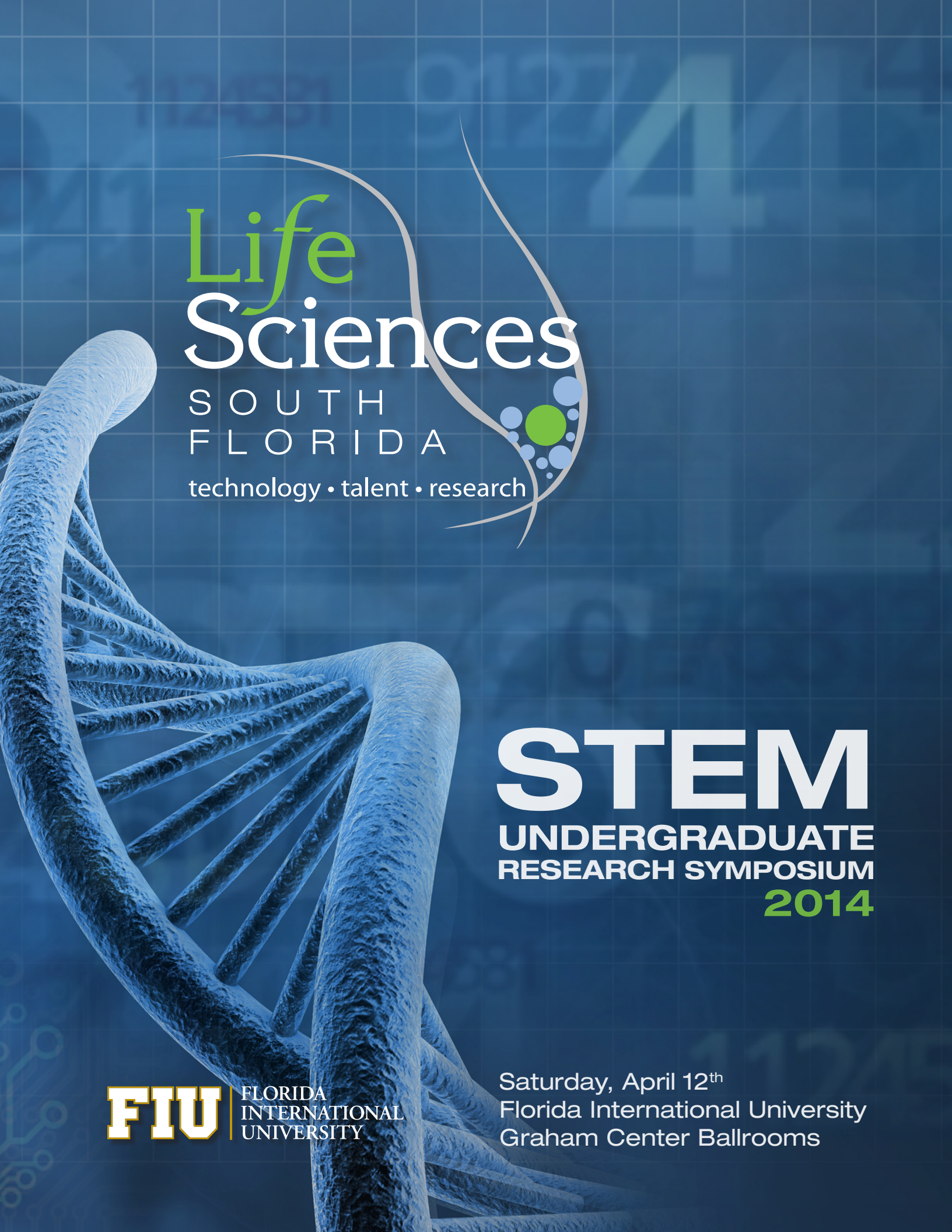

Life Sciences
SOUTH FLORIDA
technology • talent • research
STEM
UNDERGRADUATE
RESEARCH SYMPOSIUM
2014
FIU
FLORIDA INTERNATIONAL UNIVERSITY
Saturday, April 12th
Florida International University
Graham Center Ballrooms

April 12, 2014

Dear students, faculty and life science industry representatives:

Welcome to the second annual Life Sciences South Florida STEM Undergraduate Research Symposium! And, welcome to FIU!

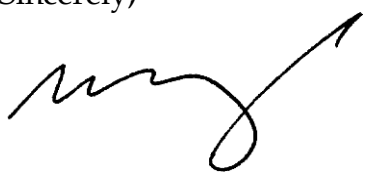
Today undergraduate Science, Technology, Engineering and Mathematics (STEM) students from across South Florida will present their original scientific research to their peers, academic and industry representatives through oral and poster presentations.

The keynote speakers for the symposium are Dr. Pedro Greer and Senator Jeremy Ring. Dr. Greer is a professor and assistant dean for academic affairs and chair of the Department of Humanities, Health, and Society at FIU's Herbert Wertheim College of Medicine. Senator Ring represents District 29 in Broward County for the Florida Senate.

Life Sciences South Florida aims to establish an industry cluster in South Florida focused on life sciences, biotechnology, pharmaceuticals, diagnostics and information technology. LSSF is committed to utilizing our collective assets to facilitate collaborations with economic development councils and regional, state, national, and international industries, governments and communities to promote innovation, investment, entrepreneurship and economic growth in the areas of biotechnology, pharmaceuticals, diagnostics and information technology to generate high-technology and high-paying employment. This symposium is a symbol of that commitment.

Congratulations to all the students selected to participate in this event. We look forward to your continued growth in STEM-related careers here in our state of Florida.

Sincerely,



Mark B. Rosenberg
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Florida International University

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KEYNOTE SPEAKER

JEREMY RING
CEO AT VIVANA
STATE SENATOR, STATE OF FLORIDA

Senator Jeremy Ring was raised in Massachusetts. Upon graduating from high school, he attended Syracuse University and received his degree in 1992.

At the age of 25, Mr. Ring moved to New York City. He joined with a small group of innovative entrepreneurs who had just formed the pioneer internet company, Yahoo! Inc. He opened the first east coast Yahoo! office out of his New York City apartment. As the original sales executive hired by the company, he was instrumental in growing sales to over $1 billion in just five years. While Internet users grew to the billions, Yahoo! changed the way we live, work and play by making it easier to access information and communicate globally. Mr. Ring's position gave him access to Fortune 500 companies throughout the world, promoting not just a product, but also the power of bold ideas. He was able to showcase the possibilities of what the future could be and ultimately, what the world has become.

After leaving Yahoo in 2001, Mr. Ring relocated to Florida, where they immediately became involved in their community. They co-founded the charity SUPERB (Students United with Parents and Educators to Resolve Bullying). The mission of SUPERB is to teach children social and behavioral skills in order to create a safe school environment enabling students to enjoy school and learn effectively. In addition to his public service, Mr. Ring remains active in the business world as the founder of Collegiate Images, a company that owns the rights to college sports media. He recently founded a ground-breaking medical company called Mercurian which focuses on autism and other related disorders using predictive modeling that helps parents chart and track their child's behavior and reactions to their environment, which leads to better and more focused care.

After his reelection to the Florida Senate in November 2010, Senator Ring spent his tenure serving on numerous Senate Committees. Sen. Ring currently serves as the Chairman of the Governmental Accountability and Oversight committee, which oversees the Florida Pension Fund, the State Board of Administrations and other government entities in the State of Florida. He served as the Co-Chair of the Joint Select Committee on Collective Bargaining. Sen. Ring was named to the Florida Statewide Passenger Rail commission by former Senate President Jeff Atwater. He has sponsored and passed several major bills over the last few years including a bill that created SunRail, a high speed passenger rail service. Another marquee piece of legislation helped to modernize Florida's State Pension System.

KEYNOTE SPEAKER

PEDRO J. GREER, JR., M.D., FACP, FACG
PROFESSOR AND FOUNDING CHAIR DEPARTMENT OF HUMANITIES, HEALTH AND SOCIETY
ASSOCIATE DEAN FOR COMMUNITY ENGAGEMENT
HERBERT WERTHEIM COLLEGE OF MEDICINE
FLORIDA INTERNATIONAL UNIVERSITY

Dr. Pedro J. Greer has an unwavering commitment to and is an advocate for those without access to health care. Throughout his career, Dr. Greer has received numerous awards and Honorary Doctoral degrees.

Most recently, he was recognized as a 2009 Presidential Medal of Freedom honoree, and in 1993, was honored as a MacArthur Foundation "Genius Grant" Fellow. Dr. Greer is board-certified in medicine and gastroenterology, and has been practicing in Miami, Florida since 1991. He established Camillus Health Concern and Saint John Bosco in Miami, Florida, both of which are health care centers for persons who are homeless, undocumented, uninsured, and/or low income. Better known as "Joe," Dr. Greer wrote *Waking up in America*, an autobiographical account of caring for persons who are homeless.

In July 2007, Dr. Greer joined the newly established Florida International University Herbert Wertheim College of Medicine as Assistant Dean for Academic Affairs. He led the creation of the Department of Humanities, Health, and Society, and in January 2009 became its Founding Chair. Dr. Greer and the department faculty have spearheaded a unique undergraduate medical education program to prepare physicians to assess and address the social determinants that affect health care access and health outcomes. The goals are to educate highly skilled, ethical, and culturally competent physicians attuned to the complex health and social needs of South Florida's diverse populations. Dr. Greer currently serves in various capacities for a multitude of national, state, and local organizations. He is a Trustee at the RAND Corporation (America's oldest and largest think tank) and is the current Chair of the Pardee RAND Graduate School Board of Governors. Additionally, Dr. Greer served as Chair for the Hispanic Heritage Awards Foundation from 2002 to 2012. He is a member of Alpha Omega Alpha National Medical Honor Society and a fellow in the American College of Physicians and the American College of Gastroenterology.

LIFE SCIENCES SOUTH FLORIDA
STEM UNDERGRADUATE RESEARCH SYMPOSIUM 2014

PROGRAM

Welcome Address and Introduction of Senator Ring	Irma Becerra-Fernandez, Ph.D. Vice President for Engagement Florida International University
Keynote Speaker	Senator Jeremy Ring Florida Senate
Introduction of Dr. Greer	Anthony Iacono, Ph.D. Vice President of Academic Affairs Indian River State College
Keynote Speaker	Pedro J. Greer, Jr., M.D., FACP, FACG Professor and Founding Chair Department of Humanities, Health and Society Associate Dean for Community Engagement Herbert Wertheim College of Medicine Florida International University
Introduction to Student Presentation Program	Susan E. Webster, Ph.D. Director, Training and International Research Initiatives Division of Research Florida International University
Oral Presentations I and II	Ballrooms
BREAK	
Poster Presentations	Ballrooms
LUNCH AND NETWORKING	
Awards Presentation	Irma Becerra-Fernandez, Ph.D. Florida International University
Closing Remarks	Susan E. Webster, Ph.D. Florida International University

ORAL PRESENTATIONS

Inhibition of Quorum Sensing by Bucida Buceras in Conjunction with Clinically-Relevant Antibiotics to Attenuate Virulence in Pseudomonas Aeruginosa

Liana Apolis[1], Rohan Batra[3], James Martin Quirke[2], Lisa Schneper[3], Kalai Mathee[3]*
[1]Department of Biological Sciences and [2]Department of Chemistry, College of Arts and Sciences
[3]Department of Molecular Microbiology and Infectious Diseases, Herbert Wertheim College of Medicine
Florida International University, Miami, FL

Abstract

Pseudomonas aeruginosa infections are often difficult to treat due to the organism's intrinsic resistance and ability to acquire antibiotic resistance. This necessitates the identification of novel therapeutic strategies that focus on other virulence mechanisms such as quorum sensing (QS). Two QS systems in P. aeruginosa, las and rhl, control virulence. Previous studies demonstrated that aqueous extracts from Bucida buceras leaves inhibited P. aeruginosa QS systems. In this study, the specificity of B. buceras aqueous extract, the combinatorial activity of B. buceras extracts with clinically-relevant antibiotics, and the identification of the active component were investigated. Methods: Direct effects on signaling were determined by measuring las- and rhl-dependent gene expression in heterologous host background in the presence and absence of B. buceras extract. The effects of interactions between B. buceras extract and azithromycin, tobramycin, ciprofloxacin, meropenem, imipenem and cefotaxime on P. aeruginosa growth were analyzed using Epsilometer tests. Results: B. buceras aqueous extract directly inhibited las and rhl QS-dependent gene expression. Also, B. buceras was antagonistic with all the antibiotics tested. Conclusion: Although our data suggests the plant extract is antagonistic with current treatments, it is possible that the compound with the antibiotic-mitigating effect is different from the anti-QS compound. Current research is focused upon purifying the quorum-quenching compound in B. buceras extract through fractionation via reverse phase chromatography. The focus of future studies demands further identification of the active anti-QS compounds, which may provide a potential solution to P. aeruginosa's remarkable resistance to antimicrobial drugs. * denotes presenter

Isolation and Identification of Pink, Feather-Degrading Bacteria from Swan Feathers

H. Apuli, K. Worsham, S. Bowen, M. Lowenberg, and T. D'Elia
Biology, Indian River State College, Fort Pierce, FL

Abstract

For the past decade, there have been an alarming number of Mute Swans that have developed a pink discoloration to their normally white plumage; a condition termed Pink Feather Syndrome. Under further inspection, the areas of discoloration were becoming brittle and degraded, and this was leading to the swans' death. This is the first report to analyze the bacterial community associated with the pink feathers, and to determine which species are capable of growing on and degrading swan feathers. White and pink swan feather clippings were collected from various sample sites in Florida and Minnesota, along with water and soil samples. A total of 23 bacteria produced pink to red colonies were isolated by culturing on TSA at 22^0C. Sequencing of the 16S rRNA genes identified a diverse assemblage of bacteria with high identity to bacteria isolated from feathers, water and soil. The 23 isolates were then screened on Feather Meal Agar (FMA) to determine if the isolates are able to use feathers as a carbon source and Dry Milk Agar (DMA) to determine proteolytic activity. Only 4 of the 23 isolates were able to grow on the FMA plates and produce clearing on the DMA plates. One of these isolates (HA-01), which originated from a pink feather, was identified as Deinococcus sp (99% identity). Nine additional Deinococcus isolates were collected from feathers, and several members of this genus have known keratinolytic activity. Keratin is a very stable protein, and is often degraded through the synergistic work of multiple enzymes produced from a diverse microbial community. These results show that degradation of swan feathers is also facilitated by a diverse composition of bacteria capable of growing on feathers and producing proteolytic damage to feather keratin.

Preparation and Optimization of Organic-Silica Hybrid Monoliths and Characterization by Capillary Liquid Chromatography

Denae Britsch and Zuzana Zajickova, Ph.D.
Department of Physical Sciences, Barry University, Miami Shores, FL

Abstract

In recent years, the development of highly permeable separation media, called monolithic columns, has immerged in capillary liquid chromatography. Monolithic stationary phases allow for high flow velocity due to low backpressure, therefore providing reduced analysis time. Traditional materials include silica-based and polymer-based monoliths. In our research, organic-silica hybrids have been prepared in-situ using [3-methacryloyloxy) propyl] trimethoxysilane as a monomer. The presence of an aqueous hydrochloric acid catalyst was necessary for hydrolysis and polycondensation of the trimethoxysilane functionality. The thermal polymerization of the methacrylate functionality was initiated using azobisisobutyronitrile. To achieve the optimum column performance, reaction conditions such as the temperature, time and concentration of catalyst and porogen (toluene) were adjusted. Progress of polymerization was followed by Fourier transform infrared spectroscopy and capillary liquid chromatography. Percent conversion of monomer to monolith was evaluated to provide information about the extent of the reaction completion. For the optimized column the following parameters were measured: efficiency of the column, selectivity for polar analytes, alkylbenzenes and steric compounds, and the degree of permeability. Minimum plate height of 9 μm (112,000 plates/m) was achieved for ethylbenzene at the optimal flow velocity of 0.27 mm/s. Methylene selectivity has been calculated as 1.28 ± 0.002, silanol selectivity as 0.13 ± 0.001, and steric selectivity as 1.70 ± 0.01. Consequently, the prepared hybrid monolithic columns prove to be efficient separation media in reversed-phase chromatography. In addition, inverse-size exclusion chromatography indicates the suitability of these monoliths towards separation of large analytes, such as proteins, and therefore possible application in proteomics.

The Importance of Voltage-Operated Calcium Channels (VOCC's) in Astrogliosis

Precious De Verteuil[1], Diara Santiago[2], Vilma Spreuer[2], Courtney Benson[2], Veronica Cheli[2], Pablo Paez[2]
[1]Department of Biology, Barry University, Miami Shores, FL; [2]University at Buffalo, Buffalo, NY

Abstract

Astrocytes are glial cells within the central nervous system (CNS) that provide trophic support of the CNS immune response. Under pathological circumstances, astrocytes respond via "astrogliosis". During astrogliosis, astrocytes exhibit changes in cellular morphology and protein expression profiles. Initially, astrogliosis is necessary to stop damage from spreading to multiple areas within the CNS; however, increased astrogliosis promotes the formation of scar tissue--preventing undamaged neurons from reestablishing connections post injury. In this study we hypothesized that L-type voltage-operated calcium channels (VOCC) expressed on astrocytes are essential for astrogliosis. We tested our hypothesis using two astrocyte cell lines that varied in expression level of VOCC. Initially, we mimic a bacterial infection by treating cells with the endotoxin lipopolysaccharide (LPS). Our results show that the astrocyte cell line with high expression of VOCC showed significantly greater expression of classical astrocyte reactivity markers (i.e. GFAP and S100β) than control cells after LPS treatment. Importantly, LPS treatment was unable to induce astrogliosis in the cell line with low levels of VOCC expression, suggesting that the presence of these calcium channels is essential for astrocyte activation. Furthermore, we treated cultures with LPS in the presence of verapamil, a specific VOCC inhibitor. As expected, the presence of verapamil in culture medium drastically reduces the number of GFAP and S100β positive cells. In summary, our results suggest that VOCC may play a fundamental role in induction of reactive astrocytes, and indicate that inhibition of these calcium channels may be an effective way to prevent astrocyte activation. (*NIH-NIGMS MARCU*STAR Grant, T34 GM008021-29, Barry University)

Chloroplast Isolation and Cloning of the Large Subunit of Rubisco of Sabal Domingenisis

S. Diaz, H. Rivera, M. Maduro, J. Vera, S. Ritter and A.J. Leon
Department of Biology, Miami Dade College North Campus, Miami, FL

Abstract

Native to the Southeastern United States, Mexico and the Caribbean Basin, Sabal genus' members thrive in Florida's subtropical climate. The majority of Sabals are not native to Florida. Home to approximately 250 different species of palms, Miami Dade College North Campus' Palmetum provides a large variety of Sabal palms to work with. The focus of this study is to identify differences in the gene that encodes for the large subunit of the enzyme rubisco (ribulose biphosphate carboxylase) between Sabal palms. Due to palms' characteristically fibrous leaves, optimization of chloroplast isolation was required because many protocols used plants that have fleshy leaves. Chloroplasts were isolated from Sabal domingensis using volumes of two sugar solutions, 0.3M sorbitol and 0.2M sucrose. Volumes of sorbitol and sucrose ranged from 20 ml to 80 ml. Data showed that the total milligrams of chlorophyll using sucrose peaked at 40 ml, and decreased using 80 ml. A similar trend was observed when using 0.2M sorbitol. Optimization of the centrifugation rates using 60 ml of sucrose increased total chloroplast yield 3 fold. Once the chloroplasts were quantified, DNA was isolated using DNeasy Plant mini kit (Qiagen) and subject to PCR. Preliminary results show an amplicon of 1.5 Kbs representative of the desired product and in accordance with other reported Rubisco genes for other palms species. Future experiments will include cloning using the TOPO cloning kit (Life Technologies) and sequence of the amplicon.

Angiotensin II Type 1 Receptor (AT_1R) Expression in the Amygdala of Fear-Conditioned Mice as an Animal Model of Post-Traumatic Stress Disorder

Elizabeth Goldberg, and Robert Speth, Ph.D.
Division of Math, Science and Technology, Nova Southeastern University, Ft. Lauderdale, FL

Abstract

Post-Traumatic Stress Disorder (PTSD) is an incapacitating psychiatric illness involving anxiety induced by traumatic events. Recent studies demonstrate that patients suffering from PTSD are at greater risk of developing hyperlipidemia, obesity, cardiovascular disease, hypertension, and adverse cardiac events. Despite these observations, the mechanistic basis for the association of PTSD with cardiovascular disease is unclear. Available treatments for PTSD are limited, prompting the need for further research in this field. One potential factor providing insight into this illness is the renin angiotensin system (RAS), a major cause of cardiovascular disease, and a facilitator of stress and anxiety. The RAS is classically defined as a circulating endocrine system that regulates several of the body's critical functions: blood pressure, flow dynamics, and fluid-electrolyte balance. This traditional view has been recently updated with studies evincing the role the RAS in neurodegenerative pathologies, founded in the observation of elevated plasma renin levels during stressful circumstances. Through a series of reactions, circulating renin generates angiotensin II (Ang II), a hormone which mainly functions through its interactions with AT_1R Ang II receptor subtype. The present investigation aims to document changes in AT1R expression in the amygdala of fear-conditioned mice as an animal model of PTSD using receptor autoradiography. The amygdala is a critical brain region for the learning and expression of fear responses. Fulfillment of this objective began with charting AT_1R binding in normal mouse amygdala, to be followed by study of AT_1R expression in fear-conditioned mouse amygdala to assess involvement of the brain RAS in PTSD.

Reversability of Zeaxanthin Aggregation

Daniel Gonzalez, John T. Landrum, Ph.D.
Department of Chemistry and Biochemistry, Florida International University, Miami, FL

Abstract

Introduction: Zeaxanthin, a natural carotenoid, has moderate solubility in organic solvents. The characteristic carotenoid UV-visible spectrum of zeaxanthin is centered at 450 nm. In mixed aqueous/alcohol solutions the absorption maximum shifts to 380 nm and is attributable to the formation of aggregates. This self-association is a model for the aggregation of carotenoids that occurs in some natural systems. Methods: Zeaxanthin was dissolved in isopropanol. Preparation of water/isopropanol mixtures produced solutions having a fixed zeaxanthin concentration but varying in the mole fraction of water. Spectra of the resulting solutions were recorded UV Visible spectrophotometer. Results: Aggregation of zeaxanthin was found to increase as the mole fraction of water, χ_{H20}, increased from 0.776-0.974. At $\chi_{H20} = 0.864$, zeaxanthin was found to be fully aggregated. As the temperature of the zeaxanthin solution, $\chi_{H20} = 0.864$, was increased, the 380 nm absorption of the aggregate decreased and the 450 nm absorbance of the monomeric carotenoid increased. Cooling this solution results in a loss of the monomeric 450 nm absorption band, is consistent with re-aggregation. Reappearance of the 380 nm absorbance band occurs only to a limited extent. Conclusions: Aggregation of zeaxanthin in isopropanol/water mixtures is a quasi-reversible process. At low concentrations, the card-stack H-aggregate does not readily reform during the time frames of the current experiments. An estimate of -9.5kJ/mole was obtained for the energy of dissociation for the card-stack aggregate in isopropanol/water mixtures, $\chi_{H20} = 0.864$.

The role of serotonin 1A receptors in cocaine induced hyper-locomotion

Douglas Kinnee-Crowley[1,2], Sunmee Wee, Ph.D. [1], Sherie Wright, Ph.D. [1], and In Jee You, Ph.D. [1]
[1]Scripps Florida Institute, Jupiter, FL
[2]Biotechnology Program, Palm Beach State College, Palm Beach Gardens, FL

Abstract

A genetically engineered mouse line was employed in this study, where transcription of the serotonin 1A (5HT-1A) autoreceptor gene in the dorsal raphe nucleus (DRN) was suppressed. This gene can be transcribed upon the introduction of doxycycline (DOXY) to the diet. The function of the 5HT-1A autoreceptor in the DRN on cocaine-induced locomotion was assessed in these mice, with and without DOXY diet. During experimentation, mice were weighed and numbered, then habituated both to the holding room and the testing chamber, to exclude novel environmental stress. Injections were prepared according to body mass (0.1 mL/g) of either saline or the stimulant drug, cocaine (7.5 mg/kg). Following injection (i.p.), mice were placed into the testing chamber and their locomotive response was measured for one hour via video-tracking software. On day one, all mice received a saline injection to identify a basal locomotive response, controlled for injection stress. Following this, mice received cocaine injections daily for 8 days where locomotive activity was recorded, followed by a 3 day withdrawal period. On day 15, mice received a final cocaine injection to induce a sensitized locomotive response. Whilst preliminary data did not show any significant changes in locomotive behavior over time, 5HT-1A receptor knockdown mice (DOXY off) showed increased cocaine-induced locomotive behavior versus wild-type (DOXYon), suggesting a correlation, however the knockdown mice are phenotypically more anxious. Testing values didn't differentiate far from control; this may be due to the strain used being known to resist cocaine.

Accelerating HIV Eradication by Defining the Contribution of Patients' Clinical, Biological, and Socio-Demographical Factors on the Size of the HIV Reservoir

Sergeine Lezeau[1,2], B.A., Rémi Fromentin[2], Ph.D., Franck Dupuy[2], Ph.D., Jessica Brehm[2], Ph.D., Jean-Pierre Routy[3], M.D., Steven Deeks[4], M.D., Moti Ramgopal[5], M.D., Rebeka Bordi[2], Ph.D., Nicolas Chomont[2], Ph.D., and Rafick-Pierre Sékaly[2], Ph.D.
[1]Florida Atlantic University, Boca Raton, FL, [2]Vaccine and Gene Therapy Institute, Port Saint Lucie, FL
[3]McGill University Health Centre, Montreal, QC., [4]University of California, San Francisco, CA
[5] Midway Immunology and Research Center, Fort Pierce, FL

Abstract

The HIV reservoir is a pool of HIV-infected cells that cannot be targeted by current antiretroviral treatments, thus enabling HIV persistence and hindering HIV eradication in treated patients. The size of this reservoir is defined by the number of cells carrying integrated, non-replicating, or latent forms of HIV. Factors that influence its size can vary in every patient and remain poorly defined. We proposed that latent HIV reservoir-size correlates with multiple factors that can affect immune function. We collected clinical, biological, and socio-demographical data sets from HIV-treated patients (n=336) and measured reservoir-size with HIV Integrated DNA PCR; we combined all of these data sets into our database. Using multivariate statistical analyses, we identified age, race, duration of infection, and the CD4 count measure of immune competence as correlates of reservoir-size for our cohort. Our results suggest that these factors may play an indirect role in reservoir-size determination. We plan to continue to increase our cohort size and to stratify our data sets to limit further confounding variables in the definition of these correlations. Defining significant correlates of reservoir-size could lead to the design of precise treatments that are adapted to these correlates, thereby accelerating HIV eradication with more effective treatments for HIV patients.

Natural Antisense Transcripts in the Mechanisms of Cocaine Addiction

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Department of Psychiatry and Behavioral Sciences
University of Miami Miller School of Medicine, Miami, FL

Abstract

Growing evidence indicates an important role of epigenetic regulation in the mechanisms of cocaine addiction. Chronic cocaine use causes long-lasting changes in gene expression in the brain's reward areas that contribute to persistent drug-seeking and drug-taking behaviors. Recent reports indicate that epigenetic processes are key factors in such neuroadaptations and behaviors. Thus, understanding epigenetic mechanisms of addiction is crucial to develop targeted therapeutic interventions. However, the majority of epigenetic regulators and associated mechanisms have yet to be investigated. Representing an unexplored yet promising addiction-related target, natural antisense transcripts (NATs), transcripts encoded on the strand opposite to the sense strand on either protein-coding or non-protein coding genes, have recently been shown by our laboratory and others to be key regulators of chromatin state. However, to date, NATs have not been studied in the context of addiction. Here, we investigated a role for NATs in cocaine addiction by injecting c57bl/6 male mice with cocaine (20 mg/kg, i.p.) or saline once a day for 10 days. The nucleus accumbens (NAc) and other brain regions were collected at multiple time points after the last injection. Of addiction-related genes investigated, many were found to have natural antisense transcripts, and the expression of several of these antisense transcripts was also found to be significantly altered following chronic cocaine administration. Thus, these data indicate that cocaine disrupts the expression of addiction-related NATs, and that NATs may play an important role in cocaine addiction. Future studies will determine the role of specific transcripts and the mechanisms of their effects.

Loss of Methionine Sulfoxide Reductase B Leads to Higher Failure Rates in Drosophila Melanogaster after Hyperthermic Stress

Syed Raza[1], Lindsay Bruce[2], and David Binninger, Ph.D. [2]
[1] Biology Undergraduate Honors Program
[2] Department of Biological Sciences, Charles E. Schmidt College of Science
Florida Atlantic University, Boca Raton, FL

Abstract

In response to hyperthermic stress, Drosophila enter a reversible protective coma called spreading depression. Methionine is a common amino acid in proteins that is easily oxidized by reactive oxygen species (ROS). Oxidized methionine can be restored to functional methionine by methionine sulfoxide reductase (Msr). The molecular basis of how Msr affects survival during hyperthermic stress is unknown. In this study I am using RNA interference (RNAi) to knock down expression of either MsrA or MsrB to better understand the role of these genes in response to hyperthermia. Results have shown that knockdown of MsrB expression in all tissues leads to a higher failure rate. However, specific knockdown of MsrB in just motor neurons did not show this effect. The reduced thermotolerance may be attributed to increased ROS, hence MsrB's anti-oxidative function may contribute to hyperthermic tolerance.

Development and Application of a Phosphorylated Peptide Enrichment Protocol for the Identification of Phosphorylated Proteins by LCMS

Catherina Scharager [1, 3], Ricardo Flefil [1], Graham M. West, Ph.D.1,
Sarbani Goshal, Ph.D.[2], Anutosh Chakraborty, Ph.D.[2], Alexandra Gorgevska, Ph.D.[3]
[1]Mass Spectrometry and Proteomics Core, The Scripps Research Institute, Jupiter, FL
[2]Department of Metabolism and Aging, The Scripps Research Institute, Jupiter, FL
[3]Biotechnology Program, Palm Beach State College, Lake Worth, FL

Abstract

Phosphorylation is a protein post translational modification (PTM) that plays an important role in many intracellular signaling pathways. Mass Spectrometry can be a powerful tool for the detection of protein phosphorylation as it requires no prior knowledge about the proteins involved and provides site-specific information, as opposed to antibody-based techniques which often provide protein- or region-specific information. However, many of these important regulating PTMs go undetected in a mass spectrometry experiment due to ion suppression by unmodified peptides that limit the detection of phospho-peptides using current proteomics platforms. Enrichment strategies enhance ionization and detection of the phospho proteome by removing interfering unmodified peptides and thus, provide an invaluable contribution to the study of many cellular processes. Here we present the development and application of a TiO_2-based phospho-peptide enrichment strategy to improve the detection of phosphorylated proteins using current proteomic platforms. The technique was initially developed using a model mixture of four peptide pairs (both phospho- and unmodified versions). Effectiveness of this enrichment was assessed using an area under the curve (AUC) approach on eluting peptides, to compare the ionization efficiency of the phosphopeptide ion signals before and after enrichment. The elution conditions were further optimized to improve recovery of the phosphorylated peptides. The technique has been applied to enhance the detection of the phospho proteome in a mammalian cell lysate and also to identify novel phosphorylation sites in IPK6, a potential target for diabetes therapeutics. Combining this phospho enrichment technique with "bottom up" proteomics has proved to increase the number of phosphorylated proteins identified by mass spectrometry analysis.

POSTER PRESENTATIONS

Transcriptome Analysis of the Entomopathogenic Oomycete Lagenidium Giganteum Reveals Putative Virulence Factors Shared by Fungal and Oomycete Entomopathogens

Sumayyah Abiff, Katrina Fins, and Aurelien Tartar, Ph.D.
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Abstract

The entomopathogenic oomycete Lagenidium giganteum is known to infect and kill mosquito larvae and, therefore, has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of L. giganteum in its mosquito host. In order to detail the molecular basis of entomopathogenicity, Expressed Sequence Tags (EST) were generated using 454 pyrosequencing. Homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins and revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that L. giganteum is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of oomycetes and fungi as control agents against insect pests, reducing the use of insecticides that can have negative impacts on the environment and human health.

Adsorption of Heavy Metal Ions by Chemically Modified Agricultural Waste Materials

Zoia Akhtar, Gabriel Pacareu, Jose Vettaparambil, and Isaiah Urhoghide, Ph.D.
School of Science, Miami Dade College North Campus, Miami, FL

Abstract

Heavy metals such as Lead, Copper, Cadmium, Arsenic, Chromium, Mercury, Cobalt, Nickel, Manganese, Tin, and Thallium have relatively high densities and are toxic or carcinogenic at low concentrations. Some heavy metals affect the central nervous system, while others affect the kidneys, liver, skin, bones, and teeth. Heavy metals are contained in waste water produced during mining and other industrial operations which could pollute streams and aquifers. For example, some pesticides used in farming contain Arsenic, which affects the central nervous system. Cadmium, which affects the kidneys, is used in batteries. Membrane, biological, oxidative, chemical, and adsorption techniques have been employed in removing harmful metal ions from industrial waste water. This research focuses on the synthesis and characterization of graft copolymers from agricultural waste materials that could be used to remove Arsenic (As), Nickel (Ni), and Manganese (Mn) ions from solution. Using ceric ion initiator in aqueous medium, Ethyl acrylate and Meth acrylic acid were each grafted onto carboxyl methyl-containing holocellulose obtained by treating corn cob-based holocellulosic materials with sodium hydroxide and sodium monochloroacetate. Ethyl acrylate and Methacrylic acid were also grafted onto corn cob pulp. The graft copolymers were characterized using FT-IR. The adsorption of As, Ni, and Mn ions were investigated using ICP-AES. The results obtained indicate that the percent ion absorbed per gram of copolymer depended on the polarity of the monomer, the polarity of the holocellulose, and the initiator concentration. 95%, 62%, and 61% of Arsenic, Nickel, and Manganese respectively were removed from solution by the copolymers.

Use of Drosophila Melanogaster Larvae to Evaluate Cardioactive Peptides

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Abstract

Conopeptides are found in the venom of marine cone snails, aiding in the paralysis of their prey, and have been shown to have potential therapeutic uses in humans. Conopressins are conopeptides that target vasopressin/oxytocin receptors in vascular smooth muscle cells, found within blood vessels. The crustacean cardioactive peptide (CCAP) is a homologous peptide found in crustaceans and has been shown to behave as a cardioaccelerator in a homologous system. This study describes the effects of CCAP in Drosophila larvae. We find that CCAP has an inotropic effect by causing a change in the contraction of blood vessels. We will further investigate the effects of another possibly cardioactive conopeptide, γ-conopressin-vil, in Drosophila larvae. Elucidating the effects of conopetides in Drosophila larvae may translate to cardioactive therapeutic uses in mammalian systems.

Mathematical Exploration of Humoral Immune Response to Repeated Viral Challenge

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Abstract

B cell and their antibodies provide an important line of protection from repeated challenges by pathogens. Vaccination strategies serve to prime virus specific antibodies, which are the best known biomarker of protection. This humoral immune response to viral infections is underexplored relative to cytotoxic responses. Our goal is to establish a minimal model of humoral immune response to influenza A virus to determine the pertinent dynamics of primary and secondary infections and humoral memory formation. We demonstrate the relative effectiveness of the humoral immune response in primary and secondary infection. We see in primary infections the humoral response is able to clear the infection; however, typically other mechanisms clear the infection prior to the full formation of the humoral immune response. In secondary infection, the humoral immune response clears virus before the other mechanisms can be invoked. For purposes of parsimony, we develop a reduced model of viral replication validated against the fitted model of Miao et al. We perform a variant of practical identifiability analysis to see that the model parameters can accurately recover from noisy data with small number of time points.

Identification and Characterization of Genetic Interactions between cdc13-1and yKU80 in Saccharomyces Cerevisiae

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Abstract

Telomeres are the physical ends of eukaryotic chromosomes that protect DNA ends from degradation and from end-to-end fusion. Telomeres consist of stretches of repeated C/G-rich DNA ending with 3' single stranded G-rich overhangs. The enzyme telomerase and accessory proteins such as Ku and Cdc13p maintain and facilitate telomere functions. In S. cerevisiae, cdc13-1 is a temperature sensitive allele of Cdc13p, an essential telosome protein that binds to single-stranded G-tails to prevent telomere degradation. The Ku heterodimer, composed of Ku70 and Ku80, functions in DNA non-homologous end joining, recombination and telomere end protection. Yeast cells lacking Cdc13p or the Ku complex have uncapped telomeres and long single-stranded G-tails. This study examines the effects of mutations in yKU80 on cdc13-1 strains. We introduced a library of 125 mutant yku80 alleles into the cdc13-1 background by plasmid shuffle and determined the effects on viability and telomere end protection of the various yku80 mutant alleles. We found that 30 out of 125 yku80 alleles tested increased the temperature sensitive phenotype of cdc13-1 strains, suggesting a telomeric end protection role for these mutant yku80 alleles. We are currently characterizing the telomere phenotypes of double mutant strains by Southern blot analysis. Initial characterization of cdc13-1, yku80 double mutant strains show that temperature sensitivity may be uncoupled from telomere shortening in cdc13-1, yku80 double mutant strains.
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Elucidating the Role of Sab during Adipogenesis-Induced Mitophagy

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Abstract

Mammalian cells degrade mitochondria by a process known as mitophagy. PINK1, Parkin, and the autophagy-related (Atg) proteins are involved in the regulation of mitophagy. Mutation of PINK1 or Parkin results in the accumulation of dysfunctional mitochondria, which is characteristic of Parkinson's disease. Recent preliminary data from our lab demonstrates that Sab, a mitochondrial scaffold protein that can regulate apoptosis, may also be involved in the regulation of mitophagy. Pre-adipocytes reduce mitochondrial density during differentiation into adipocytes and for this reason adipogenesis has great potential as a system for the study of mitphagy. After inducing pre-adipocytes to differentiate, it was discovered that at the onset of differentiation there was an increase in Sab expression followed by a gradual decrease as differentiation progressed. Subsequently, there was an increase in Atg following the increase in Sab levels. We propose that Sab-mediated signaling destabilizes mitochondrial membrane potential, which results in the upregulation of mitophagy components, including the Atg family of proteins. Based on the current data, we plan to examine our hypothesis that Sab functions in conjunction with PINK1, Parkin, and Atg to regulate mitophagy.

Phonoresponses of Females of a Moth with Two-Celled Ears to Different Acoustic Signals Emitted by Sympatric Insect Species

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Abstract

Syntomeida epilais (Arctiidae), the object of our study, is one of the moth species with two-celled ears that have been demonstrated to need acoustic communication for successful mating behavior. The aim of our study is to analyze if perched S. epilais females could discriminate the emissions produced by conspecific males from other ultrasonic stimuli present in their environment. We stimulated 20 virgin females with playback recordings of conspecific male and female signals and of emissions from another two-celled ear moth species (Empyreuma affinis). Stimulation signals were applied and recordings of phonoresponses from stationary S. epialis females were obtained outdoors during the hours of its mating behavior (3:30-6:30 AM). The responses were quantified by counting the number of modulation cycles produced by the female per applied stimuli and by measuring the latency to the first stimulus of the series. All 20 female phonoresponded to their conspecific male emissions, while 12 responded to their conspecific female emissions, 6 to the E. affinis female emissions, and only 2 to the male signals of E. affinis. After responding to the conspecific male emissions, we decapitated 3 S. epilais females. None of the headless females responded to any of the applied stimuli. This result differs radically from that obtained in another two-celled ear moth (Cycnia tenera), that continued to phonorespond headless. These results on the whole demonstrate that S.epilais females phonorespond preferentially to their conspecific male emissions, suggesting for the first time physiological adaptations for intraspecific acoustic communication in moths with two-celled ears.

Embedded Image Steganography

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Abstract

Image Steganography is the process of concealing an image within another, larger image and is considered an encryption technique. Generalized versions of the application enable the encryption of various forms of data such as text messages, files, and images. We have implemented an embedded design for the image steganography problem on a Spartan-6 programmable device. Embedded systems are dedicated to specific tasks and can be optimized to reduce the size and cost of the product and increase its reliability and performance. The design is coded in the VHDL hardware description language and the software platform used is Xilinx Webpack. During the encryption process, least significant pixels bits of the host image are replaced by pixels of the hosted image. Decryption follows the reverse process. Replacement of the least significant pixel bits yields images that are undetectable to the naked eye. Pixel mapping is performed randomly to prevent detection during cryptanalysis. A Fibonacci LFSR (linear-feedback shift register) produces the desired non-repeating random number sequence. Images are stored in DDR (double data rate) memory of the programmable device. The entire process is tested on an Atlys Spartan-6 FPGA board. Data are transmitted from/to the FPGA using a simplified USB protocol at an approximate rate of 48 Mbps. A graphical user interface allows for FPGA board initialization and programming as well as the selection of images, encryption/decryption, and bi-directional data transmission.

Using an Engineered Trojan Horse to Kill Nematodes

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Abstract

Infections due to parasitic nematodes result in nearly 125000 deaths annually. Strikingly, this rate remains nearly unchanged in the past 50 years likely owing to the fact that treatment options are either inefficient or inaccessible. Prior to infecting humans, most parasitic nematodes begin as larvae where they feed nearly exclusively on bacteria. This unique property may offer an opportunity to develop new biological control agents with the use of synthetic biology. In this study, we aim to develop gene circuit components that may be used to engineer Escherichia coli to act as a biological control agent of the model nematode Caenorhabditis elegans. We independently characterize two genetic modules: an attraction module and a killing module. The attraction module consists of genes that produce acylhomoserine lactones, which serve as natural attractants of C. elegans. The killing module consists of an inducible promoter that drives the expression of a toxin gene, cry5B. We independently characterize the response of C. elegans to each of these modules towards the ultimate goal of implementing and optimizing the function of both modules together in a single strain of E. coli. As such, our study establishes a quantitative framework for using these modules, and ultimately engineered bacteria, as a robust biocontrol agent for nematodes. * denotes equal contribution

Effects of Heat Treatment on Gene Expression of Several Heat Shock Proteins in Two Strawberry Cultivars

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Abstract

Xanthomonas fragariae causes angular leaf spot (ALS) in strawberry, which primarily affects the foliage; however, the bacterium can move systemically through the plant's vascular bundle. Heat treatment (HT) has been shown to be an effective method for reducing systemic pathogens but the process often has adverse effects on plant health. Research has shown that a brief HT at lower temperature prior to the main HT can induce heat shock proteins (HSPs) in plants, which serve to protect the plant from damage when treated at higher temperatures. The objective of this study was to determine the gene expression of ten HSP genes in two strawberry cultivars (Festival and Ventana) known to have differential tolerance to heat and included: Hsf-1 (2 genes), Hsp90 (1 gene), Hsp70 (2 genes) and sHsps (5 genes). Strawberry plants were heat treated at 37 ^{0}C for one hour to induce the heat shock response. RNA was extracted from the treated plants and control plants and qRT-PCR was used to determine the gene expression of the ten target genes encoding HSPs. Several HSP genes (one Hsf-1 and three sHsp) were up-regulated at a significantly higher fold in Festival, but only one gene, sHsp15.96, was expressed at significantly higher fold in Ventana ($P<0.05$). Results have identified genes that may confer heat tolerance in strawberry, which may be useful for selecting heat tolerant plants in breeding programs. However, additional research should quantify HSPs induced by HT at the protein level and confirm functions of these genes through "knock out"/overexpression studies.

Molecular Modeling of the Structure of Histidine Ammonia-Lyase as a Function of pH and Temperature

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Abstract

Histidine Ammonia lyase (HAL), or Histidase, is a cytosolic enzyme that catalyzes histidine into ammonia and urocanic acid. HAL is important for regulating the acid mantle and UV exposure in human skin. To complement experimental data of the effect of pH and temperature on HAL activity, we are modeling the geometric and protonation state changes at the active site. We use PROPKA, an algorithm that assigns protonation states for each atom at specific pH values, and PDB2PQR, a program that implements the PROPKA algorithm to convert a protein database file into a protein charge/radius file. We then predict the protonation states of the amino acid side chains. NAMD (Not just Another Molecular Dynamics program) is software used for the quantum mechanical and electrostatic force calculations, and the software VMD (Visual Molecular Dynamics) is used for 3D graphical representation. We are presently resolving an unusual residue found in the active site: three amino acids, alanine, glycine and serine, have bonded together forming a residue with an imidazole ring (MDO). We are currently obtaining correct electrostatic topology for the MDO/MIO residue with the Gaussian 09 software, and our results thus far include protonation states and overall charge.

The Development of Parvalbumin-Positive Neurons in Ferret Visual Cortex

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Abstract

Experience plays a critical role in maturation of cortical circuits. In visual cortex, experience-dependent development has been linked to the maturation of inhibitory interneurons. Parvalbumin-containing (PV^+) interneurons, a subtype of interneurons that target synapses onto neuronal cell bodies, play an important role in cortical circuit function. It remains unknown how visual experience shapes the organization of this subtype of GABAergic interneurons. To study the maturation of inhibitory interneurons, we used immunohistochemistry to observe the organization of PV^+ neurons in visual cortex at the onset of visual experience. Before visual experience, PV^+ cell bodies and processes are most prominent in layer 5, with some cell bodies and few processes in layer 2/3. Layer 4 generally lacks PV staining, and had less GABAergic synapses. Within 3 days of the onset of visual experience, PV^+ organization undergoes a major shift, with PV^+ cell bodies, processes, and soma-targeting synapses found throughout layers 2-6. To determine if these morphological changes are due to visual experience, we performed dark rearing that showed PV morphology remains highly laminar, unlike our "mature" pattern. This rapid change in parvalbumin organization may play a role in functional changes associated with the onset of visual experience.

Enhanced Growth of Tetracycline-susceptible Soil Bacteria when Stimulated with $FeCl_3$ and Oxytetracycline

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Abstract

Antibiotic resistance has become an important area of research because of the excessive use of antibiotics in clinical and agricultural settings driving the evolution of antibiotic resistant bacteria. However, microbial ecologists have found that antibiotic resistance is a naturally occurring phenomenon in soil communities, and often parallels with heavy metal resistance. Many of the same mechanisms, such as efflux pumps, are used to resist antibiotics and deal with heavy metal toxicity, thus the link between the two phenomena. Iron is considered a heavy metal but it is also an essential metabolic component and can regulate gene expression, yet few studies have shown iron's impact on the soil resistome. For this study, soils were collected from two locations in Miami Dade County and bacteria isolated. Antibiotic susceptibility was evaluated using the Kirby-Bauer Disk Diffusion test and selected isolates were identified using 16S rDNA sequencing. Growth responses to $FeCl_3$ and Oxytetracycline HCl were measured on 96-well microplates. Data have shown that the tetracycline-susceptible bacterial isolates, when presented with equal concentrations of $FeCl_3$ and oxytetracycline, did not have depressed growth. Instead, isolates showed improved growth relative to non-treated controls even with high oxytetracycline concentrations. Iron can regulate the expression of the Fur protein, a global regulator protein known to control siderophore production, reactive oxygen species resistance, and control of efflux pumps, all mechanisms associated with resistance. Ongoing studies to evaluate the influence of Fur on antibiotic resistance will use RT-qPCR to measure gene expression of fur and other antibiotic-resistance genes, such as tet.

Probing Sequence and Topological Specificity in the Binding of TetraN (methylpyridyl) Porphynes to DNA

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Abstract

Pyridyl porphyrins have gained attention as potential antitumor agents with anti-inflammatory properties. In this study we report the different binding affinities of tetra (methylpyridyl)porphine to relaxed and supercoiled circular PhyX174 RF DNA. Binding by this DNA was probed by using restriction endonuclease activity assays employing six restriction enzymes selected for different cleavage and flanking sequences. The restriction enzymes used were XhoI, Alw44, DraI, MluI, PstI, and StuI. The restriction enzyme assay indicate that placement of the methyl substituent alters the sequence and topological specificity of binding. Variation in the sequences flanking the restriction enzyme cleavage site affects the selectivity of the binding. Molecular modeling suggest that the methyl substituent on the pyridyl rings affects the planarity of the molecule, which may alter the binding to DNA and lead to the above observation.

The Characterization of Nitrazine Yellow as a Photoacoustically Active pH Indicator Dye

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Abstract

The photoacoustic effect results primarily from a photothermal mechanism, in which photons are absorbed by solvent molecules, converted into heat energy (resulting in a local volume expansion), and released as a pressure (or sound) wave. Previously, the photoacoustic effect has been used for studying fast reaction kinetics or long pathlength absorption measurements in gases. Within recent years, the photoacoustic effect has been utilized in biomedical imaging as it 3-dimensional imaging in turbid media. Hemoglobin has been used for non-invasively acquiring in vivo images of vasculature without the need for exogenous labels, and more recently, measuring oxygenation levels in blood vessels. Although some exogenous dyes have been demonstrated to bind to targeted proteins, the library of dyes still lacks the expansive capabilities seen in more established modalities. Our work demonstrates that the dye Nitrazine Yellow (NY) exhibits the following characteristics and is suitable for photoacoustic imaging. NY sustained photostability over a functional number of laser pulses without resulting in the creation of dangerous or unwanted by-products (e.g. reactive singlet oxygen). Additionally, NY consistently followed a direct path of heat deposition, which allows for accurate imaging. Finally, NY demonstrated desirable features for human use (i.e. rapid heat deposition, maintenance of solubility over a range of conditions). The characterization of NY as a photoacoustically active dye should allow researchers to produce non-invasive in vivo three-dimensional images of lymphatic cancers (e.g. breast cancer) or select other objects in the lymphatic system. As a pH indicator, NY also allows for simultaneous imaging and pH testing.

Tracking GUS Gene Activity in Tobacco Plants

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Abstract

The p-glucuronidase (GUS) gene was isolated in 1986 from the bacterium Escherichia coli (E. coli). Since then, due to the blue pigment it produces, it has been widely used as a reporter gene in genetically modified organisms serving to study gene expression and tissue specificity. The focus of this study is to use the GUS gene to analyze the gene expression and tissue specificity of Agrobacterium-mediated genetically modified plants. Agrobacterium is a pathogenic bacterium that has the ability to integrate a part of its DNA into a plant's DNA. The tobacco plant has been selected as a model for the study. The GUS gene was introduced into the plants using Agrobacterium tumefaciens. The plants that were confirmed to be expressing the GUS gene were grown to propagate a new generation (Tl). These Tl plants were analyzed for tissue specificity of GUS expression. Plant lines that exhibited specificity or a mutation were isolated to analyze the degree of expression, and location of the GUS gene in the plant's DNA. Results to date seem to indicate some variation in tissue specificity of GUS expression between plant lines. Specificity implies that not all the cells are producing the GUS gene product. Identifying the location of insertion will help define the mechanisms of GUS gene integration in the plant genome and possible effects on its genomic structure.

Comparison of Chaperone Tail Proteins in Subcluster K5 Mycobacteriophages

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Abstract

Bacteriophages, bacteria infecting viruses, are the most expansive and diverse organisms currently known to man. Their importance in modern scientific research encompasses having high potential for scientific use, including phage therapy against multi-drug resistant bacterial pathogens, food safety regulation, and alternative vaccine delivery. Phages have an extremely condensed genome, due to viral capsid physical packaging constraints—around 60 kilobase pairs with many genes of unknown functions. Overall genomic architecture tends to be similar across phage genomes, with "cluster" grouping organized according to genomic similarities. This project aims to identify how a K5 subcluster mycobacteriophage, isolated on FGCU campus, Omnicron, deviates from other cluster K phages and determine its evolutionary direction. Specifically, this study aims to identify the importance of the typical frame shift mutation in the gene product occurring directly upstream from the tape measure protein (TMP) in various phages, often called the tail chaperone protein. The TMP determines the length of the phage tail, which is used in host infection. In approaching the question of how subcluster K5 phages evolved from other cluster K phages and the evolutionary importance of the tail chaperone protein, similarities and unique qualities amongst K5 phages were investigated using Seaview, a bioinformatics program. The 'Pham Circles' and 'Gene Map' features of the program Phamerator were used to generate data and graphical output for various inquiries. Determining the evolutionary importance of the tail chaperone protein is significant in understanding how phages infect their hosts. Mycobacteriophages that belong to subcluster K5 are capable of infecting M. tuberculosis, an important human pathogen. Understanding how these phages evolved may lead to new insights into TB treatment.

The Synthesis and Characterization of Collagen Type II Derived Peptides for Epitope Mapping in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease that results in the degradation of collagen type II, found in the cartilage of the joints. The objective of this research was to synthesize and characterize a series of peptides derived from collagen type II to identify new antibody binding sites found in rheumatoid arthritis. By finding new epitopes in collagen type II we can advance our knowledge of rheumatoid arthritis development and ultimately prevent it by designing competitive inhibitors to prevent the degradation of the cartilage. To test new epitopes a number of triple helical (branched) peptides with general sequence $(GPO)_5$-AAA-$(GPO)_5$, where AAA represents 25 amino acid active region, were synthesized and characterized. The synthesis of the triple helical proteins was completed using Fmoc based solid phase peptide synthesis using automated peptide synthesizer and microwave conditions. To imitate the same staggering effect observed in natural collagen a specific Dde protection group (N-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl)) was employed. After synthesis the peptides were purified using preparative RP-HPLC system and analyzed using analytical HPLC. Further characterization was completed using trypsin digestion coupled with HPLC/MALDI-TOF mass spectrometry analysis and circular dichroism (CD) spectra to verify the triple-helical structure and stability of the peptides. This study demonstrates that the synthesized collagen-like peptides exhibit a polyproline type II structure, have a melting point of about 40°C, and a molecular mass of about 15kDa. The synthesized peptides will be further tested by our collaborators in Karolinska Institute (Sweden) for antibody binding using samples derived from RA patients.

Differences in Alleles of the APETALA1 Gene do not seem to be Responsible for Differences in Development among Varieties of Brassica Oleracea

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Abstract

Understanding the role of genes in plant development may result in crop improvement and be highly valuable in agriculture. Our goal is to identify the genes that are responsible for the arrest in flowering in cauliflower (Brassica oleracea var. botrytis). This arrest results in the production of the edible curd. Rbo, rapid cycling B. oleracea, flowers normally and does not produce a curd. In the current study we seek to determine which genes are responsible for the developmental differences between cauliflower and Rbo. The APETALA1 (AP1) gene has been shown to be involved in flowering in many species. Previously published results had identified differences in the sequences of two copies of the AP1 gene, AP1a and AP1c, among varieties of B. oleracea. Our hypothesis was that differences in AP1 between cauliflower and Rbo might contribute to differences in phenotype. To test our hypothesis we are determining the genotype of AP1a and AP1c in cauliflower and Rbo. We are cloning these genes from both varieties by using PCR and primers corresponding to highly conserved sequences in AP1. The deduced amino acid sequence for the first exon of AP1c from our cauliflower cultivar is identical to that previously deduced for the broccoli and kale genes. This result suggests that AP1 may not be responsible for the phenotypic differences among varieties as hypothesized. We are currently working on cloning and sequencing the AP1a and AP1c genes from Rbo to confirm that differences in AP1 alleles do not contribute to curd formation in cauliflower.

A Quantitative and Qualitative Analysis of Biofilm Production by S. Aureus

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Abstract

Biofilm formation is a survival mechanism some bacteria have evolved to adapt to host defense systems and harsh environmental conditions. As such, biofilms contribute to bacterial pathogenicity. A crucial component of biofilms is the polysaccharide matrix, which allows adherence of bacteria to surfaces. The focus of this study was to explore different qualitative and quantitative methods to analyze the biofilms formed in static cultures of Staphylococcus aureus, a clinically important pathogen. Biofilm formation in DIFCO nutrient and thioglycollate broths were examined and compared via microtiter plate assays and microscopy. A crystal violet staining method determined that only 24 hours was required for biofilm formation and that the thioglycollate broth promoted biofilm production. Live dead staining and fluorescence microscopy were also utilized to visualize cells within the biofilm matrix. Additionally, to determine the presence of a polysaccharide matrix, S. aureus biofilm cultures in a microtiter plate assay were treated with diastase. Samples treated with diastase showed less biofilm in by crystal violet staining and by microscopy than untreated controls. These data suggest that under our laboratory conditions S. aureus produces a polysaccharide rich biofilm matrix. We aim to use these methods to enable undergraduate research students to examine the biofilm inhibiting properties of various chemicals.
* denotes presenter.

Spectroscopic Detection of Metal Cations with a Novel Small Molecule Sensor

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Abstract

In recent research, the development and improvement of chemosensors capable of identifying metal cations in aqueous solutions has seen applications in biological, medical, and environmental fields of study. While most chemosensors utilize methods involving complex formation, supramolecular cages, metal coordination, and pi bond stacking to sense metal cations, this research will utilize pi-cation interactions between the organic compound benzo[1,2-b:4,5-b']dithiophene-4,8-dione ("dione") with K^+, Na^+, Ca^{2+}, and Mg^{2+} cations in a variety of solvents (water, methanol, acetonitrile, DMF, etc.). The interactions between the organic and metal cations will be studied using UV-Vis spectroscopy and titrations to ascertain the organic dione's selectivity for metal cation recognition. After determining the dione's selectivity for each metal species, concentration detection ranges will be determined. Crystallization experiments will be performed as a means to quantify the magnitude of these pi-cation interactions. Future work will include the ability of the dione to sense various transition metal cations that are detrimental to human health such as As^{3+}, Pb^{2+}, Hg^{2+}, and Cd^{2+}. Since there will be no bond formation between the dione and metal cations, the dione can be easily reused. Furthermore, this research utilizes concepts taught in undergraduate chemistry studies and could be used as a learning lab for undergraduate chemistry students to incorporate their knowledge of chemical concepts in a laboratory setting. As for the progression of the research, the spectroscopic studies of the dione species are currently underway.

Attachment Site Determination for Bacteriophage Omnicron Genome Integration

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Abstract

Bacteriophages, viruses that infect bacteria, can undergo a lytic or lysogenic life cycle. All viruses attach themselves to bacteria and inject their genomes in order to hijack the cell's machinery for their own replication. In the lytic cycle, generation and assembly of progeny viruses result in the destruction of the host and the infection of neighboring bacteria. Lysogenic phages, however, work at a much slower pace. They integrate their genomes into the host's genome. The integrated phage DNA is called the prophage. In order for the prophage to successfully integrate its DNA into its host, the phage requires the cooperation of a viral enzyme, an integrase. The integrase mediates site-specific recombination between two strands of DNA – the phage genome and the bacterial genome. It does this by identifying two attachment sites that signal where the DNA will start the integration: the attP, the phage attachment site, and the attB, the bacterial attachment site. The lysogenic mycobacteriophage Omnicron, isolated in 2013 from a soil sample at Florida Gulf Coast University, is a subcluster K5 mycobacteriophage. K5 phages are unique in that they can infect Mycobacterium tuberculosis (M. tuberculosis), an important human pathogen, in addition to the nonpathogenic laboratory strain M. smegmatis. To gain a better understanding of how Omnicron can affect and potentially manipulate it's bacterial hosts, we identified and investigated the attP and attB sites. Results of this study will provide insights into the recombination events that occur during prophage integration in the viral lysogenic life cycle.

Fitness Consequences of Female Mating Strategies in Drosophila Arizonae

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Abstract

Understanding the fitness benefits of female polyandrous mating is a widely debated subject in evolutionary biology. The costs of mating are known to be substantial in many species, yet promiscuous females gain few obvious benefits. Theory predicts that multiply mating females may receive unnoted benefits that may be indirect or genetic. Here, I investigate several potential fitness costs and benefits of multiple mating in the fruit fly Drosophila arizonae. This cactophilic species was chosen as a model because of its rapid female remating frequency and the dearth of knowledge about fitness benefits and costs of female remating. I measured the survival and lifetime reproductive success in females experiencing different mating strategies, including virgins, once-mated, monogamous, and promiscuous. Female flies (N=120) were randomly assigned to each of these four experimental groups. Monogamous and promiscuous females were exposed to males for a minimum of 2 hours daily for 11 days. Monogamous females had access to the same male each day; promiscuous females were given new males. Fly longevity and lifetime reproduction until death were monitored in vials changed daily during remating and later in cages with fly media changed every two days. Subcomponents of reproductive success were also measured, including egg hatch rate, fecundity, and pupa viability. I explore how the number of matings affect female survival and how the four mating strategies affect these fitness components. Hatching success is higher for both remating groups than for once-mated flies, while offspring production is higher in the promiscuous group than the monogamous group.

Phylogenetic Analysis of Co-Diversification between the Bacterial Order Oceanospirillales and Anthozoans

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Abstract

Associations between marine organisms and prokaryotes have been widely studied. One of these associations is the interaction between anthozoans (mainly reef-building coral species) and bacteria. One order of bacteria, Oceanospririllales, has been shown to compose a large portion of the bacterial consortium associated with many species of corals. This study analyzed the co-diversification patterns of Oceanospirillales between coral species through a culture independent method. We used 16S ribosomal DNA sequences for inferring phylogenetic relationships among the different identified strains of Oceanospirillales from both hard and soft corals. Sequences were collected from scientific literature relevant to Oceanospirillales and used to construct Maximum Likelihood and Bayesian Inference phylogenetic trees. The resultant grouping of species demonstrated a high species selectivity pattern, which suggests there is non-random assortment of Oceanospirillales depending on the coral species, additionally clades appear to be separated by a predominant coral species. Furthermore, there is a distinctive separation between hard and soft corals, which may support the hypothesis of co-diversification at higher taxonomic level.

Biochemical and Morphological Characterization of Presynaptic RIM-Binding Protein (RIM-BP) 1/2 Double Knock-Out Mice

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Abstract

Neuronal communication in the brain begins in the presynaptic terminal from where chemical neurotransmitters are released into the synaptic cleft in a calcium-dependent manner. The presynaptic terminal has been previously associated with key biochemical mutations involved in a wide array of neurological disorders. Autism Spectrum Disorders have been shown to be associated with a key active zone protein, RIM-Binding Protein (RIM-BP). In order to assess the biochemical and physiological function of RIM-BP in mammals, knock-out mice for RIM-BP 1/2 were generated. Polymerase Chain Reaction (PCR), Western blot techniques and ^{25}Iodine radioactive quantification provided insight into the activity of synaptic proteins. Transfection and fluorescent microscopy techniques were used to evaluate the general morphology of hippocampal cultures, thus obtaining a preliminary basis for the role of RIM-BP in neuronal development. Immuno-detection and radioactive quantification demonstrated that the concentration of synaptic proteins involved in synaptic vesicle docking/fusion did not significantly change between the mutants and the wild-type mice. Presynaptic calcium channels were also evaluated and the mutants did not demonstrate any up or down-regulation of these complexes. In addition, soluble and insoluble fragments of tissue homogenate were used to detect altering concentrations of the active zone proteins; however, no significant difference was seen. Visualization of hippocampal neurons did not reveal gross morphological changes in mutant neurons. These results suggest that both neuronal morphology and the biochemical composition of nerve terminals from RIM-BP DKO mice are normal. These studies are the basis for future physiological studies aimed to dissect the functional role of RIM-BP. This work was supported by NIH/NIGMS T34 GM083688 and the Howard Hughes Medical Institute (HHMI). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or HHMI.* denotes first author

Investigation of the Wound Healing Process of Adult Danio Rerio Wildtype Zebrafish

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Abstract

Danio rerio (zebrafish) share many physiological and genetic characteristics with humans, making them an attractive model system for scientific research. Zebrafish have been shown to completely regenerate significant portions of heart, fin, and tail tissues without loss of function or formation of permanent scar tissue. However, zebrafish have not yet been established as a model to study regeneration in skeletal muscle and surrounding tissues. Specifically, the healing response following deep tissue burn puncture wounds has not yet been described. We hypothesize that zebrafish should completely regenerate skeletal muscle and surrounding tissues in response to this type of injury—making them an interesting wound-healing model. Our investigations have focused on determining the length of time necessary for zebrafish to fully recover from deep tissue burn puncture wounds. We first standardized methods for introducing a hot puncture wound completely through the myotomal muscle of the zebrafish, one millimeter below the dorsal fin. Healing was tracked by photographing wounded fish daily until healing appeared complete upon gross examination. Our results indicate that deep tissue burn puncture wounds require thirty days to heal, with minimal to no external scarring visible. Our preliminary results support the use of zebrafish as a model to investigate molecular regeneration processes following deep tissue burn puncture wounds. Future studies will include time point sampling for analysis of the wound healing process at tissue and molecular levels. Findings could translate into applications for treatment of burn puncture wounds to skeletal muscle in humans, such as those inflicted during military combat.

An Identification and Comparison of Intestinal Parasites found within Gopherus Polyphemus at Two Differing Southeastern Florida Habitats

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Abstract

Gopherus polyphemus is a keystone terrestrial reptile, currently threatened in Florida due to habitat destruction. Overcrowded populations are associated with higher infectious disease transmission. Parasite roles within wild tortoise populations are largely unknown, despite growing evidence they may pose significant health risks. There is a large gopher tortoise population in the fragmented, poorly maintained Florida Atlantic University Preserve (FAUP). A separate Palm Beach County population is at Jonathan Dickinson State Park (JDSP) in a higher quality habitat. We hypothesized there would be higher intestinal parasite loads in FAUP tortoises than JDSP tortoises, due to overcrowding. Fecal flotation, sedimentation, cultivation, and a modified acid fast were used to determine parasite species and infection intensities. Fecal flotations revealed moderate to high Strongyle and Hookworm intensities in FAUP tortoises; in contrast, low Hookworm and moderate Strongyle intensities were seen in JDSP tortoises. Acid fast stains with FAUP samples revealed low numbers of organisms that may be Cryptosproidium. Preliminary crowding measurements of 5 random 50 x 50 meter quadrats in FAUP show an estimated 7.36 tortoise burrows per acre on average. If this correlates to actual tortoise numbers, this is much higher than the suggested 4 tortoises per acre limit for sustaining a healthy population. Species identification is ongoing, as it has been suggested Cryptosporidium may be an underlying problem in tortoises. This novel comparison of infection intensity to degree of crowding will be important in improving management strategies of these threatened species.

Regulation of the Mesenchymal Phenotype of Breast Cancer Stem Cells through the Adrenomedullin Pathway

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Abstract

Breast cancer stem cells are highly tumorigenic cancer stem cells that are resistant to chemotherapy and radiation and are known to cause tumor recurrence and metastases. According to recent evidence, the immune system induced epithelial to mesenchymal transition (EMT) is capable of generating the breast cancer stem cells. Adrenomedullin is an angiogenic peptide hormone that regulates the initiation of tumors and maintains the mesenchymal phenotype of breast cancer through PI3K/Akt/GSK3-β pathway activation. Adrenomedullin's stimulatory effect is mediated by its heterodimeric receptor, RAMP3/ CRLR. The aim of this study was to determine if adrenomedullin enhances the aggressiveness of tumor cells. This was done by first measuring the amount of adrenomedullin receptors found in the parental epithelial line (MMCs) versus the EMT induced breast cancer stem cells (ANV5's). Then migration and invasion was used as a measure of aggressiveness in the ANV5 cell line. Transwell methods were used with adrenomedullin as the chemoattractant. The conditions were no adrenomedullin added (control) and recombinant adrenomedullin added. The results showed that ANVs had an elevated expression of adrenomedullin's heterodimeric receptor, RAMP3/CRLR (ADMR) when compared to the MMCs. Also, when exogenous adrenomedullin was added, the cells showed more migration and invasion than cells with no adrenomedullin added. Therefore, adrenomedullin is important for migration and invasion of tumor cells and adrenomedullin signaling plays a key role in the maintenance of the mesenchymal phenotype of tumor cells.

Photosynthetic HCO_3^-- Use Mechanisms in Halimeda Discoidea under Ocean Acidification

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Abstract

Ocean acidification (OA) is increasing total dissolved inorganic carbon (DIC) in the oceans. This study examined DIC-use in Halimeda disoidea, a dominant, green, calcareous macroalga and biogenic sediment producer that is ecologically relevant in lagoon and coral reef ecosystems. It has been shown to use HCO_3^- for photosynthesis; however, the specific mechanism(s) of HCO_3^- assimilation remains unknown, creating difficulty predicting photosynthetic responses to OA. Inhibitors were used to identify which HCO_3^- mechanism is employed by H. discoidea. An external carbonic anhydrase (CA) and at least one internal CA important for photosynthesis were identified due to reduced photosynthetic rates after exposure to the inhibitors relative to uninhibited rates. Furthermore, enhanced photosynthetic rates at relatively low pH indicate that H. discoidea will increase CO_2-use and likely up-regulate HCO_3^--use through rapid CA-mediated dehydration under OA.

Role of Wnt Pathway in Determining Axial Alignment of Hindbrain and Spinal Cord Regions Relative to Mesodermal Tissue in Zebrafish

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Abstract

Vertebrate central nervous system regionalization culminates with the partitioning of the caudal neuroectoderm into anterior hindbrain and posterior spinal cord territories. While our previous work has identified Cdx transcription factors as essential for spinal cord identity specification, what remains unknown are the signaling factors regulating Cdx transcriptional domain. Using zebrafish as a model, we have investigated the regulation of one Cdx family member, Cdx4, by the signaling factor Wnt. To determine if Wnt is necessary for regulating the anterior boundary of Cdx4 expression, we blocked the Wnt canonical pathway by inducing global overexpression of the inhibitor Dkk1. Temporal control was achieved by using a transgenic line containing a heat shock inducible Dkk1 gene. When inhibition of the Wnt pathway was initiated before gastrulation, the boundary of Cdx4 transcription relative to surrounding vertebral precursors remained unchanged, suggesting that neural and mesodermal tissue alignment was not affected. However, this manipulation caused a reduction in size of the caudal hindbrain territory. Critically, inhibition at mid-gastrulation stages showed no such reduction, suggesting that Wnt specifies hindbrain size during the first half of gastrulation. Current work includes analysis of Wnt deficient hindbrain patterning as well as rescue experiments with Wnt activators. Understanding the mechanism of hindbrain and spinal cord regulation by Wnt is important for elucidating how the neuroectoderm is patterned and aligned to mesoderm tissue during early embryogenesis. This information builds on and incorporates our understanding of proper neurodevelopment in vertebrates and provides new insight into how proper tissue regionalization is affected by cell signaling pathways.

Pruritogen Actions in Drosophila Melanogaster: A Novel Model to Study Itch

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Abstract

Itch (pruritus) affects all vertebrates and can be spontaneously elicited or induced by numerous distinct stimuli. Several diseases as well as infections, insect bites and stings, and side effects of medication can elicit pruritus. Pruritus has not been documented in invertebrates; we are thus investigating if Drosophila melanogaster have an itch response by testing their response to several known pruitogens (agents that provoke itch). Pruritogen solutions were fed to starved male flies and their subsequent behaviors were analyzed for increased scratching or grooming. Preliminary data suggests that there is a response with histamine and compound 48/80, but not capsaicin and chloroquine. Thus, flies appear to exhibit itch responses to specific pruritogens. With development of a new, inexpensive model to study pruritus we can effectively characterize pruritus pathways and ultimately develop novel therapies to better treat patients with chronic pruritus that currently have no viable treatments.

Neural Stem Cells Proliferate in Vascular Niches in the Adult Mayan Cichlid Brain

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Abstract

The birth of new neurons (neurogenesis) has been observed in all adult mammalian brains examined to date. Adult neural stem cells are located close to blood vessels in each of the two neurogenic regions observed in all mammals. A regulatory role for blood vessels has recently been suggested as a mechanism to regulate neurogenesis. Postnatal neurogenesis has been reported in several regions of the fish brain including the olfactory bulb, dorsal zones of the telencephalon, hypothalamus, and divisions of the cerebellum; however, little else is known about neurogenesis in the adult fish brain. Using thymidine analog incorporation assays, cell death assays, and immunofluorescence, our preliminary observations suggest that neural stem cells in the adult Mayan Cichlid brain proliferate in vascular niches and migrate along blood vessels prior to maturing. We are currently examining the cell cycle kinetics in the different neurogenic brain regions. We have found neural stem cells migrate away from the vascular niches in Edu^+ cells on day 14 compared to those cells which are clustered in close proximity to those same vascular niches at day 1. We have found the most dense neural stem cell regions in the intermediate cerebellum and cerebellum and will quantify cell distances from vascular niches over time versus how many of those cells are proliferative versus migrating or maturing stem cells. We expect to find significant migration from day 14 to day 28 when the neural stem cells migrate away from the vasculature as they mature.

Generating Genetic Tools for Studying the Role of Oxidative DNA Damage in KRAS-Driven Lung Cancer Cells

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Abstract

Reactive Oxygen Species (ROS) are natural by-products of aerobic respiration and have significant roles in mitogenic and survival cell signaling. However, activated oncogenes in tumor cells have proven to produce excessive amounts of ROS in mammalian cells. The accumulation of high levels of ROS is shown to have deleterious effects on DNA such as base alterations, mutations, and strand breaks. The proper function of DNA damage repair proteins is essential to prevent the accumulation of such damaging effects on DNA. In oncogene-driven cancer cells, compromising such repair pathways may enhance tumor suppressors responses. Our laboratory has shown that high levels of the oxidative DNA damage product, 8-oxoguanine, can affect proliferation of cells carrying the most common oncogene, KRAS. Our preliminary data suggests that DNA damage repair proteins that detoxify 8-oxoguanine in the nucleotide pool or repair it via the base excision repair (BER) pathway may protect RAS-driven tumor cells from oxidative DNA damage-associated growth arrest. However we hypothesize the degree of protection required is higher in advanced tumors that carry high levels of KRAS oncoprotein in comparison to early tumor cells that carry low levels of oncoprotein. Accordingly, we are cloning oncogenic KRASV12 cDNA into two different backbone vectors: pBABE.hygro and pWZL.blast. We anticipate that a transduction of these two KRAS-expressing vectors into lung cancer cell lines will result in high KRAS oncoprotein expression in the pWZL.blast vector and low KRAS expression in the pBABE vector. Thus these constructs in conjunction with shRNA constructs to suppress 8-oxoguanine detoxifying repair proteins will enable us to shed light on precisely how the manipulation of these DNA repair protein affect growth of early and advanced KRAS-driven lung tumor cells.

Comparing Dendritic Arbor and Spine Density of Neurons Obtained from the Cortex and Hippocampus of Wild Type and RanBP9 Mice

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Abstract

Alzheimer's disease is the most common cause of dementia, affecting over 24 million people worldwide. In addition to dementia, Alzheimer's disease can cause seizures, severity of memory loss, and impaired language. Two features associated with Alzheimer's disease are amyloid-β plaques and neurofibrillary tangles, both of which occur in the brain. RanBP9 is a protein that, when overexpressed, increases the amount of plaques produced. Since RanBP9 increases plaques, does it correspondingly decrease dendritic arbor and spine density? The objective of this experiment is to compare the number of dendrites and spines on neurons obtained from wild type and RanBP9 mice. This was accomplished by preparing brains of mice aged 6 months and 12 months with Golgi solutions following the protocol from the FD Rapid GolgiStain Kit. The brains were frozen in OCT and sectioned at -22°C using a cryostat. The brain sections were stained using Golgi Stains and cresyl violet, and then stored at 4°C. Images were taken of the neurons, which were analyzed using ImageJ software. The results indicated that 6-month-old transgenic mice produced neurons with a similar number of dendrites and spines as the neurons of wild type mice of the same age. However, 12-month-old transgenic mice produced neurons with a significantly lower number of dendrites and spines than did the wild type mice of the same age. Overall, this study suggests that RanBP9 overexpression results in age-dependent reductions in the dendritic arbor and spine density in pyramidal neurons of the cortex and CA1 region of hippocampal brain regions.

A Novel Application of the Heck Reaction to Couple D-Limonene with Iodobenzene

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Abstract

D-Limonene ($C_{10}H_{16}$) contains two carbon-carbon double bonds and a ring made of 6 carbon atoms. Limonene, which is produced in Florida, is easily extracted from the rinds of citrus fruits and provides a relatively inexpensive raw material for flavors, fragrances, solvents and the synthesis of fine chemicals. SciFinder Scholar revealed that nearly 2,000 scientific articles were written about limonene in 2013. Most of the methods to modify the chemical structure of limonene involve the double bond inside the ring. This report details the development a novel method to modify the double bond outside of the ring of limonene, which might allow the synthesis of valuable molecules from this inexpensive starting material. The Heck reaction is a powerful method to form carbon-carbon bonds; however, the Heck reaction of limonene is not reported in the literature. We hypothesized that a novel compound could be prepared by means of the Heck reaction of D-limonene and iodobenzene. The standard conditions of the Heck reaction with a palladium (II) acetate catalyst, a base (triethylamine or potassium carbonate) under heating in dimethylformamide, with or without a triphenylphosphine ligand failed to couple the benzene group to limonene after repeated attempts. Gratefully, it was discovered that the use of silver acetate as the base did afford the target molecule. The product was purified on silica gel and the structure confirmed by infrared spectroscopy and nuclear magnetic resonance spectroscopy. Work is ongoing to optimize the reaction conditions and expand the scope of this novel application of the Heck reaction.

Whitefly Vector Dietary Conditions Associated with the Inoculation of Foregut Borne Criniviruses

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Abstract

Criniviruses, within the family Closteroviridae, are plant viruses transmitted by whitefly vectors of the Bemisia tabaci species complex in a manner that involves retention of virions in the vector's foregut, but does not require virus circulation through the vector. This study focuses on the inoculation stage of transmission for two criniviruses: Lettuce infectious yellows virus (LIYV), which is transmitted specifically by B. tabaci biotype A; and Lettuce chlorosis virus (LCV), which is transmitted by both B. tabaci biotypes A and B. This investigation seeks to identify conditions (pH and plant host components) that may play a role in mediating the inoculation of these criniviruses. Viruliferous whiteflies were fed liquid diet at pHs of 4, 7.4, and 9 to determine if and how effective viruses were inoculated under these conditions. Our experiments are aimed at testing the hypothesis that inoculation/release of criniviruses that are retained in their vectors' foreguts is influenced by pH and/or contents of the plant hosts on which the vectors feed. While the detection method for LCV is still under optimization, our results showed that purified LIYV virions were acquired by B. tabaci biotype A through membrane feeding. In addition, detection of LIYV by RT and nested PCR in the inoculation buffers at pH 4 and 9 but not pH 7.4 suggested that pH is a potential factor to trigger inoculation of virus by viruliferous whiteflies. These findings have potential consequences on the improvement of plant health and productivity upon which humans and animals depend for their survival. (NIH-NIGMS MARCU*STAR Grant, T34 GM008021-29, Barry University.)

Sensation Seeking Behavior and Aggression: Predictors of Low Physiological Arousal Under Stress

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Abstract

Using the Bimodal theory of violence, aggression can be dissected into two distinct modes: affective violence and callous-unemotional violence (i.e. predatory violence) where the aggressor is physiologically "under-aroused" (Tulogdi, et al., 2012). These individuals also demonstrate sensation seeking behaviors as a way of increasing arousal. Combined, this suggests that sensation seeking behavior can serve marker of aggression and physiological under-arousal (Wilson & Scarpa, 2011). In order to test this idea, we examined the extent to which sensation seeking behavior was associated with physiological and self-report responses to acute stress (the cold pressor test, CPT). We found that sensation seeking behavior is associated with self-reported aggression and some measures of low physiological arousal. In particular, we found a positive correlation between self-reported aggression and sensation seeking behavior ($p < 0.05$). In addition, S-IgA, a marker of immune functioning and stress level, is negatively correlated with sensation seeking behavior before ($p < 0.05$) and 1 min after ($p < 0.05$) the CPT stress. High aggression is related to low baseline and post-stress cortisol levels ($p < 0.05$). Finally, sensation seeking behavior was negatively correlated with self-reported pain perception of the CPT stressor at three different time-points: 1 minute before the CPT (anticipated pain), during the CPT (experienced pain), and 20 minutes after the CPT (recalled pain) (all p's < 0.05). Combined, these findings suggest that both aggression and SS are related to low physiological arousal, but that aggression is associated with low HPA axis activity while sensation seeking appears to involve SNS mechanisms.

A Combination of Two Tropical Spice Compounds Potentiates Chemotherapy Response in Castration-Resistant Prostate Cancer Cells

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Abstract

Patients with castration resistant advanced prostate cancer (CRPC) respond poorly to potent chemotherapeutic drugs (e.g., Docetaxel or Mitoxantrone) with survival advantages that last less than 4 months. Since chemotherapy itself has morbidity in the elderly patients, adding another such drug is unlikely to improve the outcome. We investigated the potential therapeutic benefits of treating CRPC with a combination of two natural dietary anti-proliferative compounds: Curcumin and Piperine. They are derived from the tropical spices, turmeric and black pepper, respectively. Both are orally bioavailable and can be consumed over a long period of time. The cytotoxicity of Curcumin, Piperine, and Docetaxel were individually tested on a CRPC cell line PC-3, which was incubated for 48 hours or 72 hours. Cytotoxicity was evaluated by cell counting, a colorimetric assay and by clonogenic survival, colony-formation assay. Enhancement of Docetaxel cytotoxicity by natural compounds was evaluated by treating cells with these compounds 24 hours before adding Docetaxel or with co-incubation. The potential mechanism of cytotoxicity was examined by an apoptosis assay and changes in cell cycle phase-fractions. The efficacy of the combination was evaluated by Isobologram analysis and ANOVA. All three drugs tested on PC-3 showed cytotoxicity, Docetaxel was the most cytotoxic (IC50~ 25 nM) and Piperine was the least cytotoxic (IC50~ 100 μM). Curcumin alone was significantly cytotoxic to PC-3 cells at 10 μM but it enhanced the cytotoxicity of Docetaxel. Piperine, when combined with Curcumin or Docetaxel, significantly increased the cytotoxicity by 50%. Prior exposure to Piperine resulted in enhanced cytotoxicity of Docetaxel and Curcumin more than when in co-incubation. The increased cytotoxicity in combination therapy was mainly due to increased apoptotic activity.

Synthesis and Characterization of Oxindole Derivatives and their Antibacterial Activity on Escherichia Coli and Staphylococcus Aureus

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Abstract

In the recent years, analogues of Indolin-2-one (2-oxindole) have been found to be highly effective in inhibition of pro-inflammatory cytokine IL-2 of Multiple Sclerosis. Researchers around the world have focused on methodical modification at several key locations of the molecule with the intent of studying the biological activities. In that line, this research focuses on the synthesis of Knoevenagel adducts with a variety of aromatic aldehydes. In this research, sixteen derivatives of 2-oxindole have been synthesized and characterized by traditional techniques (melting point, FT- IR, and NMR). Furthermore, the antimicrobial efficacy against gram-negative (Escherichia coli) and gram positive (Staphylococcus aureus) bacteria was evaluated by the disk diffusion method. The potential use of these derivatives is initially to study the anti-bacterial and anti-mitotic effects and then to explore broader biological studies.

Induction of Apoptosis by Phycocyanin in LNCaP and A549 Cancer Cells

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Abstract

Phycocyanin is a pigmented protein found in cyanobacteria produced by aphanizomenon flos-aquae (a freshwater species of blue-green algae) and spirulina. Phycocyanin (PC) has been researched extensively in the last twenty years because of its anti-cancer properties. Despite numerous critical findings, the anti-cancer mechanism for how PC induces apoptosis in cancer cells is still unknown. The purpose of our experiment was to determine the uptake of PC by cancer cells and then study different mechanisms of apoptosis. PC naturally emits red florescence, which was utilized to verify the uptake by LNCaP prostate cancer cells. We were able to determine that PC only binds to the cell membrane; therefore we suspected that PC must interact with a membrane protein for inducing apoptosis. Since apoptosis mechanisms are regulated by an anti-apoptosis protein Bcl-2, we examined the effect of PC treatment on Bcl-2 levels. Electrophoretic separation of proteins from the LNCaP cells and a western blot analysis showed PC down-regulated Bcl-2. Topotecan (TPT) an anti-cancer drug was used as a positive control to down-regulate Bcl-2 levels in A549 lung cancer cells. The combination of both PC and TPT resulted in greater down-regulation of Bcl-2 that might be triggering apoptosis in cancer cells. Understanding the complete mechanism inducing apoptosis is critical for PC to be considered as a possible complimentary agent during cancer treatment. (This project was supported by the Esther-King Biomedical Research Program of the state of Florida).

Effects of Caffeine on C2C12 Myoblast Differentiation

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Abstract

During pregnancy, caffeine is known to pass through the placenta and reach the fetus. Whether caffeine has adverse effects on fetus development remains to be controversial. The American Congress of Obstetricians and Gynecologists concluded in 2010 that caffeine consumption is safe up to 200 mg per day in pregnant women. Here we examine the effects of caffeine on myotube differentiation using a myoblast cell line, C2C12. We induced C2C12 myoblast cell differentiation in the presence of various concentrations of caffeine. Using our high content imaging system, we followed myotube formation for seven days by measuring length and breadth of myotubes. The expression of mygenic markers, MyoD and myogenin are typically analyzed using in situ immunostaining. Our preliminary data indicated that at millimolar concentrations, caffeine inhibited C2C12 myoblast differentiation by reducing mygenic fusion, thus generating shorter myotubes than control cells.

Particulate Matter Induces Opportunistic Emergence of Antibiotic Resistance

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Abstract

It is becoming increasingly recognized that particulate matter in the atmosphere is a significant global health threat and environmental concern. Byproducts from burning of biomass or fossil fuels, polyaromatic hydrocarbons (PAHs), comprise a substantial fraction of particulate matter in many areas. Previous studies have indicated that many PAHs are carcinogenic in mammalian cells and have mutagenic potential in microorganisms such as bacteria. However, the consequences of such mutations in bacteria remain relatively unexplored. In this study, we examine the ability of selected compounds commonly found in particulate matter to generate antibiotic resistant bacteria. We observed that separate treatment with an individual PAH - 1- nitropyrene, benzo(a)pyrene, pyrene and 2-nitrofluorene - can significantly increase the occurrence of Escherichia coli that are resistant to rifampicin and kanamycin. Furthermore, we observed that treatment with two of these compounds together increases the frequency of rifampicin resistant bacteria in an additive fashion. Finally we found that rifampicin resistant bacteria obtained after treatment with 1-nitropyrene or 2-nitrofluorene can resist rifampicin up to the solubility level of rifampicin in growth medium, suggesting mutants derived under these conditions may be highly resistant to antibiotics. Preliminary results from the treatment of E. coli with ambient aerosol samples will also be discussed. Overall, our study presents evidence that ubiquitous compounds in particulate matter may serve to increase the rate at which antibiotic resistance evolves in the environment.

Function Visualization: 3D Plotting of Continuous Functions and their Derivatives using OpenGL.

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Abstract

Scientific visualization is very important in supporting data modelling and management, as well as computer-aided instruction for better understanding of abstract mathematical structures. We have created a tool that visualizes continuous functions of the form z = f(x, y) and their partial derivatives $\frac{\partial f}{\partial x}$ and $\frac{\partial f}{\partial y}$ in three-dimensional space. The prototype utilizes library routines of the OpenGL system for rendering and transformational purposes. Functions are user-defined and employ a variety of common arithmetic operators and trigonometric functions. Functional expressions undergo multiple stages of scanning and parsing to check for errors A binary expression tree T_f is constructed for a correct functional expression. T_f is traversed and differentiation rules are used to construct binary trees $T_{\partial f/dx}$ and $T_{\partial f/dx}$that realize the corresponding partial function derivatives. Optimization techniques eliminate redundant expressions in the derivative trees. An in-order traversal of each tree is used to evaluate the underlying expression and produce a collection of three-dimensional points. Re-evaluation of expressions occurs only on newly defined functions, thus reducing execution time substantially. An OpenGl framework allows for the definition of the system's viewpoint, bounding volume, surface characteristics, and projection method. Functions and partial derivatives are plotted together for comparison purposes or separately for clarity. The system is capable of dynamic, speed-controlled plotting through motion. A frame per second rate is displayed to measure the efficiency of the algorithm.

Advanced Adaptive Torque Control of In-Stream Hydrokinetic Turbines

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Abstract

Diversifying US energy production to include renewables has been a popular topic of discussion in recent years. In-stream hydrokinetic energy, electricity production from moving currents without the use of dams, has potential for significant power production with technically feasible US electricity production estimated at 14 GW from rivers, 50 GW from tides, and 19 GW from ocean currents; which is equivalent to approximately 17% of 2011 US power production. This work focuses on improving the power production from in-stream hydrokinetic turbines using adaptive torque control, and quantifies increased energy production by comparisons with standard fixed-gain torque control. This research uses numerical modeling to acquire power production estimates under simulated conditions. With these results we can quantify potential energy gains for three representative in-stream hydrokinetic rotor designs.

Effect of Non-spatial Cues on Adult Mental Rotation Performance

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Abstract

Research suggests that color affects the mental rotation performance of individuals with low spatial ability (Khooshabeh & Hegarty, 2008). In the present study, we use the Shepard & Metzler (1971) figures to examine the effect of color on mental rotation performance. We compared participants across three conditions (1) Monochromatic (no color) (2) Facilitating-cue and (3) Inhibiting-cue. In the facilitating-cue condition, the red cubes in both images correspond, possibly facilitating mental rotation. In the inhibiting-cue condition, the red cubes do not correspond, possibly inhibiting mental rotation. We hypothesized that participants in the facilitating-cue condition would score significantly higher in the mental rotation task than those in the monochromatic and inhibiting-cue conditions. Participants in the inhibiting-cue condition would score the lowest of the three conditions. A Tobii X60 eye-tracker system was used to record the gaze patterns of participants during the mental rotation task. Preliminary results show that participants in the inhibiting-cue condition scored significantly lower than participants in the facilitating-cue ($p<.001$) and monochromatic conditions ($p<.001$). There was no significant difference between the facilitating-cue and monochromatic conditions. Interestingly, the significant sex difference in mental rotation performance found in the monochromatic condition ($p<.05$) disappears in the facilitating-cue ($p=.437$) and inhibiting-cue ($p=.573$) conditions suggesting the equal salience of color during mental rotation tasks for males and females. The findings of this study contribute to theories of cognitive strategy selection by suggesting different information processing of spatial and non-spatial information. They also have important implications for research on effective spatial training techniques.

Base Excision Repair Protects Telomeres from Activation-Induced Deaminase During Antibody Class Switch Recombination

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Abstract

Immunoglobulin (Ig) class switch recombination (CSR) is an essential mechanism for the diversification of the humoral immune response through efficient generation of antibody isotypes that mediate the elimination of pathogens. CSR is a programmed deletional recombination event between DNA double strand breaks (DSBs) at switch (S) regions in the Ig heavy chain gene locus (Igh). These DSBs are initiated by the mutagenic enzyme activation-induced cytidine deaminase (AID), which converts the cytosine (C) base of deoxycytidine into uracil (U). For reasons still unclear, many uracil moieties at the S-regions are processed by base excision repair (BER) factors, initiated by uracil-DNA glycosylase (UNG), and either fixed as mutations or converted into the DSBs needed for CSR. Nevertheless, a fraction of the uracils are also faithfully corrected by canonical BER. AID preferentially localizes at the Igh loci during CSR but it also exhibits off-target activity to other genomic regions. Here we report that AID associates transiently with telomeric DNA at times when it is also found at the Igh locus in B cells undergoing CSR. Interestingly, we observed an AID-dependent accumulation of gammaH2AX and RPA when UNG is inhibited. These results suggest that during CSR, telomeric DNA is deaminated by AID and repaired by the BER pathway. This conclusion is supported by the AID-dependent loss of telomeric C-rich DNA in UNG-deficient cells. Altogether, our results show that telomeric DNA is a target of AID and that the BER pathway protects the stability of the telomeres during CSR.

Culture, Isolation and Comparison of the Microbiome of Dissected Guts from Opiliones, Leiobunum sp. and Gasteracantha Cancriformes

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Abstract

Harvestmen (Order Opiliones, Class Arachnida) arachnids are closely related to "true spiders" (Order Araneae). While harvestmen are scavengers who masticate and digest solid food, true spiders use venom to externally pre-digest prey. To date, classification of the normal digestive flora of Opiliones has not been published. Our overall hypothesis is, given their internal digestion process, harvestmen will exhibit greater diversity of digestive microbiota in contrast to true spiders. We performed a screen to investigate this hypothesis by conducting aseptic dissection and isolation of the guts from 10 organisms of each species. Aerobic non-fastidious culture isolates from Opiliones Leiobunum sp. guts exhibited much greater diversity than those from our representative "true spider", Gasteracantha cancriformes. From Leiobunum, we identified eight species including Pseudomonas luteola, Staphylococcus aureus, and Enterococcus faecium. Four others were presumptively identified based on colony morphology and gram stains. In stark contrast to this diversity, we only isolated six different aerobic, non-fastidious colony types from dissected guts of Gasteracantha cancriformes. Of these, we identified Staphylococcus hominis, Staphylococcus epidermis and Micrococcus spp. Thus, our culture results have indicated a much more diverse microbiota in the gut of harvestmen when compared to that of true spiders. Our results contribute to current understanding in Opiliones ecology, and are important for expanding the relatively small body of knowledge published about these fascinating arachnids. We hope that results from this project might introduce these organisms as a new invertebrate model for translational studies in gut microbiome research.

Chloroplast Isolation and Cloning of the Large Subunit of Rubisco in Sabal Palms

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Abstract

Miami Dade College North Campus Palmetum houses approximately 250 different species of palms. The collection includes several Sabal species most of which are native to the Caribbean and Central America. The focus of this study is to identify the differences in the gene that encodes for the large subunit enzyme Rubisco (ribulose biphosphate carboxylase) in Sabal species. The Rubisco gene is responsible for catalyzing the process of carbon fixation through which carbon dioxide is incorporated into energy-rich molecules. To isolate chloroplasts most procedures utilize spinach, but the procedure proved unsuccessful with palms. After optimization procedures, chloroplasts were isolated from four different species of Sabal palms (S. mexicanus, S. domingenisis, S. maurittiformis, S. yappa) using volumes ranging from 20 to 80 mL of 0.3 M sucrose. Once the chloroplasts were quantified, DNA was isolated using DNeasy Plant mini kit (Qiagen) and subject to PCR. Preliminary results show an amplicon of 1.5 Kbs representative of the desired product and in accordance with other reported genes for other palms species.

Endocrine Disruptors (BPA) in Saliva as an Indicator of Autistic-Like Social Behaviors

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Abstract

Recent research suggests that the chemicals in plastics may be linked to serious health problems. Bisphenol A (BPA), an endocrine disruptor, can be found in a variety of products containing polycarbonate plastics and epoxy resins (Anirban, Bauer, & Lawerence, 2012; Kim, Han, Lyoo, Min, Kim, & Renshaw, 2010; Miodovnik, et al., 2011; Saal, et al., 2007; Walsh, 2010; Yang, Morgan, Nguyen, Moore, Figard, & Schug, 2010). For instance in the more than 31 million tons of plastic generated in 2010 most exposures came from food and drink containers, furniture, clothing, medical supplies, cleaning supplies, shampoo and conditioner bottles, and even trash bags (Environmental Protection Agency, 2012). In addition, BPA can be found in dental fillings and sealants, leachates from landfills, and from off gassing found in indoor air studies (Saal, et al., 2007). Perhaps because of this pervasive exposure, BPAs have been found in 93% of surveyed Americans over the age of six (Walsh, 2010). The exposures to environmental toxins, such as BPA, are also considered to be potentially associated with symptomatic behaviors of children with Autism Spectrum Disorders (ASD) (Kim, Han, Lyoo, Min, Kim, & Renshaw, 2010).

Imaging Autoimmune Medicated Destruction of Tumors

Syed Hamad Sagheer, Jason Miska, Zhibin Chen, M.D. Ph.D.
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Abstract

Cancers can be difficult to kill but one line of defense are immunomodulatory agents, which signifies the notion that autoimmunity can be an effective tool to eradicate cancer. To further explore this interplay, the behavior of autoimmune cells at the target tissue of the tumor was examined. The anterior chamber of the mice eye model was used to study the motility behavior of immune cells, and the T-cells used were from a line of cells; the utilization of these methods allowed for the most controlled setting to run the experiment. Splenocytes from a diabetic mouse are efficient at clearing an islet derived tumor (NIT-1) and CD4+ restricted TCR, specific for beta-cells, are alone capable of destroying the tumor. A step further was taken to look specifically at the role of the receptor CTLA4, and the central question developed as the following: How will the reduction in the expression of CTLA4 affect the destruction of the tumor mass? Through the examination of the data, CTLA4 modulates the anti-tumor response, which is correlated with specific changes in cellular motility. By modestly reducing its expression, there is a greater efficiency in the killing of the tumor mass. The presence of antigen affects motility in the control mice, however in CTLA4 RNAi T-cells their movement is independent of whether or not it's in the tumor, which indicates a critical role for intrinsic behavior of CTLA4 on T-cell motility.

Examining the Relation between Pre-Kindergartner's Spatial and Early Numeracy Skills

Daniela Salazar, Michael Lopez, Carla Abad, Rosalie Odean and Shannon M. Pruden, Ph.D.
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Abstract

Children's spatial skills can predict future success in Science, Technology, Engineering, and Mathematics (STEM) fields. Research has found that children's spatial ability improves their development of numerical knowledge (Gunderson, Ramirez, Beilock, & Levine, 2012). Using longitudinal data sets Gunderson et al. found that children's spatial ability predicted improvement in linear number line knowledge throughout the school year. The current study aims to explore the relation between early numeracy and spatial reasoning ability. Our prediction is that children's early numeracy and early spatial skills will be related. Forty-nine children from13 pre-kindergarten classrooms participated in this study. The children completed a spatial assessment battery which assessed their ability to: (1) reconstruct patterns using colored blocks (WPPSI-III's Block Design); (2) make analogies between two images depicting spatial information (Spatial Analogies); (3) comprehend words for a variety of spatial concepts (Boehm-3); (4) understand early numeracy (TEMA-3). Our results show significant, positive correlations in children's performance on numeracy tasks as assessed by the TEMA-3 and spatial skills as assessed by the Boehm-3 ($r(43)=0.470$, $p=.001$), the Spatial Analogies task ($r(43)=0.465$, $p=.001$), and the Block Design subtest ($r(41)=0.498$, $p=.001$). Although correlational, the results imply that early numeracy and spatial reasoning are related. These findings have important implications for science and mathematics education in the early school years. Presenter: Daniela Salazar

Synthesis, Characterization and Antifouling Potential of Functionalization of Gold Nanoparticles

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Abstract

In recent years, nanoparticle/polymer composites have become important owing to their small size and large surface area. The surface modification of nano-particles leads to an even greater range of possible applications. For example, there have also been several reports on the antimicrobial activities of surface modified metal nano-particles. In addition, biofouling prevention remains a major challenge and as such there is a need for antifouling agents that have minimal ecotoxicty, active durability, and affordability. In addition there is some evidence that surface modified gold nano-particles may be useful in this regard. Our interests are in working with a series of disulfide derivatives that can be produced in lab and whose structures may be altered easily. Currently, a model disulfide has been produced and is being structurally characterized. If successful other derivatives can be produced and used as controls in nano-particle by changing the end groups on the disulfide. These derivatives can then be characterized by nuclear magnetic resonances as well as infrared spectroscopy. This paper reports details of the synthesis and characterization of one model disulfide.

Isotopic Analysis of Dietary Variation in Formative Period Chile

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Abstract

Northern Chile's Atacama Desert is one of the driest environments on Earth. In fact, it has been suggested that the region serves as a good model for living conditions on Mars. By employing a number of resource management strategies including complex systems of trade, humans have lived in the inhospitable region for millennia. Here we present the results of stable isotope analysis of seven Formative Period (1500 B.C.-A.D. 500) humans from the Ancachi site near the modern town of Quillagua. Analysis of carbon and nitrogen isotopes from human bone collagen and hydroxyapatite, as well as floral and faunal remains, allows us to study the variability in protein and carbohydrate components of these individuals' diets. These data, as well as the comparison of burial methods between Ancachi and several coastal cemeteries, allow us to examine patterns of exchange and social mobility on an individual level. By comparing these data to those of hundreds of other individuals in a broader ongoing study, we can examine patterns of dietary variation in the region that indicate systematic regional exchange of food and other goods.

Evaluating the Efficacy of Treatments for Social Anxiety Disorder; A Patient's Reflective Review of Pharmaceutical, Therapeutic, and Mixed-Method Options

Kristiana Stevens and Teresa E. Thornton, Ph.D.
Oxbridge Academy of the Palm Beaches, West Palm Beach, FL

Abstract

Those who suffer from Social Anxiety Disorder (SAD) have been exposed to a plethora of treatments, yet research regarding their preference among therapies is lacking. Although treatments may affect each individual differently, a survey of patients' opinions with respect to efficacy, long-term response, and the severity of adverse effects may enable research to focus on what benefits the larger population. This is especially important given that 20% of people with SAD admit they self-medicate in addition to sanctioned treatments (Richards, 2009). Additionally, the discipline itself needs to streamline evaluation methods in order to better compare results. It is expected that this research will find unknown parameters that only a patient can provide.

Antagonistic Interactions Structure Coral Microbiome of Porites Astreoides

Daisy Vargas, Ariane Martin, Mauricio Rodriguez-Lanetty, Ph. D.
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Abstract

The microbial community associated with reef-building corals exists as a complex consortium of interacting bacteria. These bacteria have been shown to display antagonistic behavior by growth inhibition. Whether or not these inhibitory processes play a role in regulating the abundance of species within the coral associated microbial community remains unanswered. In this study we tested the hypothesis that more abundant bacteria possess higher antagonistic capabilities that rare bacteria found in association with corals. Using data from high throughput sequencing of 16rRNA, we ranked several cultured bacteria isolated from the scleractinian coral, Porites astreoides by their relative abundance within the microbiome. We selected three common (>20%) and three rare (<2%) isolates, and performed pairwise inhibitory experiments among them. Results show that some rare bacteria are indeed inhibited by high abundance bacteria. Findings from this study suggest that inhibitory processes modulate in some degree the abundance patterns of the microbial consortia associated with corals.

Transforming Growth Factor-Beta (TGFβ) Regulates Activity of CDKs at Post-Transcriptional Level

Daria Vasilyeva, Alejandra Toro, Talia Guardia, Tamara Guardia, Jazmine Duran, and Xiaotang Hu, Ph.D.
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Abstract

The cell cycle progression is controlled by cyclin-dependent kinases (CDKs). CDK-Activating Kinase (CAK) is an enzyme complex that is capable of phosphorylating (activating) all CDKs. CAK is composed of CDK7, cyclin H, and Mat 1. Dephosphorylation of CDK1 and CDK2 on tyrosine 15 by cdc25c can also activate CDK activity. In this study, we investigated the effect of TGFβ on activity and expression of CAK and cdc25 in human myeloid leukemia cells. TGF-β inhibited the proliferation of TF-1 and MV4-11 accompanied by an increase in p27 level and decrease in CDK1, CDK2, CDK4, cyclin A, cyclin D3, and cyclin B. However, TGF-β had no effect on the expression or phosphorylation status of CDK7, CDK9, CDK11, cyclin H, Mat1, and their complexes (CDK7-cyclin H and CDK7-Mat1). In contrast, these cells showed marked decrease in the levels of MAT1 and CDK7 in response to retinoic acid stimulation. On the other hand, TGFβ significantly downregulated expression of cdc25c starting from 3h and with a maximum inhibition being observed at 72h. Taken together, our data suggest that TGFβ-induced growth inhibition of human myeloid leukemia cells is CAK-independent but is linked to inhibition of early entry into mitosis of the cells by downregulating cdc25c-CDK1 pathway. Most likely, the downregulation of multiple CDKs and cyclins by TGFβ is regulated at translational or posttranslational but not transcriptional levels, because TGFβ had no any effect on the expression and phosphorylation of the CDKs that are involved in regulation of transcription factors TFII and TAK/P-TEFb, respectively.
Supported by Faculty Incentive Grant and NIH-NIGMS MBRS RISE: R25 GM059244-13, Barry University.

Theoretical Interaction of Collagen-Q with Complement Receptor Type 1 Provides New Evidence on Novel Non-Cholinergic Functions of Collagen-Q

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Abstract

Acetylcholinesterase (AChE) is an enzyme localized at the endplate of the neuromuscular junction (NMJ); there, it hydrolyzes acetylcholine terminating neurotransmission at cholinergic synapses. The major form of AChE found at the NMJ is the asymmetric form, which consists of up to three catalytic tetramers and a collagenic tail (ColQ). The catalytic subunit of AChE contains a C-terminal region of 40 amino acids known as the Tryptophan Amphiphilic Tetramerization Domain (WAT) that is critical for hydrophobic interactions with the Proline-Rich Attachment Domain (PRAD) of ColQ. The PRAD is 17 amino acids long and is within the N-terminus of ColQ. The WAT_4PRAD interactions are required for AChE localization at the NMJ in vivo. ColQ is expressed in organs other than skeletal muscle including kidney, testis, and pancreas; therefore, we searched across the NCBI protein sequence database for WAT-like domains present in extracellular or membrane-bound proteins. We found that WAT-like domains are present in Complement Receptor Type 1 (CD35), expressed in glomerular podocytes, and Fibronectin, which is broadly expressed. The interaction with CD35, if demonstrated, might uncover important facts related to kidney disorders that are linked to CD35 deregulation. In addition, by interacting with Fibronectin, ColQ might participate in the organization of the extracellular matrix and signaling pathway regulation in tissues other than Skeletal Muscle.

Optimization of Chloroplast Extraction from Sabal Domingensis

Joseph Vera, B. Lilavois, F. Rodriguez, H. Rivera, S. Ritter Ph.D, A.J. Leon
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Abstract

Optimization methods for extraction of chloroplast has been done extensively with spinach. Little work has been done with palms. Due to palms' characteristically fibrous leaves, optimization of chloroplast isolation was required. Several extraction methods were tried to isolate chloroplast from Sabal domingenisis. In order to optimize the yield of chloroplast centrifugation rates and sugar concentration were examined. Two concentration of sugars were used, 0.3 M sorbitol and 0.2 M sucrose. After reviewing the absorbance data from 0.3M sorbitol and 0.2M sucrose. Concentrations had little effect on the data. The centrifugal force from the spinach extract was altered to compensate for the fibrous leaves of S. domingensis. The increase of centrifugal force was 1,000 G, 20,000 G to extract the chloroplast. The extraction's data shown an increase of chloroplast in excess of 400 percent.

Synthetic Methods to Produce Analogues of Resveratrol that Contain a Cyclopropyl Ring

Gerry Victor and James J. Ley, Ph.D.
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Abstract

Resveratrol (3,4',5-trihydroxystilbene) is made by plants and is biologically active. Both cis- and trans-resveratrol are produced by plants in vivo, but the importance of these isomers on biological activity is not completely understood. Furthermore, the synthesis of pure diastereomers of resveratrol can be complicated by the interconversion between isomers. This report presents the attempts to prepare novel analogs of cis- and trans-resveratrol that contain a 3-carbon-atom cyclopropyl ring. Strategies and tactics evaluated for the preparation of these conformationally-restricted analogs of resveratrol included the stereospecific cyclopropanation of stilbenoids by means of Shi's carbenoid ($TFA{-}Et_2Zn{-}CH_2I_2$) or triisobutylaluminum, the Simmons-Smith reaction, the Corey-Chaykovsky reaction and reactions with ethyl diazoacetate. Products were characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY and HETCOR spectroscopy. Unfortunately, all of these methods failed to produce the target molecule. This has led to the hypothesis that the cyclopropanation of electron-poor stilbene molecules, which can then be transformed into electron-rich resveratrol analogues, may be a more promising route. Recent results of the synthesis and attempted cyclopropanation of nitro-stilbene molecules are reported.

Phylogenetic Analysis of the Integrase Gene of the Bacteriophage Omnicron

Rachel S. Walter, Renee M. Deneweth, and Sharon Isern, Ph.D.
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Abstract

Bacteriophages are viruses that infect bacteria through utilizing parts of the host biosynthetic apparatus. Phages are the most abundant organisms in the biosphere and a ubiquitous feature of prokaryotic existence. Bacteriophages are useful to the scientific community through innovations such as phage therapy and the treatment of pathogenic bacterial infections. Omnicron, discovered at Florida Gulf Coast University in 2013, is a subcluster K5 mycobacteriophage. In this study, using a bioinformatics approach (Seaview phylogenetic analysis program) we compared the integrase gene of Omnicron with other related cluster K phages and more distantly related mycobacterium phages. Integrases are enzymes that are necessary for integration of the phage into the host genome by site-specific recombination. In conjunction with excisionase, integrase is also necessary for excision of the prophage from the host genome. Studies of integrase are essential in understanding host-pathogen interactions. For example, integrase enzymes are a target of antiretroviral therapy primarily for HIV. A better understanding of integrase genes in bacteriophages and their evolution and relatedness to other viruses may lead to applications in gene therapy, construction of transgenic organisms, and facilitating gene function studies.

Biosynthesis of Neuropeptide Q

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[2]Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL

Abstract

Neuropeptide Q (NPQ) is a new neuropeptide involved in anti-nociceptive, kidney and cardiovascular functions. In many cases, neuropeptides transmit extracellular signals to the intracellular environment in order to regulate diverse biological systems. So far, the regulatory mechanism of NPQ production within cells has yet to be clarified. Obtaining the correct information regarding molecular properties of NPQ is important to understand how these biological phenomenon are regulated by NPQ. To address this question, we first aimed 1) to develop the expression system using two cell lines, HEK 293 cells, a cell line commonly used for protein expression, and AtT-20/PC2 cells, an endocrine cell line; and 2) to clarify the localization of proNPQ in AtT-20/PC2 cells. We first constructed a plasmid encoding preproNPQ_myc/His and then transfected the plasmid into HEK cells. The result from immune-blotting showed that the expression of proNPQ was successfully detected in HEK cells. We also carried out a metabolic labeling experiment with [^{35}S]methionine/cysteine and AtT-20/PC2 cells in order to confirm the expression and processing of proNPQ. Pulse-chase experiment showed that proNPQ was indeed expressed in AtT-20/PC2 cells, also likely cleaved by PC2. In addition, immunocytochemical experiments also revealed the co-localization of proNPQ with PC2 in AtT-20/PC2 cells. These results support the idea that PC2 would represent the natural enzyme to cleave proNPQ intracellularly. We will further characterize the posttranslational modifications of proNPQ using AtT-20/PC2 cells in future study. This information will also provide mechanistic explanations and help us to develop therapeutic substances for the currently reported functions.

Neurogenesis in Octopus Vulgaris

Jelileh Whitmore, Jaime L. Tartar, Ph.D., and James R. Munoz, Ph.D.
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Abstract

Cephalopods, particularly the octopus, have the largest and most complex invertebrate brains and show markers of behavioral complexity. Several mammalian studies have demonstrated a correlation between learning and neurogenesis (the birth of new neurons) in the hippocampus of the adult brain. The vertical lobe of Octopus vulgaris is thought to be analogous to the hippocampus of mammalian brain; however, neurogenesis has never been reported in the octopus. We used thymidine incorporation assays and immunostaining to demonstrate neurogenesis in the developed octopus brain. We are currently examining the proliferation, differentiation, integration, and cell death of newly born neurons in the octopus brain. Future studies will test for concomitant changes in behavioral learning and neurogenesis.

Analysis of Green Turtle and Loggerhead Facial Scutes under Ultraviolet Light

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Charles E. Schmidt College of Science, Florida Atlantic University, Boca Raton, FL

Abstract

Hatchling marine turtles can detect UV light and use it to orient from their nest to the sea. However, the use of UV light by these animals in other behavioral contexts is unknown. In this study, I investigated whether reflected UV light might enable marine turtles to identify one another as individuals. To find out, I compared facial patterns of UV and visible light reflected from juvenile loggerheads (Caretta caretta) and green turtles (Chelonia mydas). Individually different patterns were reflected from the heads of green turtles but not from loggerheads. However, in green turtles there were no differences between the visible and the UV reflected patterns. I conclude that the use of UV light to distinguish between individuals is unlikely, but UV light might be used to distinguish between species. The next step is to determine whether UV light is used by marine turtles in other contexts, such as detecting jellyfish prey that are transparent in visible light or even predators such as sharks

Defining "Pretty" Through a Child's Eye

Jason Zolanka
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Abstract

The effects of popular culture are far reaching, especially among our impressionable children. The proposed experiment is an adaptation of the Clark doll experiment conducted in 1939. Children were presented with two dolls, only varying in skin tones and hair color, and asked which one they preferred to play and interact with. Results from the Clark experiment demonstrated patterns of self-hatred and racism. In the current experiment, we wish to investigate if the cultural diversity of Florida will impact a child's perception of a stereotypical "pretty" girl. The media and popular culture tend to promote Caucasian, thin and blonde. The experiment will be conducted on kindergarten-aged students, and nine dolls will be presented. The dolls will have identical facial features and clothing, but will vary in skin tones (pale, medium and dark) and hair color (blonde, brown and black). The children will be asked to point to the "prettiest" doll and results will be recorded.

ACKNOWLEDGEMENTS

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