dichloromethane yielded solids from otherwise intractable oils formed in some of the aliquat reations. The overall yields for the second set of products is 60-80%. Oxygen charge-transfer complexes are apparently formed by some of these compounds. Physical chemical experiments to verify this phenomenon and use it to predict antioxidant activity will be performed.

Modeling pesticide induced effects on intracellular calcium oscillations

Salter, D. [1], Borges, A. [2], Symington, S.B. [1], and Kádár, S. [2]

[1]Department of Biology and Biomedical Science, Salve Regina University Newport, RI
[2]Department of Chemistry, Salve Regina University Newport, RI

[1]RI-INBRE Summer Undergraduate Research Fellowship Program
[2]RI EPSCoR Summer Undergraduate Research Fellowship Program

The aim of the project is to experimentally (calcium imaging) and theoretically (mathematical modeling) describe the intracellular calcium dynamics of PC12 cells to assess the potential adverse effects of environmental toxicants. PC12 cells were passively loaded with the fluorescent calcium indicator dye, calcium green to monitor intracellular calcium influx. Preliminary experimental evidence indicates that the intracellular calcium concentration of PC12 cells is 180 nM (± 19.4) and oscillates at a period of approximately 30 seconds (± 3.0). The dynamics of this oscillation was modified by the addition of extracellular dopamine and potassium chloride which resulted in varying amplitude of the oscillation. This experimental data was used to construct a comprehensive multi-cell mechanism describing calcium dynamics and a possible mechanism for information transmission between cells. Our model was constructed by combining the previously reported Cuthbertson and Chay model that accounts for G-protein signal transduction and the Borghans, Dupont, and Goldbeter model which emphasizes calcium-induced calcium release with biphasic regulation. Both models assess fundamental characteristics of calcium dynamics; however do not comprehensively discuss the overall process. Preliminary theoretical data based on the newly combined model indicates that the intracellular calcium concentration of PC12 cells oscillates at a period of between 25 and 33 seconds. With further slight refinement, our new combined model can be used as a predictive tool to assess the potential adverse effects of various environmental toxicants.

Characterization of Kinesin-3 cargo and the mechanism of cargo/motor interaction

Samoriski, C.M., Sarkis, J.P., and DeGiorgis, J.A.

Biology Department, Laboratory of Neurobiology, Providence College, Providence, RI
Marine Biological Laboratory, Woods Hole, MA

RI-INBRE Summer Undergraduate Research Fellowship Program

The squid giant axon and the development of video-enhanced DIC light microscopy provided the first direct observations of axonal transport. Conventional kinesin, the founding member of the kinesin motor family, was discovered in squid and recently we identified a second kinesin, Kinesin-3, in the squid giant axon that moves a subset of organelles towards the plus-ends of microtubules (DeGiorgis et. al., 2008). This Kinesin-3 localizes to a foci between the motor's organelle cargo and the microtubule substrate. Here, we set out to determine the number of Kinesin-3 at the foci, to determine the molecular anatomy of the membrane-bound Kinesin-3 cargo, and to determine how the motor associates with the organelle surface. To these ends, we have isolated Kinesin-3 organelles from squid axoplasm as well as squid optic lobe. These organelles co-purify with Kinesin-3 by Western blot and antibodies to Kinesin-3 decorate the organelle surfaces by immuno-electron microscopy. These organelles bind to exogenous bovine microtubules in the absence of ATP. By EM tomography the association of Kinesin-3 organelles to microtubules cause dynamic shape changes to the microtubule suggesting a multiple Kinesin-3 association. Kinesin-3 organelle/microtubule complexes will be used in mass spectral analysis to determine the molecular components of the kinesin-3 cargo and to identify a putative Kinesin-3 receptor.

Characterization of the *LtLIP3* genetic locus encoding a secretory lipase in the non-pathogenic parasite *Leishmania tarentolae* using southern hybridization and restriction map analyses

Santos, C.L., Fraulino, D.A., and Shakarian, A.M.

Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania tarentolae are model systems used to study human parasitism and to investigate the cell and molecular biology of this important group of pathogens. The focus in our lab is to identify and characterize secreted proteins towards defining the roles these molecules play in parasite survival and development. We hypothesize that secretory proteins such as secretory lipases help *Leishmania* survive the hostel environments it encounters throughout the parasite lifecycle. Since little is known about the function of lipases in these organisms, in the current study, we began a characterization of the genetic locus of the *LtLIP3* gene that encodes a secretory lipase in *Leishmania*. *Leishnmania tarentolae* promastigotes were cultured in M199+ media at 26^0C. To characterize the lipase locus, cells were harvested by centrifugation and genomic DNA was isolated using standard protocols. Southern hybridization of the gDNA from *L. tarentolae was* carried out to determine the structure of the *LtLIP3* lipase gene locus. For these experiments 5µg of the gDNA was digested with various restriction endonuclease (*Sal* I, *Kpn* I, *Mlu* I, and *Hind* III) and restriction fragments were separated by agarose gel electrophoresis. The DNA fragments were visualized by staining with ethidium bromide and subsequently transferred by capillary action to a positively charged nylon membrane. Membranes were hybridized with a full length *LtLIP3* gene probe to detect restriction fragments containing the lipase gene. In continuing studies, the data obtained by exposure to X-ray film will be used to generate a restriction map of this locus in *L. tarentolae*.

Evaluation of biofilm development in clinical *Staphylococcus aureus* bacteria that colonize the blood, nares, tissue, urine, and catheters

Sarkisian, S.A.[1,2], Alshammari, T.M, [1,2] and LaPlante, K.L.[1,2]

[1]University of Rhode Island, Kingston, RI
[2]VA Medical Center Infectious Diseases Research Laboratory, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: *Staphylococcus aureus* bacteria can cause persistent infections by producing extracellular slime matrixes known as biofilms. It is currently unknown if biofilm forming bacteria are associated with an infection type. Therefore, we set out to quantify, and classify biofilm production in clinical *S. aureus* isolates; then to identify a relationship between biofilm producing strains and a patient's infection source.

Methods: A random sample (n=189) of unique clinical *Staphylococcus aureus* bacteria were taken from patients at the Veterans Affairs Medical Center (VAMC) in Providence, Rhode Island over five years (June 2004 through May 2009). The source of each isolate was documented (i.e., blood, nares, tissue, or urine) and biofilm production was quantified. Known biofilm producing *S. aureus* (ATCC 35556) & *S. epidermidis* (ATCC 35984) & non-biofilm producing staphylococci (ATCC12228) were used as controls. Biofilm production was determined by Christensen's method & quantification was determined by validated methodology of Stepanovik. The optical density (OD_{610}) of bacterial biofilm were classified as no biofilm production. Chi squared or Fishers exact test was used to compare biofilm vs non-biofilm production. The statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS).

Results: Known biofilm producing *S. aureus* (ATCC 35556) produced strong biofilm formation with an average OD_{610} reading of 1.78 ± 0.65, and *S. epidermidis* (ATCC 35984) a moderate to strong biofilm producer with an average OD reading of 1.67 ± 0.70. The non-biofilm producing staphylococci (ATCC12228) produced with an average OD reading of 0.29 ± 0.08. There was statistical significance shown in the nares and urine sources compared to the other sources. Quantification of results is as follows:

Source(n = 189)	No Biofilm % (n)	Weak Biofilm % (n)	Moderate Biofilm % (n)	Strong Biofilm % (n)	P value*
Blood (n = 47)	68.1%, (32)	31.9%, (15)	0%, (0)	0%, (0)	0.132
Nares (n = 50)	96.0%, (48)	4.0%, (2)	0%, (0)	0%, (0)	0.0001
Tissue (n = 46)	76.1%, (35)	21.7%, (10)	2.2%, (1)	0%, (0)	0.985
Urine (n = 40)	62.5%, (25)	37.5%, (15)	0%, (0)	0%, (0)	0.022
Catheter (n = 6)	66.7%, (4)	33.3%, (2)	0%, (0)	0%, (0)	0.630**
Total:	76.2%, (144)	23.3%, (44)	0.5%, (1)	0%, (0)	

* P value is source vs. others, i.e. blood vs non-blood.
** Fisher exact test

Conclusion: The results indicate that "strong biofilm" production of *S. aureus* is not evident in patient blood, nares, tissue, urine, or catheter samples. However, cultures taken from tissue were more likely to produce biofilm. Overall, cultures taken from urine produced significantly more biofilm (P=0.001) compared to the other sources. Therefore, a correlation may exist between biofilm production and urine as the source. This may be explained by high likelihood of *S. aureus* urinary cultures are taken from urinary catheters. More research should be conducted to discern the origin of *S. aureus* in patient urine.

Evolutionary history of *Pet*E, a host-derived photosynthetic gene, in marine viruses

Schonwald, J. and Marston, M.

Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Synechococcus spp. are unicellular photosynthetic cyanobacteria that are at the bottom of the oceanic food web. Like all photosynthesizing organisms, *Synechococcus* contains the petE gene which codes for Plastocyanin, a key protein in the photosynthetic electron transport chain. Viruses that infect *Synechococcus* often carry copies of host-derived photosynthetic genes including petE. These extra genes allow viruses to keep the infected cell alive longer, which may increase viral fitness. In this study, Rhode Island myoviruses were probed for the presence of petE genes. We then compared the evolutionary history of viral-encoded petE genes with the evolutionary history of core-viral DNA polymerase genes. Several PCR primer sets were developed to target segments of the petE gene. One of the primer sets was successful in amplifying petE sequences from 20 viral isolates out of the 31 viral isolates tested. Although in some cases nonspecific binding was a problem, we were able to directly sequence the petE gene from over 10 viral isolates. The divergence among viral-encoded petE genes ranged from 5 to 37%. Phylogenetic trees constructed using petE sequences were compared to trees constructed with viral DNA polymerase sequences. Our preliminary results reveal that the evolutionary history of viral-encoded petE genes is different than the evolutionary history of core-viral genes. This suggests recent genetic exchange of petE genes among viruses or between viruses and their hosts. The next steps of this study include the amplifying, purifying and sequencing of more viral petE genes, in order to better determine the differences in evolutionary history.

Unusual sequence effects on conformational heterogeneity in arylamine adducted DNA

Scimeca, J., Jain, V., Patnaik, S., and Cho, B.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Undergraduate Research Fellowship Program

Arylamine-modified DNA adducts exists in two distinct conformers, B and S. The B conformer binds in the major groove, while the S ("stacked") conformer interrupts the Watson-Crick base pairing of the helix. The NarI sequence (5'-GGCG*CC-3'; G*= dG-C8-FABP) in E. coli is a well known hot spot for frame shift mutations, and previous studies have shown the effect of the next flanking base on conformational heterogeneity of arylamine adducts; the adducted sequence G*CT displays both B and S conformers, while G*CA displays only B. In the present study, we sought to elucidate the structural basis of conformational heterogeneity by simulating the process of translesion synthesis in a 19-mer oligonucleotide (5′- CTTA CCATCG*CTACC ATTC -3′) containing the same GCT context. FABP modified sequence was annealed with six different complimentary strands (n-1, n, n+1, n+2, n+3, and full) for conformational analysis of the adduct using CD spectropolarimetry and ^{19}F NMR. We found that the FABP-DNA adduct exists in the S conformer until the lesion site has been passed, after which it exists in equilibrium as both B and S. This suggests that the S conformer is induced by the presence of thymine, while interaction with the complementary strand pushes the carcinogen back to the B conformer and gives rise to a mixture of B and S.

Elucidating the molecular mechanisms of sigma-1 receptor modulation of breast cancer proliferation

Shaya, J. and Bowen, W.

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

RI EPSCoR Summer Undergraduate Research Fellowship Program

The sigma-1 receptor is an ER transmembrane receptor that has been implicated in a variety of conditions including schizophrenia, cocaine addiction, depression, and, most relevant to this project, cancer. While little is known about the involvement of the sigma-1 receptor in cancer, it has been shown that the sigma-1 receptor is upregulated in a variety of cancers. Previously, *Wu et. al.* have developed a stable transfected cell line termed line 41 from the MCF-7 breast cancer cell line, that expresses high levels of the sigma-1 receptor compared to the MCF-7 cell line which barely expresses any of the receptor. It was noted that upon stable overexpression of the sigma-1 receptor, the transfectant, line 41, experienced an enhancement in growth rate, further implicating the role of the sigma-1 receptor in cancer. The goal of this project was to examine how the sigma-1 receptor is able to cause an increased rate of cell proliferation in comparing line 41 to MCF-7. Using a standard Promega AQuone Proliferation assay, it was shown that regardless of media serum content, line 41 grows at a much higher rate than MCF-7. Given this, the possibility that the normal growth signaling pathways for MCF-7 might be amplified upon transfection of the sigma-1 receptor was then tested using a defined serum-free media and the mitogen Insulin-Like Growth Factor-1 (IGF-1). Using Genistein and GFX109203A, inhibitors of different parts of the receptor tyrosine kinase (RTK) growth pathway, it was shown that line 41 possesses a higher activity of this pathway compared to MCF-7. Moreover, it was shown that line 41 experiences activation of IGF-1R signaling even in the absence of IGF-1, meaning that line 41 is able to activate the IGF-1R growth pathway in a ligand-independent manner. This suggests that the sigma-1 receptor is endogenously activating the RTK growth pathway to stimulate proliferation. In examining how line 41 can endogenously stimulate proliferation, it was discovered that phenol red, the cell culture media pH indicator, was the mitogen that resulted in line 41's ability to proliferate in serum-free media while having little to no effect on MCF-7. It was also shown that the removal of phenol red results in a loss of line 41's ligand-independent IGF-1R activation. This data suggests that the mechanism through which phenol red is promoting proliferation is the key to understanding how the sigma-1 receptor stimulates proliferation via RTK signaling. It has been previously shown that phenol red has weak estrogenic activity and so, 17-β-Estradiol, the active form of estrogen, was tested to see if it could mimic the effect of phenol red, yet it, alone, was unable to reproduce the dramatic effects of phenol red on line 41 proliferation. While, the exact mechanism is still unclear, it is clear that sigma-1 receptor overexpression in breast cancer cells results in an amplification of proliferation via an enhancement of Receptor Tyrosine Kinase signaling and it seems that the Estrogen Receptor is a potential link between the sigma-1 receptor and activation of receptor tyrosine kinases.

Expression of $Ca_v3.2$ in HEK cells

Shea, A.M., and Symington, S.B.

Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The goal of the project is to develop a human embryonic kidney (HEK) cell line with stable expression of human $Ca_v3.2$ (a T-type voltage-sensitive calcium channel) along with a GFP marker. Two different strategies to establish a stable $Ca_v3.2$ HEK cell line were attempted. In one setup, the plasmid containing $Ca_v3.2$ was co-transfected into a HEK 293 cells along with a separate plasmid containing the GFP marker using PEI. Both vectors containing $Ca_v3.2$ and GFP, respectively, are under control of the CMV promoter. We also have transfected HEK cells with a $Ca_v3.2$ fusion protein. Homologous primers from $Ca_v3.2$ were used to PCR amplify the target gene and then inserted into the pcDNA3.1 vector. The newly ligated construct was cloned to *E. coli* and the integrity of the clone verified by restriction mapping. Expression of $Ca_v3.2$ in HEK cells was identified using a combination of fluorescence microscopy and using whole cell voltage clamping.

The role of p21 in the Fanconi anemia pathway

Shen, M. and Howlett, N.

Department of Cell and Molecular Biology, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi anemia (FA) is a rare disease characterized by congenital abnormalities, progressive bone marrow failure, and increased cancer susceptibility. FA arises from biallelic mutations in any one of at least thirteen genes that encode proteins involved in a DNA damage repair pathway. The hallmark of FA patient cells is hypersensitivity to DNA crosslinking agents. Despite extensive research into the FA pathway, much is still unknown about its exact mechanisms. Recently, we have observed that the monoubiquitination of the FA protein FANCD2, a key event in the activation of the FA pathway, is impaired in cells lacking the p21 protein. The p21 protein is a member of a family of cyclin-dependent kinase (CDK) inhibitors that regulates cell cycle progression in two ways: by binding CDKs via a CDK-binding domain and by binding PCNA via a PIP-box motif. Here, we have further explored the role of p21 in the proper functioning of the FA pathway. We have investigated whether FANCD2 monoubiquitination and p21 expression are coordinately regulated following DNA damage, whether p21 null cells display a sensitivity to DNA crosslinking agents similar to that of FA patient cells, and whether p21 is required for the localization of FANCD2 to discrete nuclear foci necessary for its participation in DNA repair. Furthermore, we have begun to examine whether the CDK-binding or PCNA-binding function of p21 is important for its role in the regulation of the monoubiquitination of FANCD2.

The effects of *Entamoeba* spp. kin recognition on pathogenesis

Silva, L. and Espinosa, A.

Department of Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica affects 50 million people worldwide. It is one of the leading causes of death from protozoan diseases. *Entamoeba histolytica* cells are highly motile and this is an essential feature of the pathogenesis of amebiasis. It has been implied that negative chemotaxis may play an important role in *E. histolytica* pathogenesis. *Entamoeba dispar* does not shown chemokinesis in response to conditioned medium meaning that the difference between pathogenic and nonpathogenic amoebas could be rooted in alternative behavior, rather than in particular proteins or molecules that harm the host. *E. invadens* IP1 and *E. invadens* VK were tagged with Green CMFDA and daily observations were made focusing of the aggregation or death to see if there was kin preference for each strain. A dichotomous key was created for various strains based on daily observations to differentiate each strain morphologically, as it has been suggested that all *Entamoeba* spp. cells are unrecognizable microscopically.

Examination of the role of muscle specific isoforms of Ufd2a during myoblast differentiation

Solares, J. and Spinette, S.

Biology Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Ufd2a is an E3/E4 ligase which has three alternatively spliced isoforms, two of which (Ufd2a II & III) are uniquely expressed at different times during the differentiation of skeletal muscle myoblasts. In order to determine the significance of these muscle-specific isoforms, their expression was blocked in a murine myoblast cell line using morpholino oligonucleotides prior to their differentiation in culture. Differentiation was monitored, using immunofluorescence microscopy to observe the expression of the differentiation marker, muscle myosin heavy chain along with DAPI stained nuclei to observe the levels of myoblast fusion. The data obtained thus far has allowed the initial quantification of cell fusion events in murine myoblasts expressing normal levels of each Ufd2a isoform and those in which the muscle specific isoforms have been blocked. Do to various difficulties with the current methods; experiments were performed to optimize the cell staining and adherence to the glass cover slips. Addition, while it appears that Ufd2a II and III may be required for cell fusion the mechanism of action remains unknown. Since cell cycle exit is required prior to cell fusion we wished to develop protocols to robustly monitor cell cycle exit during differentiation through analysis of p21 expression and DNA content analysis using Flow Cytometry.

In order to verify that the phenotype is specifically due to a lack of Ufd2a II and III expression, cells were transfected with exogenous human Ufd2a III tagged with GFP after blocking the endogenous muscle isoforms with morpholinos. The expression of the exogenous forms were viewed using fluorescence microscopy throughout the differentiation process. These experiments showed that transient expression lasted only 48 hours while full differentiation required 3-4 days. Therefore, a stable line of cells expressing the GFP-tagged UFd2aIII is being developed.

Defining the role of Ufd2a in myoblast fusion may contribute important information for developing future methods of muscle regeneration for patients with injuries, or muscular dystrophy since myoblast fusion is an early essential step in muscle repair.

Transporter expression changes in liver after high cholesterol feeding in gallstone susceptible and resistant mice

St. Germain, A.L., Paranjpe, M.A., and Slitt, A.L.

Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The western diet is considered to be a diet high in saturated fat (i.e. cholesterol), sugar, and sodium with low nutrient density. Chronic consumption of a western diet is associated with obesity, heart disease, high blood pressure, and fatty liver. Transporters are protein “pumps”, which are important for uptake and efflux of drugs into and out of cells. Specifically, Oatp1a1 is localized to the basolateral membrane and transports certain drugs (i.e. statins) into hepatocytes. Mrp2 and bcrp are localized to the canalicular membrane of hepatocytes and mediate ATP-dependent active transport of organic ions such as glutathione, glucuronide- and also sulfate- conjugated drugs into bile. Mrp3 and 4 are localized to the basolateral membrane of hepatocytes and mediate the efflux of glucuronide conjugated drugs and purine analogs, respectively. Because cholesterol consumption results in fatty liver, we hypothesize that cholesterol feeding will cause transporter expression changes in liver. In this study, male C57Bl/6 and AKR mice were fed a standard or lithogenic diet (19% protein, 5% fat and 5% fiber) for two, four, or 8 weeks weeks. At the end of each time point, serum, gallbladders, and livers were collected. As anticipated, histopathologic assessment of the livers indicated that the lithogenic diet resulted in increased presence of fat in livers. Total RNA was isolated from liver using phenol-chloroform extraction and mRNA expression of Oatp1a1, Mrp2-4, and Bcrp drug transporters was determined using the branched DNA amplification assay. Data presented in the poster will display Oatp1a1, Mrp2-4, and Bcrp mRNA expression and immunohistochemical staining in liver.

The regulation of the Ser/Thr phosphatase protein phosphatase 1 by the inhibitor DARPP-32 and the targeting protein PNUTS

Stein, B. and Peti, W.

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

RI EPSCoR Summer Undergraduate Research Fellowship Program

Protein Phosphatase 1 (PP1, 330 amino acids, 37.4 kDa) is a ubiquitous, essential serine/threonine phosphatase that is highly conserved across many organisms. PP1 is involved in a variety of cellular processes including cell cycle progression, transcription and neuronal signaling and is responsible for approximately 33% of all the dephosphorylation reactions in humans. Moreover, PP1 is known to be regulated by more than 200 inhibitor and targeting proteins. Most of these PP1 regulatory proteins contain a conserved PP1 binding site commonly referred to as the RVxF motif, which is necessary for the stable interaction with PP1, but is not enough to account for the specificity of the newly formed holo-enzymes. PNUTS (PP1 Nuclear Targeting Subunit) and DARPP-32 (Dopamine- and cyclic AMP-regulated phosphoprotein) are both examples of these PP1 regulatory proteins. PNUTS is a PP1 targeting protein, whose expression is up-regulated during hypoxic conditions and may affect the p53 apoptotic pathway through interactions with PP1. DARPP-32 is a PP1 inhibitor that is important for integrating signaling pathways in the brain. The first purpose of this study was to determine whether the complex formed from $PP1_{7\text{-}330}$ D208A mutant and DARPP-$32_{1\text{-}118}$ RVxF mutant (RVxF motif mutated from KKIQF to KKVTF, 17 kDa) is more stable and effectively purified than that formed by wild-type PP1 and DARPP-32. A stable complex must be produced in order for its properties to be studied. The second purpose of this project was to determine whether a complex of $PP1_{7\text{-}330}$ and $PNUTS_{309\text{-}433}$ (13.5 kDa) could be successfully purified. $PP1_{7\text{-}330}$ (wild type and mutant) and $PNUTS_{309\text{-}433}$ were grown using an *Escherichia coli* based expression system. $PNUTS_{309\text{-}433}$ was purified using His_6-tag affinity chromatography and heat purification. Protein complexes were formed using a Ni^{2+}-NTA column purification and purified further with size exclusion chromatography. Although the PP1 D208A DARPP-$32_{1\text{-}118}$ RVxF mutant complex was successfully formed, it was not stable. Most of the complex was lost during purification and what was obtained was impure and had a maximal concentration of only 22 μM. This finding suggests that the mutations in PP1 and DARPP-32 did not enhance binding enough to produce a stable complex. A purification of the $PNUTS_{309\text{-}433}$:$PP1_{7\text{-}330}$ complex yielded seemingly pure and stable complex. These preliminary results suggest that it is possible to produce a stable PP1:PNUTS complex, which will allow for further biophysical analysis.

Comparison of coastal cyanophage communities from Bermuda and Rhode Island

Taylor, S. and Marston, M.

Department of Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Cyanophages infecting *Synechococcus* spp. are abundant and genetically diverse in coastal environments. Despite the tremendous amount of marine viral diversity, previous studies have shown that viruses with almost identical gene sequences can be widely distributed. More recently, however, several studies have suggested that viruses may exhibit biogeographic patterns. In this study, we examined the community composition of cyanophages from the coastal waters of Bermuda and Rhode Island to assess if any viral genotypes were common to both communities. Viruses were isolated from Bermuda or Rhode Island water samples using three *Synechococcus* strains (WH7803, WH8101, and WH8012). After plaque purification, PCR was used to amplify segments of six viral genes (g20, portal protein; g43, DNA polymerase; psbA, a cyanobacteria-derived photosystem II D1 reaction center protein; psbD, a cyanobacteria-derived photosystem II D2 reaction center protein protein; cobs, a cofactor of ribonucleotide reductase; and phoH, a protein induced under phosphate stress). from each viral isolate. To date, over 100 cyanomyoviral isolates have been characterized, and these can be grouped into over 60 distinct genotypes. Based on phylogenetic analyses of g20 sequences, these genotypes fall into three main clades and are related to all other known viral isolates infecting *Synechococcus* spp. Isolates from Bermuda and Rhode Island do not form separate clusters within the tree, but are interspersed throughout the three clades. Although multiple viral isolates with >99% sequence similarity at the nucleotide level can be found within the same community, there was no overlap of genotypes between the two communities. Regardless of the gene used in the analysis, all of the isolates from Bermuda differed by at least 4% at the nucleotide level, although most differed by over 10%, when compared to any of the Rhode Island isolates. These preliminary results suggest that the cyanophages in each of these two communities may be genetically unique. Further characterization of these communities, and others, will help to establish the biogeographic distributions of coastal marine cyanophages.

Identification, cloning and sequencing of an actin gene from the lizard parasite *Leishmania tarentolae*

Tobin, M. [1], Shakarian, A.M[2], and Symington, S.B.[2]

[1]The Metropolitan Regional Career and Technical Center, The MET School for High School Students, Newport, RI

[2]The Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

Salve Regina University High School Outreach Program

In the current study, the a portion of the gene for actin was identified, cloned and sequenced from *Leishmania tarentolae*, a lizard parasite used as a model system for human disease. For these experiments, *L. tarentolae* promastigote cells were grown in M119+ media and harvested by centrifugation. The genomic DNA (gDNA) was isolated and used as template in PCR amplifications using primers based on actin genes from other *Leishmania* species. Agarose gel electrophoresis of the PCR reactions showed an expected ~300 bp amplified product which was then ligated into the pTOPO plasmid vector and transformed into *E. coli*. Transformed colonies were selected for on ampicillin agar plates. Colony PCR and agarose gel electrophoresis was used to identify those colonies that contained plasmids with actin inserts. Sequence analysis of the purified pTOPO::actin plasmid revealed a 300 bp portion of the actin gene which codes for 100 amino acids of the actin protein. Taken together, this data constitutes the first report of the identification, cloning and sequencing of an actin gene in *Leishmania tarentolae*.

Deltamethrin inhibition of human T-type voltage-sensitive calcium channels isoforms

Valentine, Z., Mutanguha, E.M., and Symington, S.B.

Department of Biology and Biomedical Science, Salve Regina University Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroids are a class of insecticides naturally derived from pyrethrin I and are commonly used in agricultural and vector control programs. The purpose of this research was to determine the effects of deltamethrin, a potent pyrethroid insecticide, on current characteristics of the human T-type voltage-sensitive calcium channels, $Ca_V3.1$ and $Ca_v3.2$. Human calcium channel cDNA was transcribed into cRNA using the mMessage mMachine *in vitro* transcription kit and injected into defollicated *Xenopus* oocytes. Human Ca_V3 currents were electrophysiologically characterized using the two-electrode voltage clamp technique with Ba^{2+} as the charge carrier. Pyrethroid effects on the overall peak current were determined following perfusion with various concentrations of deltamethrin. Our results indicate that deltamethrin reduced peak current of $Ca_v3.2$ in a concentration-dependent manner and had an approximate EC_{50} value 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). These results indicate that pyrethroids are potent and stereospecific inhibitors of human T-type voltage-sensitive calcium channel.

Regulation of *c-myb* by NF-κB family members during murine erythroleukemia differentiation

Ross, M.[1], Vincent, N.[2], and Toth, C.[2]

[1]Department of Biochemistry, Providence College, Providence, RI
[2]Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

c-Myb is a transcription factor and proto-oncogene that is important for hematopoietic cell development. The regulation of the c-Myb protein and its down stream targets is well studied while the regulation of expression of the *c-myb* gene is not well understood. *c-myb* expression is controlled by a conditional block in transcriptional elongation in the first intron of the gene. Previous studies have shown that NF-κB family members are involved in regulating the activation of *c-myb* by binding to Rel-Related Proteins Binding Elements (RRBE) in the first intron. This leads to the hypothesis that NF-κB regulates expression of *c-myb* during development of hematopoietic cells. Here we show using chromatin immunoprecipitations (ChIPs) of murine erythroleukemia cells that a block in pre-mature transcription arrest caused by hexamethylene bisacetamide (HMBA)-induced differentiation can be over-come by stimulation of adenyl cyclase by forskolin. ChIP analysis of NF-κB occupancy of the two RRBE elements in the first intron show that p50 and RelB bind to the 5' RRBE site exclusively. This study demonstrates for the first time a correlation between NF-κB occupancy of RRBE sites and *c-myb* expression *in vivo* in hematopoietic cells.

Internet-based addiction counselor education study (IBACES)

Warot, S.S., Russo, E.L., Desjarlais, J.L., and Montvilo, R.K.

Department of Psychology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Within the field of behavioral health care, there is a somewhat limited tendency of professionals to avoid implementing evidence-based research into practice. Many behavioral health practitioners, including addiction counselors, are exposed to evidence-based practice through only infrequent experiences with continuing education events, such as forums, seminars, or workshops. Continuing education serves as the primary modality for updating information presented to substance abuse treatment providers. Many providers have been found to attend these continuing education sessions only because they are required to, and use these sessions as opportunities to socialize rather than learn. Because of this, addiction research remains largely isolated from those it is intended to inform. In the study, a series of two experiments will be performed to determine whether continuing education courses for addiction counselors Have more of an impact if they are presented online as interactive courses rather than if they are presented in the typical conference format. Qualitative as well as quantitative data will be analyzed in making this determination. By implementing an internet-based learning modality as a continuing education option for behavioral health practitioners, we hope to help bridge the gap between the findings of effective treatment in evidence-based practice, to the actual dissemination of such knowledge within the community.

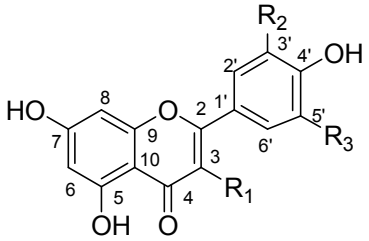
Isolation and structural elucidation of compounds from Fraser's Marsh St. John's Wort *(Triadenum fraseri)*

Woolridge, D.B.C.[1], Li, L.[2], Jean-Gilles, D.[2], MacDonnell, M.[2], Henry, G.E.[3], Slitt, A.[2], Chichester, C.[2], and Seeram, N.P.[2]

[1]Division of Biology and Medicine, Brown University, Providence, RI
[2]Bioactive Botanical Research Laboratory, Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI
[3]Department of Chemistry, Susquehanna University, PA

RI-INBRE Summer Undergraduate Research Fellowship Program

St John's Wort (*Hypericum perforatum*) is a medicinal plant widely used as a botanical dietary supplement for treating depression. Despite extensive chemical investigation into the bioactive constituents of St. John's Wort, its close relative, Fraser's Marsh St. John's Wort (*Triadenum fraseri*), has not been previously investigated. This plant is a perennial native to North American marshes. As part of our ongoing research to identify bioactive natural products from plants, we initiated the current study. A methanol leaf extract of *T. fraseri* was subjected to a series of isolation procedures including silica gel and LH-20 column chromatography, as well as semi-preparative high performance liquid chromatography (HPLC). Four pure isolates were obtained and identified as the flavonoids myricitrin (**1**), quercitrin (**2**), rutin (**3**), and nicotiflorin (**4**) by ^{1}H and ^{13}C-NMR methods. The crude plant extract and purified compounds were evaluated for antioxidant activity in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay. This is the first report investigating the antioxidant activities and isolation of chemical constituents from Fraser's Marsh St. John's Wort.



1 R_1=O-rha; R_2=OH; R_3=OH
2 R_1=O-rha; R_2=H; R_3=OH
3 R_1=O-glu-rha; R_2=H; R_3=OH
4 R_1=O-glu-rha; R_2=H; R_3=H

AUTHOR LIST

AUTHOR LIST (CONTINUED)

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