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(Listed alphabetically by first author's last name

ROLE OF AHR LIGANDS IN MICRORNA-MEDIATED TH17/T REGULATORY CELL DIFFERENTIATION IN DELAYED TYPE HYPERSENSITIVITY

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The aryl hydrocarbon receptor (AHR) is known to have an impact on immunomodulation. Recent data showed that TCDD, an exogenous AhR ligand, tends to induce T regulatory cells (Tregs), while FICZ, an endogenous AhR ligand, induces Th17 cells. The aim of this present study is to investigate the effects of TCDD and FICZ on microRNA profile in delayed type hypersensitivity (DTH). Treatment of C57BL/6 mice with TCDD attenuated DTH responses to methylated bovine serum albumen and induced Tregs. Focusing on the Treg subsets, we found that there was a significant increase in inducible peripheral, natural thymic, and Th3 T regs. In addition, there is increase in TGFβ levels in the draining lymph node, as well as increased expression of TGFß and Treg transcription factor, Foxp3. In contrast, treating DTH mice with FICZ induced inflammatory Th17 cells and increased the expression of IL-17 and Th17 transcription factor, RORγ. Analysis of microRNA (miR) profiles from draining lymph nodes showed differential regulation between TCDD and FICZ groups. Specifically, miR-132, which was overexpressed in TCDD groups, leads to downregulation of gene targets HMGB1. Downregulation of these gene targets leads to an increase in Treg differentiation. In contrast, FICZ treatment caused a downregulation of miR-132, which leads to an upregulation of HMGB1. In summary, this study demonstrates that TCDD and FICZ have divergent effects on miRNA modulation in a DTH model, and both ligands differentially regulate miR-132, which targets key components involved in Th17 and Treg development.

ANGIOGENESIS AND MAST CELLS IN PRECANCEROUS PROSTATE

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Prostate cancer (PCa) is an adenocarcinoma that constitutes the second main cause of death due to cancer among men in the USA. Almost half of men display Prostatic Intraepithelial Neoplasia (PIN) by the age of 50. High grade-PIN (HPIN) is considered a precancerous stage, although most cases will not advance to cancer. Angiogenesis or the generation of new blood vessels from pre-existing ones, is a hallmark of solid tumors, as they need blood supply to grow. Vasculature formation is promoted by vascular endothelial growth factor (VEGF). Mast cells (MC) are prostate resident cells, with cytoplasmic granules harboring many mediators, including VEGF and tryptase protease. Thus, we hypothesized that MC-mediated angiogenesis drives prostate transformation. We used a transgenic mouse model C3(1)/SV40Tag that mimics the human disease progression to PCa with age. We developed a computer-assisted quantitative imaging method to measure morphometrics to quantify the number and activation of MC in microscopy sections. A similar approach was optimized for angiogenesis through quantification of CD31, an endothelial cell marker. Our preliminary data indicated that Low (L)PIN/C3 prostate sections showed higher numbers of total and activated MC than normal/C3 (N/C3) mice (63.6 vs. 27 MC/mm2 and 44.2 in LPIN vs. 10.7 MC/mm2, respectively). Microvasculature analysis revealed higher density of new capillaries in LPIN/C3 than in N/C3 or WT mice, scoring 0.03 vs. 0.01 for CD31-IOD/total image area ratios, respectfully. In conclusion, increased angiogenesis and MC activation could serve as a predictor for prostatic transformation. Supported by NIH/NIAID R01 AI095494, NIH/NIAMS R21 AR067996 and NIH/NIGMS P30 GM103336 (Pilot Project) to CAO.

COMBINATION OF CANNABINOIDS, Δ9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD), AMELIORATE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY PROMOTING CELL CYCLE ARREST AND APOPTOSIS IN ACTIVATED T CELLS THROUGH MIRNA SIGNALING PATHWAYS

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Multiple sclerosis (MS) is a chronic and disabling disorder of the central nervous system (CNS) characterized by breakdown in the blood- brain barrier and demyelination. Finding a cure for MS remains challenging, and most treatments involve the use of immunosuppressive drugs that have toxicity. The marijuana plant, Cannabis sativa produces phytocannabinoids that relieve nausea, pain, and inflammation. In the current study, we investigated the effects of using a combination of the psychotropic Δ9-tetrahydrocannabinol (THC) and non-psychoactive cannabidiol (CBD) on the regulation of activated T-cells during the development of experimental autoimmune encephalomyelitis (EAE), a murine model of MS. We demonstrated that administration of THC+CBD ten days after EAE induction was effective at ameliorating the disease, including inflammation and CNS cellular infiltration. MicroRNA microarray analysis revealed altered miRNA profile in brain infiltrating CD4+ T cells following THC+CBD treatment of EAE mice. In addition, mice treated with THC+CBD showed decreased levels of brain-infiltrating CD4+ T cells, pro-inflammatory cytokines interleukin17(IL-17) and interferon-gamma (INF-γ) and increase in the levels of brain -infiltrating Forkhead box protein P3(FoxP3)+ CD4+ T cells and anti-inflammatory cytokine interleukin 10(IL-10). Further evidence indicated that THC+CBD treatment significantly downregulated several miRNAs (miR-21a-5p,miR-155-5p, miR-146a-5p) in brain CD4+ T cells that target genes associated with cell cycle arrest (Cyclin-dependent kinase inhibitor 1B (CDKN1B) and Cyclin-dependent kinase inhibitor 1A (CDKN2A) and apoptosis Bcl-2-like protein (BCL2L11).

Collectively, these studies demonstrate that THC+CBD treatment leads to the amelioration of EAE development by suppressing T cell responses through the induction of select miRNAs that control cell cycle progression and mediate apoptosis. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, R01AI123947, R01AI129788 and P20GM103641)

EFFECT OF RESVERATROL ON GUT MICROBIOME IN TNBS-INDUCED COLITIS

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Colitis is an inflammatory bowel disease of unknown etiology characterized by acute or chronic inflammation of the large intestine. Currently there is no cure for patients suffering from colitis, and most treatments involve the use of immunosuppressive drugs that can have adverse side-effects or increased toxicity. In the current study, we investigated the effects of resveratrol, a natural component found in grapes, strawberries, and raspberries, on murine TNBS colitis model. Our data shows that administration of resveratrol alleviates symptoms associated with colitis in this model, which includes reversal of weight loss and colon shortening. In addition, mice treated with resveratrol showed decreased levels of circulating inflammatory biomarkers like serum amyloid A, myeloperoxidase and lipocalin 2. Flow cytometry data showed significant increase in in mesenteric lymph node CD3, CD4 T cells population and INF gamma in TNBS group while showed significant increase in Foxp3 T cells in treatment group. Endoscopy and histopathology also showed decreased tissue damage and cellular infiltration in the colon. In order to better understand the beneficial effects of resveratrol against colitis, we performed 16S rRNA metagenomic sequencing to investigate alterations in the gut microbiome after induction of colitis by TNBS and treatment with resveratrol. Analysis of cecal flushes revealed that TNBS administration leads to increase in several Firmicutes ,Tenericutes and Bacteroidetes. However, mice that were treated with resveratrol showed a remarkable reversal in these gut microbial alterations caused by TNBS colitis induction, having gut microbiome similar to that of vehicle-treated control mice. Collectively, these data suggest that resveratrol is able to ameliorate colitis by preventing pathogenic gut microbial dysbiosis and restoring gut microbiome composition to a more homeostatic state. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

GENERATING A HIV-1-DEPENDENT CHIMERIC VECTOR TO DELIVER A PRO-APOPTOTIC GENE

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A potential method to reduce HIV-1 replication may be to induce apoptosis in HIV-1-infected CD4+ T-Helper lymphocytes, the primary virus target. The pro-apoptotic Bcl-2 associated X protein (Bax) gene has been shown to initiate cell death when over-expressed in cells and may be effective in these studies. In order to restrict expression of pro-apoptotic Bax to only HIV-1 positive cells, an HIV-dependent lentiviral was created to express a Renilla luciferase (LucR)/nuclear localized eGFP fusion gene from the HIV-1 promoter/enhancer. This vector, pLTNG(INS2)R also includes a transcription inhibitory sequence from the HIV p24 gag region (INS) and the Rev Response Element (RRE). Co-transfection of pLTNG(INS2)R and pNL4-3.Luc.R-.E-, a replication incompetent HIV-1 genomic clone, into 293T and HeLa cells, showed LucR and eGFP expression to be highly dependent on the presence of both HIV-1 tat and rev. In addition, confocal microscopy imaging of eGFP-positive cells indicated eGFP localized to the nucleus. Expression of pro-apoptotic genes using a pLTNG(INS2)R-based vector is problematic because the generation of recombinant retrovirus using this vector requires activation by HIV-1 tat and rev, likely resulting in the death of the virus producer cell. To circumvent this problem, the Sleeping Beauty (SB) transposon, pT2/BH, which functions to deliver genes without the need for gene expression, will be modified to deliver the LTNG(INS2)R element into target cells. For this, the SB inverted repeat/direct repeat sequences, IR/DR(L) and IR/DR(R) will be individually amplified and cloned into pLTNG(INS2)R at locations upstream and downstream of the LTNG(INS2)R element. Once completed and tested for HIV-dependent expression, the transposon will be used to test various pro-apoptotic genes for the ability to target the induction of apoptosis.

ACCLIMATION TIME OF EASTERN PAINTED TURTLES (*CHRYSEMYS PICTA PICTA*) TO A NOVEL EXPERIMENTAL ENVIRONMENT

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Laboratory experiments using animals often assume that subject behavior is consistent throughout the experiment. However, exposure to novel conditions may cause temporary disruptions to behavior and impact results. A period of time may be required before animals settle into a consistent behavior pattern. We tested the hypothesis that basking behavior of Eastern painted turtles (*Chrysemes picta picta*) changes as individual turtles become acclimated to a novel experimental environment. Individual turtles were placed in an environmental chamber with water and a fixed platform for basking. Turtle basking on the platform was video recorded for up to 14 days. Carapace temperature was recorded at three minute intervals using iButton thermochrons. Analysis of video and temperature data was used to evaluate daily basking patterns. We present information on the frequency and duration of basking events and assess the time required for *Chrysemys picta* acclimation to our environmental chamber. Determination of acclimation time will facilitate design of future experiments to further assessing basking behavior.

RECURSIVE RESETTING IN NEURAL NETWORKS

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Neurons are excitable cells that are silent most of the time and only briefly produce a burst of electrical activity called action potentials (APs) in response to inputs received from other neurons. The main mechanism used by neurons to respond and adapt to environmental stimuli is through changing their firing frequency proportional to inputs received. The relationship between the external stimulus timing and the change in the firing rate of the neuron is called a phase resetting curve (PRC). Our work will focus on investigating numerically the relationship between the shape of the external perturbation and the PRC. For this purpose, a model neuron will be used to map the effect of external perturbations, such as the amplitude, duration, rate of change of inputs from other neurons, and the PRC. The objectives of the project are to investigate the relationship between the PRC and biologically relevant control parameters, such as the amplitude, duration and rate of change of external inputs in a realistic model neuron.

EPIGENETIC MODIFIERS 5-AZACYTIDINE AND TRICHOSTATIN A ALTER ADIPOSE-DERIVED STEM CELL GENE EXPRESSION

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Adipose derived stem cells (ADSCs) are multipotent, mesenchymal stem cells that are found within the microvasculature of adipose tissue. While ADSCs have the potential to differentiate into multiple cell lineages, they cannot match the differentiation potential of pluripotent stem cells. ADSCs can be epigenetically manipulated in order to increase their developmental potency. An enhanced state of ADSC developmental potency could be particularly beneficial in efforts to drive the cells into specific lineages, like skeletal muscle, that are not among those most readily produced by ADSCs. If successful, such a method could provide an easily-accessible source of autologous myogenic cells for skeletal muscle regeneration and tissue engineering. We hypothesized that exposure to the histone deacetylase inhibitor trichostatin A, and prevention of DNA methylation by 5-azacytidine, would alter the epigenome of ADSCs in a way that enhances developmental potency and enables more efficient myogenic differentiation. Our results suggest that the epigenetic modifiers did indeed alter gene expression in ADSCs. We observed changes in the expression of genes associated with enhanced differentiation potential as well as genes associated with the myogenic lineage. In the future, we would like to optimize the combination of epigenetic modifiers in order to generate ADSCs with the most myogenic potential. We will then combine those cells with a porcine acellular muscle matrix scaffold to study the potential of ADSCs to be used in skeletal muscle tissue engineering and regenerative medicine.

DEVELOPMENT OF A BICISTRONIC VECTOR SYSTEM TO TEST ANTI-HIV 1 SIRNAS THAT TARGET THE ACCESSORY PROTEIN VIF

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The Human Immunodeficiency Virus (HIV) is a retrovirus that lowers the competency of the immune system by infecting and destroying CD4+ T-helper lymphocytes. The HIV genome is composed of nine genes, one of which encodes an accessory protein called the Viral Infectivity Factor (Vif), which inhibits an innate anti-retroviral immune response by facilitating the ubiquitination and degradation of a host protein called Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G). In the absence of Vif, APOBEC3G is packaged into progeny virions and, upon infection of the next host cell, causes hypermutation of the viral provirus during reverse transcription. This buildup of mutations inhibits provirus function and stops virus replication. Vif function may be inhibited by using the RNA interference (RNAi) pathway to silence the gene. We have previously created a series of retroviral vectors to express small hairpin RNAs (shRNAs) targeted to nucleotides 5111-5121, 5522-5242, and 5551-5571 of the Vif gene within the HIV-1 NL43 genomic clone (Accession number M19921). The goal of this study is to create a bicistronic expression plasmid to test the efficacy of anti-Vif shRNAs. This plasmid will allow for both indirect and direct measures of RNAi effects by expressing a Renilla Luciferase (LucR) and a HA-epitope tagged Vif fusion gene linked by the Thosea asigna T2A polypeptide cleavage sequence.

MARIJUANA Δ9-TETRAHYDROCANNABINOL INDUCES UNIQUE CHANGES IN THE MURINE GUT MICROBIOME THROUGH INDUCTION OF MYELOID-DERIVED SUPPRESSOR CELLS AND T HELPER 17 CELLS.

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Δ9-tetrahydrocannabinol (THC) is the main psychoactive ingredient found in the Cannabis plant. THC exerts its effects through binding to both cannabinoid CB1 and CB2 receptors as a partial agonist. Synthetic THC is currently being used to treat anorexia in people with HIV/AIDS, and has been approved for use in people with multiple sclerosis, neuropathic pain, and spasticity, among others. Thus, despite its illicit status in many countries, the therapeutic potential for THC is high.

The mammalian intestine harbors a diverse array of bacteria that are constitutively referred to as the microbiome. As such, the gut microbiome has gained attention in recent years for its vast implications regarding human health and disease. Here we use an acute and chronic model of murine THC administration to study the effects of THC on the naive murine immune

system, and to see how these immune changes relate to the flux of intestinal bacteria. Our lab has shown previously that a single dose of THC causes a migration of myeloid-derived suppressor cells (MDSCs) from the bone marrow to the peritoneal cavity. Recent work demonstrates that these MDSCs remain in the peritoneal cavity throughout chronic THC administration, where they proliferate and produce IL-6. The peritoneal IL-6 leads to an increase in T helper 17 (Th17) cells in the mesenteric lymph node (mLN). The alteration in the gut immune cells occurs in tandem with an increase in the number of Alphaproteobacteria in the cecum and feces of mice treated with THC compared to vehicle and naÃ¯ve mice.

DETERMINING THE SEQUENCES INVOLVED IN MPING TRANSPOSITION

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Transposable elements (TEs) are segments of DNA that are mobilized from one location to another within a genome, often creating mutations. The TE we study is a 430 base pair element called *mPing*, which requires three components to be mobilized: transposase proteins (TPase and ORF1), terminal inverted repeats (TIRs) located at its extreme ends, and target site duplications (TSDs) flanking the element. The transposase proteins bind to the TIRs and TSDs of the transposable element to form the transposition complex. A mutant version of *mPing*, called *mPing20*, was discovered from a mutagenesis strategy and has a nearly 1.5x higher transposition rate than that of mPing, suggesting that some or all of the seven base pair changes to the middle of the element function to promote transposition.

The goal of this project is to identify the TIR sequences required for mPing transposition as well as determine which of *mPing20*'s base changes are responsible for its increased transposition. ADE2 reporter constructs containing mutant and control elements were assayed in yeast to determine the transposition rates. We found that for *mPing*, all TIR bases are not equally necessary for transposition to occur. Highly conserved bases are more critical to the formation of the transposition complex. We expect that *mPing20* transposition rates will be adversely affected after mutation of any of its transposition promoting base pairs. Combined, these results assist in providing a clearer picture of the role of the TIR and internal sequences in formation of the active transposition complex necessary for mPing transposition

A 1ST GENERATION AMPEROMETRIC GALACTOSE BIOSENSOR

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Biosensors are analytical devices that can be used to detect markers implicated in various disease states. 1st generation amperometric biosensors rely on a chemical reaction between an analyte and a specific oxidase enzyme that results in the production of hydrogen peroxide (H_2O_2.) The resulting peroxide is subsequently oxidized at a working electrode, and this oxidation generates a current that is proportional to the amount of analyte present.

We present our current findings toward the development of a 1st generation biosensor for the detection of galactose, a sugar molecule implicated in the disease galactosemia. These findings will include the advantages of using silane-generated xerogels and a urethane layer for improving the selectivity and increasing the linear sensing range of the biosensors. The responses of the biosensors to common interferents will also be presented. By targeting the sugar, the biosensor could offer a new clinical method for the detection and diagnosis of galactosemia, and serve as a model system for the detection of other clinically relevant molecules through a similar design.

DEVELOPMENT OF PRUSSIAN BLUE MODIFIED ELECTROCHEMICAL SENSORS STABILIZED WITH NICKEL HEXACYANOFERRATE.

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Prussian Blue modified electrodes have been shown to be useful in the detection of hydrogen peroxide. While the Prussian Blue sensors are sensitive and have a large linear range, the lifetimes of these sensors are limited. An initial investigation of the inclusion of nanoarrays of Prussian Blue within a matrix of nickel hexacyanoferrate has been undertaken. The sensitivity and lifetimes of these modified electrodes has been compared to electrodes with both complete and partial coverage with Prussian Blue.

SOLVENT-ISOTOPE EFFECTS ON THE 2,4'-DIHYDROXYACETOPHENONE DIOXYGENASE REACTION

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Oxidation of 2,4'-dihydroxyacetophenone (DHA) by the enzyme, 2,4'-dihydroxyacetophenone dioxygenase (DAD), produces benzoic acid and formic acid in the presence of oxygen. The DAD reaction is unique in that it cleaves a carbon-carbon bond of the alkyl group of the aromatic ring of DHA, instead of directly on the ring as seen in the intradiol and extradiol dioxygenases. The mechanism for this reaction is currently unknown. Possible mechanisms for the oxidative cleavage of DHA by DAD can be narrowed down by measuring solvent isotope effects; specifically, observing the effect that heavy water (D_2O) has on the reaction rate. The rate of the reaction in water will be compared to the rate in D_2O. If the rate of the reaction in D_2O is found

to vary from that in water, this will indicate that a solvent-exchangeable proton is involved in the rate-determining step of the reaction. The observation of a solvent isotope effect and any associated pK_a will assist in the interpretation of the reaction mechanism.

DEVELOPMENT OF AN MPING-BASED ACTIVATION TAG FOR ZEBRAFISH MUTAGENESIS

Tiana Chandler and C. Nathan Hancock
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Transposable elements (TEs) are DNA sequences that move from one location in the genome to another. A transposable element used frequently for mutagenesis is the element known as *mPing*, first discovered in rice (*Oryza sativa*). In order for *mPing* to transpose from one area of the genome to another it must be provided the proteins ORF1 (Open Reading Frame 1) and Transposase (TPase). This element also preferentially inserts upstream or downstream of genes. This preference can be advantageous in regards to inducing mutations that affect gene expression. One technique of mutagenesis utilized is the use of activation tags, which is an insertional sequence that contains enhancer elements thereby inducing overexpression of nearby genes. To make *mPing* into an activation tag, we inserted the enhancer sequence from the *Xenopus laevis* Elongation Factor 1 promoter into a hyperactive version of *mPing, mPing20*, creating *mPing20X*. A yeast transposition assay showed that *mPing20X* transposes at rates similar to *mPing*. *mPing20*X was then inserted into the reporter gene mCherry to function as a visual marker for transposition. Along with the mCherry:*mPing20X* reporter, a separate construct containing an ORF1 ONE and TPase genes fused together using a T2A peptide was made. To test these constructs in vivo, they will be injected into zebrafish (Danio rerio) an excellent model organism for vertebrate biology. Fish displaying mCherry expression will indicate that transposition of *mPing20X* is occurring.

3D IMAGING SYSTEM FOR SCREENING AND DIAGNOSING CERVICAL CANCER

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Cervical cancer is the third most common cancer affecting women worldwide. We present prototype hardware and software for a low-cost novel 3D imaging system for screening cervical cancer. Our system uses a small pen camera and a focus-tunable liquid lens to construct 3D endoscopic images from a series of 2D images taken at different focus settings. Our software is able to extract depth information from what is "in focus" in each image and process this with a constructed all-focus image to produce the final 3D result. In developing the system, we encountered problems related to image distortions, temperature changes, and image stability but were able to find solutions to them based on developed software and adjusted hardware parameters.

EXPRESSION OF HEART-SPECIFIC FLUORESCENT REPORTER PLASMIDS IN CIONA INTESTINALIS JUVENILES

Brooke Davis and Heather Evans-Anderson
Winthrop University

Ciona intestinalis, more commonly know as sea squirts, are sessile invertebrates that have many benefits as a model system to study developmental biology. Our focus is on heart development and in this study we will utilize three different plasmids to generate transgenic embryos with hopes of getting the Ciona intestinalis to express a fluorescent reporter in the developing juvenile heart. Plasmids will be delivered to Ciona embryos via electroporation immediately following fertilization and dechorination. Fluorescent reporter expression will be driven by the well characterized Mesp promoter, which has been shown to be expressed in the heart progenitor cells in Ciona larvae (Stolfi A et al. 2010). After metamorphosis, the heart progenitor cells become differentiated into cardiac cells that form the heart in the early juvenile stage. Expression of the Mesp-driven reporter genes has not been examined in the post-metamorphic stages of Ciona development. Three different fluorescent reporters will be tested: Mesp-H2B-cherry, Mesp-H2B-venus, and Mesp-H2B-GFP in order to determine which one will remain detectable in the juvenile stage when the heart has formed. Once the conditions for expression of a fluorescent reporter in the juvenile heart are established, this will allow for further studies in which the growth of the heart can be quantitatively analyzed via fluorescent microscopy.

CLONING A SIRNA TARGETED TO HIV-1 VIF

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The Human Immunodeficiency Virus (HIV-1) infects and destroys CD4+ T-lymphocytes resulting the gradual loss of immune function and the appearance of the Acquired Immunodeficiency Syndrome (AIDS). HIV-1 expresses a small accessory gene known as the viral infectivity factor (Vif), which functions to ensure viral replication by blocking the function of Apolipoprotein B mRNA editing enzyme-catalytic polypeptide-like 3G (A3G). In the presence of vif, A3G polyubiquitinated and degraded by proteasome; however, in the absence of Vif, A3G is packaged in progeny virions and induces hypermutation of the viral genome in the subsequently infected cell. One method to inhibit Vif function is the use of small interfering RNAs (siRNAs), which target and induce mRNA degradation through the RNA interference (RNAi) pathway. To test this hypothesis, a short hairpin RNA (shRNA) was designed to target Vif mRNA at nucleotides 5111-5131 in the HIV-1 NL43 genomic clone (Accession number M19921). The resulting shRNA was synthesized as dsDNA and cloned the shuttle vector, pSRNG, to

generate an expression cassette that expresses the shRNA from the RNA Polymerase III H1 promoter. The H1si5111 expression cassette was amplified from the shuttle vector and cloned into the retroviral vector, pLGN which expresses two selectable markers: eGFP and neomycin phosphotransferase. Future tests will analyze the ability of Vifsi5111 to inhibit HIV function.

EXPLORING THE THERMAL AND VISCOUS INSTABILITIES IN RELATIVISTIC, RADIATIVE, VISCOUS HYDRODYNAMIC SIMULATIONS OF THIN DISKS

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Many analytic, semi-analytic, and even some numerical treatments of blackhole accretion parametrize the stresses within the disk as an effective viscosity, even though the true source of stresses is likely to be turbulence driven by the magneto-rotational instability. Despite some attempts to quantify the differences between these treatments, it remains unclear exactly what the consequences of a viscous treatment are, especially in the context of the temporal and spatial variability of global disk parameters. We use the astrophysics code, Cosmos++, to create two accretion disk simulations using alpha-viscosity, one thin and one thick. These simulations are then compared to similar work done using MHD in order to analyze the extent of the validity of the alpha-model. One expected result, which we, nevertheless, demonstrate is the greater spatial and temporal variability of MHD. In addition, we also examine potential evidence for the viscous instability seen within our 3-D simulations using MHD, of which has yet to ever be seen in numerical simulations. The instability is a consequence of alpha when it becomes a non-monotonic function of the surface density, and is thought to be important in the explanation of accretion disk behavior. We create 2-D and 3-D simulations recreating this viscous instability to examine the structure, evolution, growth rate and saturation amplitude.

SIRNA MEDIATED DOWNREGULATION OF HIV-TAT IN ANTI-TAT SIRNA PROTECTED LYMPHOCYTE POPULATIONS

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The Human Immunodeficiency virus (HIV-1) targets and kills CD4+ T-lymphocytes. The gradual destruction of CD4+ cells causes a generalized loss of immune function, eventually leading to increased infections by a number of opportunistic pathogens, which is characterized as Acquired Immunodeficiency Syndrome or , AIDS. HIV-1 is a lentivirus that expresses a number of regulatory and accessory genes, which function in virus replication. One such gene encodes the regulatory proteins called the transactivator of transcription (Tat). Tat is a small protein that is among the first expressed during virus replication and functions to upregulate RNA Polymerase II transcription from the viral promoter through its interaction with the viral transactivation response element (TAR) , which is responsible for upregulation of virus production. In the absence of Tat, viral transcription is poorly initiated and viral replication is inhibited. One way to inhibit Tat is through the use of a retroviral vector to express small interfering RNAs (siRNA) that target and direct mRNA cleavage through the RNA Interference (RNAi) pathway. To test this hypothesis, we have designed short hairpin RNAs (shRNAs) to target three Tat sites within the HIV-1 NL43 genomic clone (Accession number M19921). Each of these shRNAs were synthesized as dsDNA and cloned into the retroviral vector pLGN, in which they are expressed from the RNA Polymerase III H1 promoter. In order to assess the anti-Tat activity of these shRNAs, recombinant retroviral particles will be generated and used to transduce CD4+ T cell lines. Following selection of stably transduced cells, HIV challenge assays using a Renilla luciferase expressing HIV-1 genomic clone (pNL43.T2A.Luc) will be carried out using the siRNA-protected CD4+ T cell populations. The ability of each siRNA to inhibit HIV replication will be assessed by luciferase assay, quantitative polymerase chain reaction, and p24 assay.

PASSIVE ACOUSTIC MONITORING OF BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*) - FIELD AND LABORATORY CALLING PATTERNS

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The acoustic monitoring of underwater fauna primarily focuses on various marine species. Sonar is used by military and fishing industries for detection, classification and tracking of marine organisms. Passive sonar (listening) proves valuable in understanding organisms within the planet's waters; yet, few records of freshwater fish sounds have been documented. The following research supplements call data of the freshwater sunfish *Lepomis macrochirus*, commonly known as bluegill. Bluegill are North American natives, no larger than 2kg, found in freshwater bodies from California to South Carolina. The few existing studies of freshwater fish acoustics suggest that bluegill primarily call during breeding season, however this research reflects their nonbreeding calling patterns. Field diurnal calls were recorded within isolated-species-ponds of The Cheraw Fish Hatchery in SC. A hydrophone-equipped recording unit was programed to collect acoustic data at 30-minute intervals and was set beside the bluegill pond for one-week-periods in two separate months. Calls are typically short; low frequency "grunts" under 1 KHz, having one to many palpitations per call. Preliminary analysis indicates bluegill call most actively between noon and dusk, with a shorter stretch of activity at dawn each day. In the Francis Marion University greenhouse, eight adult-sized bluegill from the hatchery shared a 190-liter tank and were recorded during select 24-hour

periods. This on-campus laboratory data is progressively being reviewed, but preliminary analysis shows similarity to the field data. Hopes to build on this work in the future include: breaking down calls by type and understanding their role in nonbreeding and breeding communication.

GENERATING MEF2CA AND MEF2CB TRANSGENIC ZEBRAFISH LINES USING BAC-MEDIATED RECOMBINATION

Kenneth Glenn and April Delaurier
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The purpose of this research is to study the dynamic role of mef2ca and mef2cb in craniofacial skeletal development and muscle development. We plan to make transgenic lines that will express fluorescent transgenes in an endogenous fashion to both mef2ca and mef2cb, allowing tracking of gene expression in living fish, which can be correlated with connective tissue and heart patterning. The mechanism for this project involves the transformation of bacterial artificial chromosomes containing wither mef2ca or mef2cb into DY380 E.coli cells. Then, Phusion PCR products will be electroporated into the transformed DY380 cells. Once integration has been confirmed, the construct will be injected into single-celled zebrafish embryos which will then be screened for the expression of these fluorescent transgenes. The patterning of these areas can be studied because the family of Myocyte enhancing factor 2 (Mef2) transcription factors are important regulators of muscle formation. Although mef2ca and mef2cb are both orthologues of the human MEF2C gene, mef2cb is more closely related to MEF2C. Of the thirteen closest genes surrounding MEF2C in humans, mef2cb is proximal to twelve of them. This is significant because studies have shown that mutations of the MEF2C gene are linked with heart defects in humans and craniofacial defects in zebrafish. Therefore, tracking gene expression of mef2ca and mef2cb could be advantageous for the study of both craniofacial and heart development in zebrafish and in elucidating the cell types and timeframe in which these genes are expressed.

THE INFLUENCE OF PERCEIVED CONTROL OVER TASK DIFFICULTY ON COPING WITH MATH ANXIETY

Christine Hartmann and Keri Weed
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The purpose of this study was to examine the relationship between self-reported math anxiety and performance on an addition verification task (AVT). The sample for this study consisted of 19 right-handed female undergraduates, with a mean age of 18.76 (range = 18-23). We employed a 2 (math anxiety level: high, low) by 2 (condition: choice, no choice) between subjects factorial design. Participants in the choice condition chose the difficulty level of their AVT, those in the no choice condition were not given a choice. Participants were assigned to the high or the low anxiety group based on a self-report math anxiety rating scale. Dependent variables were monitored through emotional, physiological, and behavioral measures. We hypothesized accuracy would be greater and reaction time shorter in groups that perceive they have control of their AVT difficulty because participants who perceive they have control of their AVT difficulty will be able to better allocate psychological coping resources during the math task. Through analysis of perceived difficulty of the AVT between the choice and no choice conditions a nonsignificant trend in support of our hypothesis which showed that the no choice group felt that the AVT was more difficult ($M = 3.71$, $SD = 1.98$) than the choice group ($M = 2.43$, $SD = 2.15$). Participants with high math anxiety reported more perceived anxiety than those with low math anxiety on the AVT, $F(1, 11) = 14.77$, $p = .05$. Preliminary analysis of EDA was insignificant. Data collection for this study is ongoing.

KARYOPHERIN DISTRIBUTION AND EXPRESSION IN THE EARLY DEVELOPMENT OF THE SEA URCHIN

Devon Hathaway, Paul Siegwald, Greg McFadden, Melanie Overcash, and Christine Byrum
College of Charleston

Importins (IPOs) and transportins (TNPOs) are karyopherin-beta (KAP-beta) proteins that move important cargo such as transcription factors into and out of the nucleus. Recent research indicates that some karyopherins are misexpressed in late stage cancers and Alzheimer's disease. Expression of KAP-beta proteins is likely integral to early development of the sea urchin, *Lytechinus variegatus*. Partial sequences of seven IPOs and two TNPOs were cloned from *L. variegatus* embryos at six developmental stages, ranging from the two-cell to pluteus stages. Using wholemount *in situ* hybridization (WMISH) we will produce a map depicting distribution of IPO and TNPO mRNA. Our research has shown that IPO11 expression is highest in the gut, oral, and vegetal regions while IPO5, IPO9, and Transportin 1/2 (TNPO1/2) are expressed ubiquitously throughout the embryo. We are interested in how this may impact cell fate specification by limiting nuclear localization of TFs.

PRODUCT INHIBITION IN THE REACTION OF 2,4'-DIHYDROXYACETOPHENONE DIOXYGENASE

Ineisha Herrington and Kenneth Roberts
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The enzyme, 2,4'-dihydroxyacetophenone dioxygenase (DAD), converts 2,4'-dihydroxyacetophenone (DHA) into benzoic acid and formic acid by insertion of molecular oxygen. Under saturating conditions, as the reaction progresses, the rate of formation unexpectedly decreases over time. One explanation for this decrease in rate is that the reaction is inhibited by a product. To understand the decrease in rate, product inhibition studies are being investigated. This study will evaluate the kinetics of the reaction across different concentrations of benzoic acid and/or formic acid. The rate of the reaction will be measured using UV-VIS spectroscopy, as the UV absorbance spectrum changes from substrate to product. If the product is in

fact inhibitory, a decrease in rate is expected. If the product is not inhibitory, the rate will remain the same. Analysis of product inhibition could provide insight into why the reaction slows down over time as well as information into allosteric effects of the reaction.

CHARACTERIZATION AND CLONING OF A DEVELOPMENTAL MUTANT ALLELE IN C. ELEGANS

Taylor Hinds and Daniel Williams
Coastal Carolina University

Development of an animal from zygote to adult with the correct body plan is a complex process that encompasses cell fate specification and cellular and tissue morphogenesis. The genetic model organism *C. elegans* is well suited to address developmental questions because of their defined cell lineage, quick growth rates, and high number of offspring. Our lab has isolated a mutant allele (*myr1*) with an incompletely penetrant notched-head phenotype that resembles Eph receptor tyrosine kinase signaling mutants with epithelial morphogenesis defects. Current work is focused on characterizing *myr1* and mapping it to one of the 6 *C.elegans* chromosomes in an effort to identify the mutated gene. This work could increase our understanding of Eph signaling in epithelial morphogenesis.

A 1ST GENERATION AMPEROMETRIC GALACTOSE BIOSENSOR

Gillian Horn, Amanda Burton, and Will Case
Converse College

Biosensors are analytical devices used to detect specific molecules that serve as potential markers in disease detection. 1st generation amperometric biosensing has become a promising strategy for the detection of clinically relevant molecules. In 1st generation biosensing, an analyte reacts with its specific oxidase enzyme to generate hydrogen peroxide (H_2O_2). The peroxide molecules are then oxidized at a working electrode and generate a signal that is an indirect measure of the amount of analyte present.

This poster presents our current findings toward the development of a 1st generation amperometric biosensor for the detection of galactose, a molecule linked to the disease galactosemia. Our research investigated the use of silane-based xerogel as enzyme immobilization scaffolds and their effect on sensitivity and linear stability. The benefits of incorporating an outer layer membrane were also studied as well as the ability of the biosensor to discriminate against common interferents. Xerogel-based amperometric biosensors could provide a new method for diagnosing galactosemia and may lead to the development of an adaptable template capable of signaling an array of diagnostic molecules.

COMPUTATIONAL METHODS FOR PREDICTING AEROELASTIC FLUTTER

Katelynn Huneycutt, Spencer Wilder , Jason Howell, and Justin Webster
College of Charleston

Aeroelastic flutter is a self-excited instability which can occur when a thin elastic object is immersed in a fluid flow. We considered axial flow flutter of a cantilevered beam. In this configuration, flutter occurs for lower fluid velocities, with larger characteristic beam displacements in comparison with the well understood panel models. Using finite difference simulations on a linear piston-theoretic beam, we ascertained relationships between key parameters and the onset of instability (flutter); our results were corroborated with a modal stability analysis. We also examined the effect of including an extensible nonlinearity in the model.

LOW- DIMENSIONAL CHAOS IN ONTOGENETIC MICE INJECTED WITH COCAINE

Julia Imperatore and Sorinel Oprisan
College of Charleston

We used optogenetic mice to investigate the response of the medial prefrontal cortex (mPFC) local network to light stimuli delivered by a 473 nm laser through a fiber optics. Local field potential (LFP) recordings obtained with an optrode were band-pass filtered online between 0.1 and 130 Hz. The entire experimental protocol consisted of two successive two-second long recordings in response to (1) a 40 Hz, 10-pulses train, that lasted 250 ms with 10 ms pulse duration followed by a 15 ms break, and (2) a single pulse with 10 ms duration. We analyzed the response of the network to a single 10 ms duration light pulse using delay embedding method on optogenetic mice prior to and after cocaine administration. We found that the dynamics could be reconstructed in a three-dimensional space. Our results open the possibility of designing a low-dimensional model for optical stimulation of the local network.

EFFECT OF BOT FLY (*CUTEREBRA FONTINELLA*) PARASITISM ON THE MOVEMENT AND MICROHABITAT SELECTION OF WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*)

Allison Johnson and Jonathan Storm
University of South Carolina Upstate

White-footed mice (*Peromyscus leucopus*) are the preferred host for the bot fly (*Cuterebra fontinella*). As part of a mark-recapture study, we assessed the movement and microhabitat selection of white-footed mice infected with bot flies. We found no difference in the rate of infection between male and female mice and there was no correlation between the density of mice and bot prevalence. Bot-infected mice did not differ from uninfected mice in their mean squared distance from center of activity (MSD) and there was no shift in their center of activity between the May (bot-free) and August (bot-infected) periods. During May, mice that became infected did not differ in MSD from mice that did not become infected, suggesting that large movements do not increase the risk of infection. We also found no difference in stem density and downed woody debris (DWD) around traps that captured infected and uninfected mice. Our data suggest that bot infection has little impact on the movements of white-footed mice.

MPING AS A TOOL FOR TRANSPOSON MUTAGENESIS IN ZEBRAFISH

Alec Jones and April Delaurier
University of South Carolina Aiken

The goal of this project is to demonstrate the successful in vivo transposition of the mobile element mPing, from Oryza sativa (rice), in zebrafish. mPing is a 430-bp, class II miniature inverted-repeat transposable element (MITE), which is mobilized by two enzymes: ORF1, which contains a DNA recognition domain, and TPase, which contains a catalytic DDE domain. mPing, like many invertebrate transposons, has yet to be tested for activity in a vertebrate organism, yet may serve as an effective tool for transposon mutagenesis in vertebrates, such as zebrafish. A single iTol2 expression vector, containing the CMV immediate early promoter driving expression of mmPing20x-interrupted mCherry, will be co-injected with both Tol2 transposase mRNA and mRNA of ORF1-T2A-TPase. The expression vector also contains a cmlc2:eGFP transgenesis marker labelling cardiac cells, to check for plasmid integration. Successful rates of transposition will be determined in injected F0 fish by the ratio of mCherry expressing fish to the number of fish with cardiac eGFP expression. This will also permit us to determine the rate of transmission among F1 fish, and to potentially establish a line of fish containing mmPing20x, and remobilize this element in subsequent generations via injection of ORF1-T2A-TPase mRNA. The results of this study will form the basis to future research to use mmPing20x containing a Xenopus-derived EF2Î± enhancer as an activation tag in zebrafish as a tool for novel gene discovery.

ABUNDANCE AND DISTRIBUTION OF MICROPLASTIC PARTICLES IN WINYAH BAY, S. C.

Dillon King and George Boneillo
Coastal Carolina University

Plastics are durable synthetic organic polymers that are found in consumer products such as plastic bags, toys, facial scrubs and monofilament line used to manufacture fishing nets. Microplastics are generally defined as plastic debris ranging from 0.33 to 5 millimeters in size, while macroplastics are defined as plastic debris greater than 5 millimeters in size. Microplastic pollution in the environment is a global concern. These small particles float near the surface of the water and do not degrade rapidly. Microplastics can carry toxic contaminants throughout ecosystems and are easily ingested by aquatic organisms. The consumption of these particles can be harmful to organisms by causing endocrine disruption, slowed growth rates, blocking of the digestive tract and entanglement. Winyah Bay is a large estuarine system that receives freshwater input from the Waccamaw River, Sampit River, Black River, and Great Pee Dee River. Winyah Bay flows into the South Atlantic Bight which borders the subtropical North Atlantic Gyre. The objective of this study is to quantify the abundance of microplastics in Winyah Bay and the surrounding rivers within an 8-month time span. Additionally, this study will compare microplastic sampling techniques. Water samples will be collected using plankton nets with two different mesh sizes, as well as whole water samples to determine microplastic size fractions that could be underestimated using net sampling techniques. We will present preliminary results from the first series of sample collections.

SYNTHESIS OF PHIDIANIDINE ANALOGS CONTAINING 1,2,3- TRIAZOLES

David Laws and Bryan Wakefield
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Phidianidine is a compound that has been isolated from the sea mollusk Phidianis militaris. Phidianidine contains a 1,2,4-oxadiazole ring and exhibits interesting biological activities such as cytotoxicity in rapidly replicating cells and partial agonism of the μ-opioid receptor. The phidianidines have three distinct regions: the indole, heterocycle and linker. The goal of this project is to synthesize analogs of phidianidine where the 1,2,4-oxadiazole ring is replaced with a 1,2,3-triazole with varying indole and linker regions. This can be accomplished through the copper-catalyzed cyclization of 3-propargylindole with azide. This reaction has been realized with benzyl azide and now work is ongoing to construct fully functionalized azido-linkers for this reaction.

EVALUATION OF THE TOXIC EFFECTS OF GLYPHOSATE, 2,4-DICHLOROPHENOXYACETIC ACID, AND THEIR COMBINED FORMULAS ON EARTHWORMS (*EISENIA FETIDA*)

Caitlin Lazurick, Nicole Lidzbarski, Rachel Owings, Jeff Brotherton, and Edna Steele
Converse College

The emergence of genetically engineered crops has dramatically increased herbicide use as farmers are able to crop dust instead of spot treat weeds. Among the widely-used active ingredients of herbicides are glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D). Such chemicals are the main ingredients of Roundup® and Trimec®, respectively and are now formulated in a combined form called Enlist Duo® to control resistant weeds. However, these herbicides are considered possible carcinogens by the International Agency for Research on Cancer (IARC). Therefore, it is concerning that the herbicides could negatively impact organisms that come in contact with treated plants and soil. For example, earthworms are essential to the decomposition, aeration, and nutrients of soil. Ill effects from herbicide use could result in nutrient deficient soil and earthworm dependent species declining. We investigated the effects of 2,4-D and glyphosate at various concentrations (27-270 µg/cm2) and their combined formulas on the mortality, reproduction, and weight of earthworm populations. We conducted tests in both sterilized and unsterilized artificial soil in order to determine the effects of the herbicides on a scale with and without the influence of bacteria that are capable of breaking down 2,4-D. However, despite seeing ill effects in direct contact trials at the same 2,4-D concentrations, we saw no significant differences in the populations treated with the tested concentrations or combinations. This research indicates that the herbicides, used together or individually, could not reach the worms in the soil. This might be important in determining how we use herbicides in the future.

INVESTIGATING THE TOXICITY AND ACCUMULATION OF A MEDICALLY IMPORTANT HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) USING EARTHWORM AS A MODEL SYSTEM

Nicole Lidzbarski, Caitlin Lazurick, Rachel Owings, Jeff Brotherton, and Edna Steele
Converse College

Glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D), the active ingredients in Roundup® and Trimec®, are herbicides widely used in agriculture and in many other areas including residential. A new, combined formulation known as Enlist Duo® is especially effective in controlling tough broadleaf weeds and grasses. Food crops are being genetically modified to resist both of these herbicides, and there will likely be a significant increase in the agricultural use of Enlist Duo® to control herbicide-resistant weeds. As both chemicals will be applied directly to leaves, the safety of other organisms that come into direct contact with vegetation or contaminated soil needs to be investigated. This might include humans since some reports suggest that 2,4-D is possibly carcinogenic, and glyphosate is probably carcinogenic.

Earthworms are an important part of the soil ecosystem. Not only are they in direct contact with the soil, but they also ingest the soil containing organic matter along with any contaminants. As earthworms constitute a food source for other organisms, bioaccumulation is possible. In this toxicity study, we investigated the responses of earthworms exposed by direct contact or by ingestion of treated organic material to various concentrations of 2,4-D, glyphosate, or both. HPLC methods to detect and quantify 2,4-D and glyphosate in worm extracts were developed and were used to determine possible bioaccumulation of 2,4-D and glyphosate.

Our results show 50% mortality in earthworms exposed to 6.5 mg/mL of glyphosate or 1.0 mg/mL of 2,4-D. However, when tested with both herbicides in a ratio similar to Enlist Duo®, we observed 50% mortality in earthworms exposed to only 0.8 mg/mL of glyphosate and 0.7 mg/mL of 2,4-D. This indicates that under direct contact conditions, there is a synergistic effect of the herbicides on earthworms. HPLC analysis demonstrated that uptake of both 2,4-D and glyphosate is detectable in earthworms exposed by direct contact. Furthermore, we detected glyphosate uptake in soil-treated and plant-treated worms. There was no evidence that the presence of 2,4-D changed the uptake of glyphosate. Results of this study may provide valuable information for future toxicity studies on a larger scale.

UNDERSTANDING THE FUNCTION OF KDM1A USING CRISPR/CAS-9 IN ZEBRAFISH

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University of South Carolina Aiken

The purpose of this research is to understand the function of the lysine demethylase 1a (kdm1a) gene of the PHF21A complex in human Potocki-Shaffer Syndrome (PSS) using the clustered regularly-interspersed short palindromic repeat (CRISPR) and associated Caspase 9 (Cas9) system in zebrafish. PSS is a genetic disorder that follows an autosomal dominant inheritance pattern in which symptoms include craniofacial abnormalities and intellectual disabilities. These anomalies are caused by a mutation on chromosome 11, resulting in the deletion of the p11.2 p11.12 band. One goal of this research is to create a stable line of zebrafish carrying a mutant form kdm1a in order to study the phenotype-genotype correlation of loss of kdm1a. Targeted mutagenesis in this gene was completed by co-injecting a guide RNA (gRNA) targeting kdm1a and nuclear-localized nCas9n mRNA into the one cell stage of zebrafish embryos. These embryos were screed using a T7E1 assay for mutations. Founder fish were identified, raised, and outcrossed in order to test germline transmission to the F1 generation via T7E1 assay. Positive F1 fish were sequenced in order to establish the nature of mutations. To date, we have two lines showing frameshift mutations in the kdm1a gene. These fish will be in-crossed and F2 offspring will be screened for phenotypes. Ultimately, we want to establish the effect a kdm1a knockout can have on craniofacial development and development on other organs such as the brain and spinal cord, with a goal of understanding the role of kdm1a in PSS.

PROTEIN-PROTEIN INTERACTIONS ASSOCIATED WITH A PUTATIVE CHLOROPLAST SPLICEOSOME

Alexandra Margets and Michelle M. Barthet
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Many may think that spliceosomes are present only in the eukaryotic nucleus but the chloroplast of land plants possibly have their own spliceosome. A spliceosome is a large complex of proteins and RNAs required for the removal of introns from premature RNA transcripts. Maturase (MatK) is a group IIA intron maturase found in the chloroplast of most land plants, and is postulated to have a critical role in chloroplast intron excision. Other proteins such as that of WTF1 and RNC1 have been shown to interact with the same target introns as MatK suggesting a putative chloroplast spliceosomal complex. Co-immunoprecipitation followed by immune-detection methods were used to define protein-protein interactions associated with the MatK maturase in an aim to discern associated factors of chloroplast intron excision and existence of a chloroplast proto-spliceosome. Preliminary findings from interaction studies will be discussed along with a model of proteins involved in group IIA intron excision in the chloroplast of land plants.

CULTURING MURINE ADIPOSE-DERIVED STEM CELLS AS SPHEROIDS IN THE PRESENCE OF TRICHOSTATIN A AND 5-AZACYTIDINE ALTERS GENE EXPRESSION

Elizabeth Mcabee and Matthew Stern
Winthrop University

Stem cells are undifferentiated cells that have the capability to differentiate into one or more cell lineages. Adipose-derived stem cells (ADSCs) are multipotent, mesenchymal stem cells that are located within the microvasculature of adipose tissue. Although multipotent ADSCs can differentiate into several cell lineages, they cannot match the differentiation potential of pluripotent stem cells such as ES and iPS cells. However, previous research in our lab shows that culturing murine ADSCs as three-dimensional spheroids can induce the expression of genes associated with pluripotency. We hypothesized that the combination of culturing ADSCs as three-dimensional spheroids and treatment with compounds that manipulate the epigenome can 1) upregulate the expression of several genes associated with enhanced potency and 2) improve the efficiency of myogenic differentiation by ADSCs. Our results support our hypothesis that culturing ADSCs as spheroids in combination with treatment with trichostatin A, a histone deacetylase inhibitor, and 5-azacytidine, an inhibitor of DNA methylation, all impact the expression of genes associated with ADSCs™ potency and/or myogenic potential. Future work includes identifying the combination of culture conditions that most efficiently enhances the myogenic potential of ADSCs. This can be tested by recellularizing porcine acellular muscle matrix scaffolds with these enhanced ADSCs in order to assess their myogenic potential. Maximizing the myogenic potential of ADSCs would allow ADSCs to serve as a plentiful source of myogenic cells for skeletal muscle tissue engineering and regenerative medicine applications.

THE CORRELATION BETWEEN ENVIRONMENTAL HYDROCARBON CONTAMINATION AND PARASITIC INFECTION IN *FUNDULUS HETEROCLITUS*

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Fundulus heteroclitus is a common estuarine fish located along the east coast and is adaptable to a wide range of temperatures, salinities, and levels of pollution. Therefore, it makes an ideal indicator of environmental health. Fifty fish were collected from the polluted waters around the Georgetown, South Carolina harbor. Each fish was weighed, measured, bled, necropsied and observed for parasites. Tissue samples from the liver, gills, and GI tracts were preserved in 10% neutral buffered formalin. Immunohistochemistry was performed to determine the presence of the aryl hydrocarbon receptor (using mAb 5B6) which served as an indicator of environmental exposure to polychlorinated biphenyls (PCBs). mAb CX5-3 was used to probe for the pro-inflammatory COX2 protein. Tissues were observed under a light microscope and compared to a control group of *F. heteroclitus* previously collected from pristine sites. In the gills of fish with no parasites present at dissection, tissues around microscopic parasites were up-regulated for one or both proteins of interest. This was not the case for the gills of fish with visible parasites at dissection. Livers from the polluted site exhibited fattier livers than the control fish. In the gastrointestinal tracts, AhR2 was more up-regulated compared to fish from pristine sites.

EXPRESSION OF VIF-RESISTANT APOBEC3G FROM A HIV-1-DEPENDENT LENTIVIRAL VECTOR

Erin Mclaughlin and William Jackson
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While current HIV treatments may reduce viral load in HIV-positive individuals, these treatments are not ultimately curative. Gene therapy has the potential to be a more effective and permanent method of controlling HIV infection. One gene therapy approach involves the delivery of anti-HIV genes to infected cells using lentiviruses. This project explores the delivery of the innate anti-retroviral protein human Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (A3G), which works to induce mutations in the HIV provirus during reverse transcription. HIV encodes the protein Vif (viral infectivity factor) that blocks A3G™s antiviral effects. This project uses a Vif-resistant human A3G that has a single amino acid change, D128K that renders A3G resistant to Vif™s effects. We have previously created a lentiviral vector, pLTG(INS2)R, which expresses Renilla lucifersase and eGFP in a HIV-1-dependent manner. D128K A3G was cloned in place

of the Renilla luciferase gene in this vector producing pATG(INS2)R. Successful transfection of pATG(INS2)R with the helper plasmids pMD2.G and pCMVR8.74 into HEK 293T cells produced recombinant virions. Currently, A3G is being modified to express an influenza hemagglutinin tag (HA tag) on its N-terminus to allow for easy detection of A3G using antibody-based assays. Once cloned, the vector will be used in challenge tests to analyze the anti-HIV activity of this reagent.

IMPROVED ALGORITHMS FOR THE THERMAL IMAGING OF EXTRASOLAR PLANETS WITH SPITZER/IRAC AND HUBBLE/NICMOS

David Melnick and Joseph Carson
College of Charleston

Understanding the orbital parameters of directly imaged exoplanets is crucial for unlocking their formation and evolution history. But such orbital information is often severely limited by an inadequate time baseline between detections. To improve this situation, we are working in parallel with the VLT/SPHERE Exoplanet survey in an attempt to achieve Hubble NICMOS archival pre-detections. The complementary Hubble analyses offer a potential 10+ year baseline between detections, while also helping to constrain the planetâ€™s spectral energy distribution. In addition to potentially enhancing sensitivities, our analysis of Spitzer IRAC Fomalhaut-b images provides a test case for our image processing pipeline and statistical method for image quality evaluation.

MICRORNA-30 MODULATES METABOLIC INFLAMMATION BY REGULATING NOTCH SIGNALING IN ADIPOSE TISSUE MACROPHAGES

Kathryn Miranda, Prakash Nagarkatti, and Mitzi Nagarkatti
University of South Carolina School of Medicine

Macrophages are innate immune cells that play integral roles in maintenance of adipose tissue homeostasis. Visceral obesity stimulates pro-inflammatory macrophage accumulation in adipose tissue causing chronic low-grade inflammation that can lead to insulin resistance and cardiometabolic disorders. Notch signaling is elevated in obesity and serves as a form of communication between macrophages and adipocytes. Blockade of this pathway promotes adipose tissue browning while reducing inflammation and therefore, has therapeutic potential. MicroRNAs (miRNA, miR) are non-coding RNAs that bind the 3'UTR of target mRNAs to repress their translation and miRNA based therapies are presently being developed for clinical purposes. In the current study, we identified differentially expressed miRNAs in adipose tissue macrophages (ATMs) between lean and obese mice by microarray. Array analysis and PCR validation revealed miRNAs -30a-5p, -30c-5p, and -30e-5p were downregulated in obese ATMs suggesting the miR-30 family plays an important role in macrophage phenotype. Pathway analysis demonstrated that miR-30 targeted Notch signaling genes including Delta-like ligand-4 (DLL4), a previously identified therapeutic target for cardiometabolic disorders. It was noted that DLL4 expression was increased on obese ATMs and in vitro miRNA transfection studies further demonstrated that miR-30 modulates Notch signaling-induced inflammation. These data demonstrate for the first time that the miR-30 family may play a critical role in the regulation of DLL4-mediated Notch signaling in ATMs, thereby modulating metabolic inflammation. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755 and P20GM103641).

INVESTIGATION OF Δ9-TETRAHYDROCANNABINOL (THC)-MEDIATED REGULATION OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS) IN MICE

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Delta-9-Tetrahydrocannabinol (THC) is the major bioactive component of marijuana. Cannabinoids produce a wide spectrum of central and peripheral effects. THC has been shown to cause immunosuppression and the immunosuppressive property of THC can be attributed, at least in part, to its ability to induce MDSCs. Myeloid-derived suppressor cells (MDSCs) are immature, potent T cell-suppressive cells of myeloid origin. They are believed to regulate immune responses in normal state as well as during inflammation, infection and cancer. They can suppress the adaptive immune response mediated by CD4+/CD8+ T cells. Additionally, MDSCs can secrete immunosuppressive cytokines and induce regulatory T cell development in certain cases. MDSCs can be of granulocytic origin, also known as granulocytic MDSCs (G-MDSCs) that are the largest population of MDSCs in tumor-bearing mice, representing >75% of all MDSCs. They suppress antigen-specific T cell responses, primarily via release of reactive oxygen species (ROS). G-MDSCs have also been found in cancer patients and as are circulating granulocytes. In the current study, we plan to investigate THC-mediated regulation of MDSCs in mice. We will further characterize MDSCs and examine their functions. In addition, we will also perform microRNAs (miRNAs) arrays and examine their regulation in G-MDSCs and Granulocytes and examine unique miRNAs.

ROLE OF MIR-34A IN AMELIORATION OF SEB-INDUCED LUNG INJURY TREATED WITH TETRAHYDROCANNABINOL (THC)

Amira K. Mohammed, Prakash Nagarkatti and Mitzi Nagarkatti
University of South Carolina School of Medicine

Staphylococcal enterotoxin B (SEB) is a highly potent CDC select agent that can trigger acute lung injury. SEB induces immune dysregulation leading to robust T cell proliferation and differentiation, as well as massive cytokine and chemokine release. Δ9-Tetrahydrocannabinol (THC) is a psychoactive ingredient found in marijuana, *Cannabis sativa*. we investigated the effects of treatment with THC of SEB-induced acute lung injury. To this end, acute lung injury was induced by a dual dose of SEB in C3H/HeJ mice, which were treated with vehicle or THC. THC-treatment led to survival of all the SEB-administered mice, while all vehicle-treated mice succumbed. THC treatment decreased the CD3+, CD4+, CD8+ and NKT subpopulations and increased the number of MDSCs in the lungs. THC also induced a significant decrease in the pro-inflammatory cytokines, IFN-γ and TNF-α in the BALF and in the levels of chemokines, CCL5 and MCP-1 in the sera and BALF as well. In order to determine the epigenetic mechanisms underlying the THC-induced beneficial effects, we performed high-throughput microRNA microarrays with lung-infiltrated mononuclear cells from vehicle and THC-treated mice. Pathway analysis demonstrated that THC treatment led to immune suppression through several mechanisms including down regulation of Let7a-5p that may be responsible for increased expression of IL-10, and down regulation of miR34-5p leading to increased FoxP3. Furthermore, Validation of the expression of miR-34a by RT-PCR with lung mononuclear cells confirmed our high throughput analysis and in silico findings. Together, THC plays a major role through epigenetic mechanisms to modulate immunological pathways that suppress SEB-induced acute lung injury. (Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20GM103641 to PN and MN and MoHESR fellowship for AKM).

PROGESTERONE LEVELS IN BLOOD VERSUS SALIVA OF BEEF CATTLE

Leah Moorefield and Staci Johnson
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Several pharmaceuticals are utilized to control the estrous cycle of lab and farm animals. The development of such regimes requires knowledge about reproductive hormone levels, which are traditionally measured through the use of blood sampling. The validity of salivary sampling when compared to blood sampling has become a topic of research in several mammalian species. The objective of this study was to assess the correlation between plasma and progesterone levels in saliva of cattle. Blood and saliva samples were taken from five multiparous Angus x Herford cows every afternoon over the course of 21 days. Plasma and saliva samples were assayed to analyze progesterone levels. No correlation (P=.17) was discovered between saliva and plasma progesterone concentration in any individual animal or in total. In conclusion, the use of salivary sampling in place of blood sampling may prove ineffective. The results of previous research are contrary to these results. Therefore, additional studies are necessary to fully understand the relationship of blood and saliva hormone levels.

PORCINE ACELLULAR MUSCLE MATRIX SCAFFOLDS SUPPORT RECELLULARIZATION BY MYOGENIC CELLS

Natalie Mseis, Carolina Pham, and Matthew Stern
Winthrop University

Skeletal muscle has a remarkable, yet limited capacity for regeneration. Severely damaged skeletal muscle is incapable of full regeneration, leaving patients with few suitable options for restoring lost muscle mass and/or function. Tissue engineering and regenerative medicine offer a potential solution for individuals with severely damaged muscle. Two components required for successful skeletal muscle tissue engineering/regenerative medicine are a source of myogenic cells and a biomaterial capable of stimulating myogenesis in vivo and in vitro. In our lab, we are producing a biomaterial from decellularized porcine muscle, which we refer to as Porcine Acellular Muscle Matrix (PAMM). Our goal is to recellularize PAMM scaffolds with C2C12 myoblasts. We hypothesize that PAMM scaffolds have the ability to promote recellularization and complete myogenic differentiation by C2C12 myoblasts and other sources of myogenic cells. Our results indicate that decellularization of porcine muscle can be achieved via two different protocols: 1) a detergent-based method and 2) a method based on actin depolymerization and hypertonic/hypotonic shock. Additionally, we establish that PAMM scaffolds can support the infiltration and growth of C2C12 myoblasts. We are currently exploring the potential of adipose-derived stem cells (ADSCs) to contribute to myogenic differentiation within PAMM scaffolds following exposure to culture conditions that are believed to enhance their myogenic potential.

2,3,7,8-TETRACHLORIDBENZO-P-DIOXIN (TCDD)-INDUCED MDSCS MEDIATE IMMUNOSUPPRESSIVE ACTIVITY THROUGH MICRORNA DYSREGULATION

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Myeloid-Derived Suppressor cells (MDSCs) are a heterogeneous population of immunosuppressive cells derived from the bone marrow. MDSCs serve an important, if not paradoxical role, during early and late stages of infection and inflammation. The compound 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), one of the most potent environmental contaminants, is formed not only as an unwanted byproduct in the manufacturing of chlorinated hydrocarbons, but also in incineration processes, paper and pulp bleaching, and emissions from steel foundries and motor vehicles. TCDD is known to suppress the immune response by many mechanisms, such as induction of T regulatory cells. However, in our current study, we demonstrated that TCDD

treatment mediates immunosuppression by inducing unique cells known as MDSCs that express both the macrophage marker, CD11b and neutrophil marker, Gr-1. For this purpose, we injected C57BL/6 mice with vehicle or 10μg/kg TCDD intraperitoneally and harvested the cells from the peritoneal cavity and estimated the MDSCs and MDSC subsets by flow cytometry when we found increased numbers both monocytic and granulocytic MDSCs following TCDD treatment when compared to vehicle treated group. Further studies revealed TCDD-induced MDSC can suppress ConA-mediated T-cell proliferation, we next investigated the epigenetic mechanisms including microRNA dysregulation underlying the induction and immunosuppressive effects of MDSC induced by TCDD. MiRNA are small non-coding RNA molecules involved in transcriptional and post-transcriptional inhibition in gene expression. We performed high throughput microarray analysis of MDSC isolated from TCDD and vehicle treated groups. We found that in TCDD-induced MDSCSs, certain miRNAs such as mir-543-3p and mir-150-5p were downregulated. These miRNA target genes including ARG, IL-10, STAT-3 and, PIM1 which are involved in MDSC induction and function. In summary, our data shows that TCDD can affect MDSC induction and function through modulation of miRNA. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641).

SPECTROSCOPIC CHARACTERIZATION OF FLUTAMIDE-POLYVINYLPYRROLIDONE INTERACTION: IMPROVING BIOAVAILABILITY OF DRUG MOLECULES.

Henry Nickson and Bijoy Dey
Claflin University

The main aim of the research was to increase the solubility of flutamide, a crystalline solid drug used in treating prostate cancer, in body fluid by making it an amorphous solid drug. X-ray and IR spectrometers were some of the apparatus that aided the experiment. In an attempt to make flutamide an amorphous solid drug, flutamide was mixed with large amount of a polymer, Polyvinylpyrolidone (PVP), and the mixture was sonicated until a homogenous mixture was obtained. The mixture was heated; evaporating the ethanol and leaving behind a solid flutamide and PVP complex called amorphous solid dispersion (ASD) .The IR spectrometry and X-ray of the results (ASD) were taken and analyzed and from the analysis, our goal of making an amorphous solid drug was accomplished and successful.

EXAMINING THE ENVIRONMENTAL IMPACT OF THE 1000-YEAR FLOOD ON THE ESTUARINE FISH FUNDULUS HETEROCLITUS

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Columbia College

Fundulus heteroclitus is a fish native to the coastal waters of the eastern coast of North America. F. heteroclitus thrive in estuarine environments, however a large influx of freshwater due to historic flooding in October 2015 (1000-year flood) has possibly altered their habitat in the ACE basin, as well as impacting their health. In this experiment, 50 fish were collected from the Belle W. Baruch Marine Sanctuary (BWBMS) and the length, weight, and sex of the fish were recorded. Fish were examined for the presence of parasites, and serum, gills, liver and gastrointestinal tract were collected and examined using immunoassays such as immunoblotting and immunohistochemistry. The tissues of the fish were processed, embedded, and cut at the Histology Core Lab at Clemson University. Three different antibodies, CX5-3 (±-COX-2), M24-2 (±-lysozyme), and 2C11 (±-eosinophilic granular cells), were used to examine serum and selected tissues of fish to locate immune proteins and cells of interest. This data was compared to baseline data of F. heteroclitus collected from BWBMS prior to October 2015 from the North Inlet Estuary. Fish collected post-flood were found to have 100% parasite prevalence compared to 72% of parasite prevalence in fish collected pre-flood from same site. Fish collected were also found to have a higher intensity of infection (avg.=7.72, min.=1; max.=43) as compared to fish collected from the same site pre-flood. Higher prevalence of innate immune cells were found in post-flood fish liver tissues compared to livers from fish pre-flood research. There were no observable differences between pre-flood and post-flood gill and gastrointestinal tissues.

DEVELOPMENT OF METHODS FOR THE DETECTION OF 2,4-D AND GLYPHOSATE IN EARTHWORMS

Rachel Owings and Jeff Brotherton
Converse College

Methods were developed for the detection in earthworm tissue of the common herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and glyphosate. Worms were extracted using 50:50 ethanol: 0.1 M HCl, 10 mL per gram worm for both 2,4-D and glyphosate analysis. Untreated worm extracts were spiked with 2,4-D and glyphosate and used to develop optimum HPLC conditions, where co-eluting background peaks were minimized. Both methods used gradient programs and a C18 column with the mobile phase solvents 40 mM sodium acetate in water pH 4.0 and acetonitrile. The limit of quantification for 2,4-D in worm extracts was 2.2μg/g worm which corresponds to 2 ppm or a 2% uptake by a treated worm. The limit of quantification for glyphosate in worm extracts was 0.9μg/g worm which corresponds to 1 ppm detection or a 1% uptake by a treated worm. To increase sensitivity for glyphosate detection, worm extracts were derivatized using 9-fluorenylmethoxycarbonyl chloride (fMOC) to produce a fluorescent product. These methods were suitable for analyzing worms obtained from soil treated with 2,4-D, glyphosate, or both.

INVESTIGATING THE ACTION OF DIMETHYLFUMARATE IN NEURONS

Tulsi Patel and Norma Frizzell
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Multiple sclerosis (MS) is a chronic inflammatory condition resulting in neuronal demyelination and axonal loss. While there is no cure, a new treatment was approved by the FDA in March 2013 (Tecfidera®). The active component of Tecfidera® is dimethylfumarate (DMF), a fumarate ester resulting in significant clinical improvements, but also nausea sufficient to discontinue use in ~10% of cases. Only one beneficial mechanism of action of DMF has been studied closely, however, our laboratory studies the irreversible modification of protein cysteine residues by fumarate (succination) and we hypothesized that DMF is reacting with novel protein targets in neurons and astrocytes. This could explain the beneficial and side effects of Tecfidera®, and allow us to identify improved drug targets for MS.

I performed a proteomics-based investigation to identify new targets of DMF protein modification in neurons and astrocytes. Protein samples from untreated or 100μM DMF treated neurons were separated by gel electrophoresis. Bands were excised from the gel and processed with the enzyme trypsin, generating peptide fragments for analysis by mass spectrometry. 24 protein subunits were identified as being succinated, providing new information on the mechanistic action of DMF. I have continued to study one enzyme, Ubiquitin carboxy-terminal hydrolase L1 (UCHL-1), which hydrolyzes a peptide bond at the C-terminal glycine to release ubiquitin from monoubiquitinated proteins. Deficiency of UCHL-1 has been linked to several neurodegenerative disorders. Current investigations will elucidate if succination on cysteine 152 of Uchl1 is altering its deubiquitinase activity, potentially contributing to altered ubiquitination profiles in neurons.

IDENTIFYING WHICH SUBCELLULAR COMPARTMENTS IN THE BRAIN EXPRESS PDE9A AND HOW THAT EXPRESSION CHANGES WITH AGE

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Phosphodiesterases (PDEs) are a superfamily of enzymes that degrade cyclic nucleotides (cAMP and cGMP), intracellular signaling molecules critical for brain function. Among the PDE families, PDE9 has the highest affinity for cGMP and, thus, is a therapeutic target of interest. To better understand its therapeutic potential, we characterized the subcellular localization of PDE9A in the brain and how PDE9A expression/localization changes with age. We show that PDE9 mRNA and protein are expressed significantly higher in cerebellum versus hippocampus, with particular enrichment in the Purkinje cell layer. In both hippocampus and cerebellum, we reliably detect the previously reported PDE9A5 isoform as well as two new PDE9 isoforms at ~120X and ~100X kDa. Biochemical fractionation shows that all PDE9A isoforms localize to the nucleus and are significantly enriched in membrane vs. cytosolic fractions. This subcellular compartmentalization is consistent with the fact that PDE9 regulates pools of cGMP that are downstream of particulate (i.e., membrane), but not soluble (i.e., cytosolic), guanylyl cyclases. Interestingly, the relative enrichment of PDE9 in nuclear versus membrane fractions significantly differs as a function of isoform, brain region, and age. Not only does the subcellular compartmentalization of PDE9A5 dramatically shift between postnatal days 7-28, but expression of both PDE9A mRNA and protein significantly decrease during this time period as well. PDE9A localization and expression patterns stabilize after postnatal day 28. Together, these data suggest PDE9 is localized to preferentially regulate nuclear- and membrane-proximal pools of cGMP, and its role in brain function dramatically changes during early postnatal life.

DETERMINING THE ROLE OF HOMOLOGOUS RECOMBINATION IN REPLICATIVE TRANSPOSITION OF *MPING*

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Transposable elements are mobile segments of DNA that make up a large portion of plant genomes. Class II transposable elements use a “cut and paste” mechanism in which the element is excised and reinserted elsewhere in the genome, making them powerful agents in genome evolution. One of these elements, *mPing* has high transposition activity and despite the fact that *mPing* utilizes a “cut and paste” mechanism, its copy number has been shown to increase over generations, suggesting the presence of a replicative transposition mechanism. This experiment will test if homologous recombination (HR) repair, a mechanism in which homologous sequences from elsewhere are used to repair double strand breaks, repairs *mPing* excision sites with an *mPing* containing homologous sequence. We measured repair of *mPing* excision sites in yeast using a reporter system in which *mPing* disrupts the *ADE2* gene, preventing cell growth until excision of mPing and subsequent repair of the *ADE2* gene. Previous results showed that *ADE2* restoration was higher in haploid cells than in diploid cells, suggesting that HR repair may be occurring in the diploids. To confirm the role of HR repair, we are performing transposition assays in HR deficient strains created by knocking out the *rad51* gene. We predict that in the absence of HR repair we will see equal restoration of *ADE2* function in the haploid and diploid strains. If we can confirm that HR repair is occurring, we will attempt to directly identify cases of replicative transposition by analyzing *mPing* copy numbers in our strains.

FUNCTIONAL CHARACTERIZATION OF AN ESSENTIAL CHLOROPLAST PROTEIN

Christopher Pierpont and Michelle M. Barthet
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Maturases are a group of enzymes which catalyze the removal of introns from pre-mRNA transcripts during post-transcriptional processing. They are arguably involved in one of the most important processes of the cell, as the splicing of introns is crucial for proper protein synthesis. In prokaryotes, maturases are home-target specific in that the enzyme is normally encoded within the intron which it splices. Maturase K, or MatK, is proposed to be the only plastid-encoded maturase of land plants. An RNA-level assay has shown that MatK associates with several introns within the plastid, similar to the splicing machinery of the nucleus. Because of this, MatK could potentially be part of a proto-spliceosome. However, though it has been shown that MatK is an essential protein for plastid function, and that it does indeed associate with introns, maturase activity has not been clearly demonstrated. A direct, in vitro, protein-level assay would not only allow for the simple demonstration of maturase activity, but would also provide a certain level of manipulation for analytical testing. An assay such as this would help characterize MatK function at the molecular level, as well as aid in determining associated factors required for splicing activity in the chloroplast. The aim of this project is to successfully design this assay, and demonstrate maturase activity of MatK over the introns it associates with. Thus far, four of the intron substrates that MatK associates with have been successfully cloned into a bacterial system, and MatK expressed. Progress of activity tests will be discussed.

INVESTIGATING PHAGE ACTIVITY WITHIN THE STUDENT POPULATION AT COASTAL CAROLINA UNIVERSITY

Amy Powers, Lisa Pieterse, and Paul Richardson
Coastal Carolina University

This study is aimed to investigate the bacteriophage population occurring within the students at Coastal Carolina University (CCU). A nose and ear swabs are taken from students who volunteer. Both microbial and molecular experiments are conducted to detect the presence of bacteriophages against Staphylococcus aureus and Escherichia coli. A total of 40 students have been sampled in the 2016-2017 academic year thus far. The purpose of this study is to try to find a naturally occurring agent (bacteriophages) that will be affective against the MSRA strain of Staphylococcus aureus. The end goal of this project is to help produce an alternative medical therapy to treating MSRA since it is antibiotic resistant.

CHARACTERIZING GALACTOSEMIA IN *C. ELEGANS*

Ashley Pribble and Daniel Williams
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Galactosemia is an inherited metabolic disorder caused by the inability to metabolize the simple sugar galactose. Classic galactosemia is due to deficiency in the enzyme galactose-1-phosphate uridyltransferase (GALT) and is thought to be due to a build up of toxic intermediates of galactose metabolism. Our lab is working to better understand galactosemia by developing a *C. elegans* model of galactosemia. We have identified the worm homolog of GALT and obtained mutant alleles in this gene. Current experiments are aimed at characterizing the GALT(-) phenotype when grown under different treatment conditions. Ultimately, we aim to define the cellular pathology associated with galactosemia and use *C. elegans* genetics to identify genes that influence galactosemic conditions.

RESULTS OF A COURSE-BASED STUDY RELATING AMPHIBIAN DISTRIBUTION AND LAND USE FEATURES IN SOUTH CAROLINA

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In 2014 a course-based collaborative project was conducted to understand the role of different kinds of land use on anuran (frog and toad) distribution in South Carolina. This project, which was part of a national study, fused teaching with data-driven research and citizen science. The anuran detection data, which represented anuran distribution, came from the North American Amphibian Monitoring Program (NAAMP), a volunteer-based program taking place in South Carolina from 2008 - 2016. Anuran detections were summarized for 44 statewide routes situated within the Blue Ridge, Piedmont, and Coastal Plain ecoregions. The Quantum Geographic Information System (QGIS) application was used to analyze landscape variables within 300m, 600m, 1km, 5km, and 10km buffers at selected NAAMP survey stops. The NAAMP effort produced detections for 28 of the 30 anuran species know to occur in South Carolina, with the highest species richness occurring in the Coastal Plain. The most commonly detected species included Spring Peeper (*Pseudarcris crucifer*), Gray Treefrog (*Hyla chrysoscelis*), Green Treefrog (*Hyla cinerea*), Southern Toad (*Anaxyrus terrestris*), and Southern Leopard Frog (*Lithobates sphenocephalus*). Variables negatively correlated with anuran species richness included road density, high proportions of forest, and urbanization. Variables positively correlated with anuran species richness included wetland area, agricultural area, and survey number. General Linear Models including combinations of landscape variables produced significant estimates for species richness and selected anuran species, but further analyses are necessary to the determine the full extent of road density and landscape feature connectivity on amphibian distribution in South Carolina.

"INVESTIGATING NON-EQUILIBRIUM FLUCTUATIONS OF NANOCOLLOIDS UNDER THE INFLUENCE OF A MAGNETIC FIELD USING DIRECT IMAGING AND SHADOWGRAPHY METHODS."

Ashley Rice, Ana Oprisan, and Sorinel Oprisan
College of Charleston

Iron oxide nanoparticles are becoming an increasingly important factor in the biomedical industry-specifically in areas such as drug targeting, magnetic cell separation, immunotherapy, and others. In this project, we investigated non-equilibrium concentration fluctuations during the diffusion of iron oxide nanocolloids. These particles exhibit superparamagnetic properties as they are on the order of 1-10 nm and have a high magnetic susceptibility. Using two imaging methods, direct imaging and shadowgraphy, we recorded their diffusion process both in the presence and in the absence of a magnetic field. We then implemented the Differential Dynamic Algorithm to analyze the images and the data we were able to extract from them. From this data, we were able to gather information about their physical properties and determine both the diffusion and viscosity coefficients of the iron oxide nanoparticles.

SELF-ASSEMBLED MONOLAYERS OF ALKYLCARBOXYLIC ACIDS ON ZNO NANOPARTICLES

Aerin Richardson and Nicholas Marshall
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â€¯Zinc oxide (ZnO) nanoparticles can be modified using carboxylic acids to create a hydrophobic monolayer on the surface of the particle. Formation of this self-assembled monolayer depends primarily on the length of the chain. In this study, we prepared modified ZnO particles with a variety of substituted carboxylic acids R-COOH and characterized the resulting materials using contact measurements and UV-Vis and IR spectroscopy. We have determined the minimum chain length needed for self-assembly in this system, with detectable self assembly beginning with acid chain length of three carbons or more. These acid monolayers increase hydrophobicity of the zinc nanoparticles, with particles modified with long-chain fatty acids such as myristic and palmitic acids being highly hydrophobic.

ACOUSTIC MONITORING OF BAT POPULATIONS IN FLORENCE, SC

Aaron Robinson and Jeffrey Steinmetz
Francis Marion University

In this study an acoustics monitoring system was used to study on bat populations of Florence County. Wildlife Acoustics Echo Meter Touch provides spectrograms of bat calls and auto identification. Bat populations were monitored between the months of May 2014 to February 2017. A route was selected for repeated measurements that sampled a variety of areas including ponds, open fields, neighborhoods, and city streets. The route was driven every two weeks to record bat populations. To study the effect of time on bat activity, once every four weeks the route was monitored two times per night with an hour and thirty minutes separation. For the Pee Dee region, the Echo Meter Touch auto identifies nine species of bats. Based on the bat calls recorded, all nine species were collected. The most commonly detected species were Lasiurus borealis (Eastern red) and Nycticeius humeralis (Evening bat). When the route was monitored twice a night, preliminary data shows a decrease in bat activity later at night. The numbers of recordings collected in the study were higher in the summer and fall compared to the winter. This data will provide a baseline for a long term bat monitoring project.

TESTING STRATEGIES TO PRODUCE TARGETED INSERTION OF MPING

Mary Roby and C. Nathan Hancock
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My project focuses on mPing, a 430bp miniature inverted repeat transposable element from rice that can move from one place to another within the genome. The goal of my research is to produce targeted insertion of the transposable element mPing in yeast by connecting the TPase protein to a DNA binding domain that recognizes a specific target sequence. Our strategy is to use the dCas9 DNA binding domain because of its high specificity for the target site, which is regulated by a guide RNA. In previous research, a dCas9/TPase fusion protein did not produce targeted insertion, possibly due to protein miss-folding or steric hindrance. To test this hypothesis, we plan to use a dCas9-Gal 11P fusion protein, which will bind with a Gal4(58-97)-TPase fusion protein. Thus, targeted mutagenesis will be achieved through a guide RNA (gRNA) directing dCas9 to the target site, resulting in the dCas9/TPase complex being recruited to the desired DNA site, encouraging mPing to insert near the target site. A yeast intron was added between the dCas9 and the Gal 11P domains in order to allow propagation of the construct in bacteria. We propose that if dCas9-Gal 11P and Gal4(58-97)-TPase fold correctly and interact, they will function together to produce targeted insertion. We will insert the fusion protein constructs into yeast to test the transposition rate of mPing using a yeast transposition assay. We predict that although transposition will be lower than controls, we will see an increase of insertions of mPing into the target site.

EFFECTS OF HEAD IMPACT ON NEUROCOGNITIVE FUNCTIONS AND BALANCE

Shaquanda Ross-Simmons and Michelle Vieyra
University of South Carolina Aiken

The goal of this study was to investigate the effects of sports-related head injury on balance, attention, and memory. Reliable differences have been found using measures that directly tap into brain functioning, such as the auditory oddball task. This task requires participants to attend to two tones, one presented more frequently than the other, while EEG is recorded. The P3 wave is elicited when a subject detects the infrequent tone. We hypothesized that athletes reporting a diagnosed concussion or high-risk sports would have compromised balance and neurocognitive functioning as compared to athletes in low risk sports. Forty-five undergraduate participants were identified as concussed, non-concussed in high-risk sports, and non-concussed in low-risk sports using a survey of athletic history, head trauma and demographics. The Biopac MP36 system, a balance board, and the BESS protocol was used to measure balance. E-prime and a 32 channel electrode EEG system was used to conduct a working-memory task and an auditory oddball test. Concussed groups had significantly worse balance in comparison to the other groups. No significant differences were found for accuracy on the oddball task or working memory scores. A 3 X 3 repeated measures ANOVA was used to detect differences in latency and amplitude of the P3 wave. Preliminary analysis shows no differences in P3 latency, but a marginally significant interaction between location and group on amplitude. Consistent with prior research, no differences were found on behavioral measures, but more sensitive balance and EEG measures were able to detect subtle differences between groups.

COMPARISON OF BACTERIOPHAGE FOUND AT RESIDENTIAL AND COMMERCIAL ENVIRONMENTS

Alexis Setta, Elizabeth Christmas, and Paul Richardson
Coastal Carolina

The data to be presented was collected in efforts to assess the difference in bacteriophage population as observed in commercial and residential areas. Bacteriophage population and diversity is expected to be greater in residential areas due to the greater population and more frequent activity levels commonly observed in residential areas. Water and fecal samples were regularly gathered from each location and tested using molecular and microbial techniques in order to be compared. PCR and electrophoresis were used as a means of confirming bacteriophage presence as well as to illustrate the differences and frequencies in species among each location. Plating techniques were used as a secondary way to confirm bacteriophage presence as well as to view viral activity when exposed to several types of bacteria. Information collected in this study becomes increasingly significant based on level of human and domestic animal interaction with the water as well as during times of flooding.

EXAMINING THE INFLUENCE OF REGULATORY PATHWAYS ON IMPORTIN EXPRESSION

Ramsha Shams, Quentell Wagener, and Christine Byrum
College of Charleston

Nuclear transport proteins (karyopherins) play crucial roles in intracellular cargo transport with the nuclear pore complex acting as a bridge between cytoplasm and the nucleus. Our lab has demonstrated that karyopherin alpha importins are differentially expressed during sea urchin embryogenesis whereas many karyopherin beta forms are not. Little is known, however, about how key regulatory pathways influence karyopherin expression. We will use polymerase chain reactions (PCR) to learn more about the regulatory pathways influencing expression of karyopherins in early development of the sea urchin *Lytechinus variegatus*. This organism, a valuable deuterostome model, was selected based on ease of culture and genetic simplicity. Approaches to chemically inhibit and/or activate signaling pathways that influence formation of the oral/aboral axis in the sea urchin will be described; strategies to evaluate effects on the expression of karyopherin alpha and karyopherin beta importins will be outlined.

INTRAMOLECULAR PROTON TRANSFER DYNAMICS IN MALONALDEHYDE BASED ON HAMILTON JACOBI EQUATION

Shaquille Shaw and Bijoy Dey

The purpose of this research is to determine the reaction path for the transfer of hydrogen atom malonaldehyde molecule. In doing so, we have relied on solving a modified Hamilton-Jacobi (HJ) equation by applying fast marching method (FMM) proposed by Sethian (1996). This leads to transforming the potential energy surface (PES) (which are often complicated) to a more amenable reaction action surface (RAS). Steepest descent (also called back-tracing) on the RAS allows us to determine the reaction path. Our results on the RAS and the reaction path for the hydrogen transfer dynamics in malonaldehyde are not only significantly different from other methods they also provide new perspectives on the dynamics.

AGING AND ROS-MEDIATED NEURODEGENERATION IN *C. ELEGANS*

Chelsea Shoben and Daniel Williams
Coastal Carolina University

Aging is a major risk-factor for many neurodegenerative diseases and reactive oxygen species (ROS) have been implicated in the degeneration process. However, the interplay of aging and ROS, as well as their relative influence on neurodegeneration have not been established. Our lab investigates ROS-mediated neurodegeneration using the fluorescent photosensitizer KillerRed in select neurons of *C. elegan*s. Light activation of KillerRed produces ROS, which induce neurodegeneration of GABA neurons, and results in a characteristic "shinker" phenotype. We are currently inducing neurodegeneration at different age-points with hopes of uncovering age dependent differences in the degeneration process.

TESTING A SIRNA TARGETING HIV-1 VIF

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The Viral infectivity factor (Vif) is a HIV-1 accessory gene that assists in viral replication by facilitating the degradation of the host cytosine deaminase Apolipoprotein B mRNA editing enzyme catalytic subunit 3G (A3G). A3G, in the absence of Vif, is packaged into progeny virions and, following virus entry into a host cell, results in virus inactivation by causing cytosine to uracil mutations during reverse transcription of the viral mRNA. An effective way to down-regulate HIV replication is to use small interfering RNAs (siRNA) that target mRNA degradation through the RNAi pathway. To take advantage of this pathway, a small hairpin RNA (shRNA) targeted to HIV-1 Vif located at nucleotides 5551-5571 of the HIV-1 genomic clone NL43 (Accession number M19921) was created and cloned into the retroviral vector pSRNG, placing it under the control of the RNA Polymerse III H1 promoter. The goal of this project is to test the activity of Vifsi5551 using a Î²-galactosidase reporter assay. For this assay, the Vif NL42 target sequence from nucleotides 5500 to 5600 was amplified from pNL4-3 and cloned into the 3â€™ untranslated region (3â€™ UTR) of Î²-galactosidase expressed from the CMV promoter (pCMVÎ²gal) creating pCMVÎ²galvif55-56. This reporter plasmid will next be used to measure the ability of Vfsi5551 expressed from pSRNGVifsi5551 to induce target cleavage.

CTSK:MCHERRY-ITOL2 - A TRANSGENIC CONSTRUCT TO STUDY THE ROLE OF OSTEOCLASTS DURING ZEBRAFISH DEVELOPMENT

Brianna Snelling and April Delaurier
University of South Carolina Aiken

The goal of this project is to use transgenic lines to study the activity of osteoclasts (bone-resorbing cells) in the developing zebrafish. Fluorescent reporter lines that tag specific genes in cell populations allow for specialized study of cells and cell functions during development. This project aims to use mCherry as a reporter gene for cathepsin K (ctsk), which is a gene specifically associated with osteoclasts. This fluorescent tag will allow observation of ctsk and osteoclast activity in the developing embryo. The genetic construct will be made containing the upstream regulatory elements of ctsk to drive the expression of mCherry. The completed construct will be injected into 1-cell stage zebrafish embryos to generate germ lines of fish expressing mCherry. This will allow us to observe the role of osteoclasts during development. These lines can then be crossed with a previously constructed transgenic line that labels osteoblasts (bone forming cells; sp7:EGFP), and we can use the resulting transgenic lines to study how osteoclasts and osteoblasts work together during development and through adulthood. Understanding this mechanism has implications for future study of the role of osteoclastic resorption during development, and forms a model for studying human diseases involving resorption, such as osteoporosis.

CLONING A HIV-1 VIF-RESISTANT A3G GENE INTO A LENTIVIRAL VECTOR

Mckenzie Spires and William Jackson
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HIV-1 is a retrovirus encoding 15 proteins, which include three structural proteins (Gag, Pol, and Env), two regulatory proteins (Tat and Rev), and four accessory proteins (Nef, Vif, Vpr, and Vpu). This study focuses on the Viral infectivity factor (Vif), and how it interacts with Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (A3G). A3G induces extensive cytosine to uracil mutations, resulting in guanine to adenine substitutions, while Vif prevents A3G incorporation into virions by inducing A3G ubiquitination and proteasomal degradation. My project is to create a fusion gene incorporating the Vif-resistant D128K A3G and the selectable marker puromycin-N-acetyltransferase using the Thosea asigna virus T2A peptide cleavage sequence. This fusion gene will then be cloned into the lentiviral vector, pLRed(INS2)R, which we have shown to express a Renilla luciferase/eGFP fusion gene in a HIV-dependent manner. Once cloned, we hypothesize that this lentiviral vector will block HIV replication.

ASSESSING THE FUNCTION OF KLEPTOPLASTY WITHIN FORAMINIFERA

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The specificity of diatom chloroplast organelles participating in the biological phenomenon of kleptoplasty is identified using nuclear and chloroplast genes. Functionality of the kleptoplastic condition within single-celled marine foraminiferal hosts is investigated using molecular data. In contrast to the broad diversity of the plastids they retain kleptoplastic foraminiferal hosts have limited diversity. The expression of rbcL genes determined from cDNA recovered from plastids retained within foraminiferal hosts supports their potential functionality in carbon fixation.

CHANGES IN THE VASCULAR PLANT DIVERSITY OF CUTTYHUNK ISLAND, MASSACHUSETTS

Richard Stalter
St. John's University

Cuttyhunk Island, Dukes County, Massachusetts, comprising 235 ha, 41 25' N, 70 56 W, was formed by a ressional moraine during the Wisconsin Glacial Stage approximately 14,000 years ago. The island was surveyed for vascular plants on September 30, 2016. The island's flora, 1923 to 2016, is comprised of 71 families, 181 genera, and 283 species, of which 227 species (80%) are native and 56 (20%) are non-native. Herbaceous taxa compose 84% of the island's vegetation. The most species-rich families are Asteraceae, Cyperaceae, and Poaceae. The largest genera are Carex, Juncus, and Eleocharis. Floristic studies of two earlier investigators, Fogg who collected in 1923 and O'Neill who collected in 1974 included lists of 134 and 266 taxa respectively. Since no floristic or ecological studies have been conducted Since O'Neill's 12 field trips to the island in 1974, the objective of this preliminary study was to collect and identify the vascular plant species present at Cuttyhunk Island. In a preliminary 30 September, 2016 foray Stalter collected 136 species, 111 genera in 60 families. Additional collecting trips will be taken to the island during the 2017 and 2018 growing seasons. During the past 93 years the island's flora has continually changed partially in response to a dynamic landscape repeatedly impacted by coastal storms and human activity

ENDOCANNABINOID AMELIORATES ACUTE LUNG INJURY INDUCED BY *STAPHYLOCOCCUS AUREUS* ENTEROTOXIN B (SEB) THROUGH REGULATION OF MICRO-RNA

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Staphylococcus Enterotoxin B (SEB), produced by *Staphylococcus auras* causes a wide range of symptoms. SEB is a CDC select agent of bioterrorism. It is a superantigen that activates up to 30% of T cells by crosslinking the T cell receptor (TCR) to nonpolymorphic region of MHC class II on antigen presenting cells (APC). The inhalation of SEB leads to toxic shock syndrome and death. In the current study, we used an intranasal dose of SEB to induce acute lung injury (ALI) in C57BL/6 mice. Anandamide (AEA), an endogenous cannabinoid, is part of endocannabinoid system (ECs) and binds to CB1 and CB2 receptors. In our study, we found that using AEA alleviated ALI in SEB-exposed mice. Lungs were excised from naïve and SEB-treated mice administered with vehicle (SEB+Veh) or AEA (SEB+AEA) for histopathological analysis. There was a significant decrease in the infiltration of inflammatory cells in the lungs from SEB+AEA mice compared to SEB+Veh. Flow cytometric analysis demonstrated a decrease in CD4+, CD8+ and NKT cells as well as Vß8+ T cells whereas an increase in CD11b+Gr1+ myeloid-derived suppressor cells (MDSC) and FoxP3+ T regulatory cells in SEB+AEA group when compared to SEB+Veh treated mice. We next examined whether miRNA mediated the protective effects of AEA on SEB-induced ALI. Microarray analysis of lung-infiltrating cells revealed 59 up- and 77 down-regulated miRNA in SEB+AEA mice relative to SEB+Veh. Using Ingenuity Pathway Analysis (IPA), we identified target genes for miRNAs with > 1.5 fold change. We found that miR-34a-5p, miR23a and miR27a were downregulated, which target the T regulatory cell transcription factor FOXP3, Arg1, TGß2 and IL-10 genes, respectively. Also, miR-30c-5p, which targets anti-inflammatory genes, SOCS1 and SOCS3 were downregulated.The miRs and target genes were validated by RT-PCR. Thus, we have identified miRNAs that play a role in protection from SEB-induced ALI by AEA. Supported by: NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20GM103641 and Higher Committee Education Development in Iraq (HCED).

APPLICATION OF TOL2-BASED ACTIVATION TAG CONSTRUCTS FOR ZEBRAFISH MUTAGENESIS

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Transposons are segments of DNA that can move from one region to another within the genome. The Tol2 transposon from Medaka fish has successfully been used for transgenesis, integrating foreign DNA, into a wide variety of vertebrates. Our goal is to develop Tol2 into a mutagenesis tool for gene discovery. Mutagenesis by transposon insertion, called transposon tagging, enables the discovery and analysis of gene function by causing mutations. Activation tagging, a type transposon tagging, is when a strong enhancer is positioned within the transposon. Activation tagging is used to learn about the function of genes by inducing overexpression. This is significant because many genes may otherwise be hard to study because of lethality or redundancy. Activation tagging has never been used for zebrafish, but it is commonly used for gene discovery in plants.

Zebrafish can serve as vertebrate development models, therefore activation tagging within zebrafish allows for the discovery of genes that are important for vertebrate development. A Tol2-based activation tag, with a h2afx promoter sequence inserted in the middle of Tol2 terminal inverted repeats (TIRs), was engineered using various molecular biology techniques (PCR, digestion, and sequence analysis). Additionally, a DNA construct encoding Tol2 transposase, which will allow transposition of the activation tag to occur, was produced. The integration of both constructs into zebrafish embryos is being performed to measure transposition rates and look for altered gene function. To develop more active constructs for zebrafish mutagenesis, yeast transposition studies are also being performed in order to identify methods to increase transposition rates.

ANALYSIS OF THE MOLECULAR WEIGHT DISTRIBUTION OF POLYHEXAMETHYLENE BIGUANIDE USING EQUILIBRIUM DIALYSIS, SIZE-EXCLUSION CHROMATOGRAPHY, DYNAMIC LIGHT SCATTERING, AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Diverse cationic biocides are used in low (ppm) concentrations in personal care products such as multipurpose contact lens solution (MPS) to inhibit bacterial growth. Characterization of these compounds is essential to understanding their efficacy, particularly for complex mixtures that incorporate a broad range of discrete forms. The biocide polyhexamethylene biguanide (PHMB) is incorporated as a polycationic, polydisperse additive in MPS and other sanitary solutions. Recently, we developed a method using ultra high performance liquid chromatography (UPLC) coupled with electrospray (+) mass spectrometry (ESI-MS) to elucidate the structures of multiple oligomers from commercially available PHMB, which comprise a significant size range. To better investigate the distribution of oligomeric species for purposes of ascertaining biological efficacy and contact lens adsorption, we have performed equilibrium dialysis using membranes of various sizes (MWCOs). By using multiple membranes followed by size exclusion chromatography (SEC) on dialyzed samples, we have successfully isolated PHMB samples of a range of molecular weights. This distribution is confirmed via dynamic light scattering (DLS). Re-injection and analysis of isolated samples via UPLC-MS provides a mechanism to correlate MS fragment analysis of oligomers with size estimation provided by SEC/DLS.

THE EFFECTS OF CAFFEINE AND A HIGH SUCROSE DIET ON ADIPOSE TISSUE ACCUMULATION, MEMORY, AND ANXIETY IN RATS

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The purpose of this study is to look at the relationships between beverages containing sugar and/ or caffeine and obesity, short term memory and anxiety. Over 38% of the US population is obese and a contributing factor is Americaâ€™s insatiable thirst for sugary drinks. Evidence suggests that the levels of obesity may be lower due to caffeine in most beverages. Caffeine has been shown to lower weight gain through thermogenesis and fat oxidation. Caffeine has been found to prevent cognitive decline related to aging, and improve performance on cognitive tests. Unfortunately, both caffeine and sugar have been shown to increase anxiety in moderate to high doses. This study used rats as a model to test these relationships. Rats were randomly placed into 4 groups; control, sugar only, caffeine only, and sugar + caffeine. After 16 weeks the rats were given short term memory and anxiety tests, weighed and then sacrificed and dissected to determine body fat accumulation. The rats fed sugar alone accumulated the most fat followed by the sugar/ caffeine group, despite the sugar/ caffeine group consuming a significantly higher amount of calories. In the anxiety tests the sugar fed rats showed the least amount of anxiety with both caffeine groups showing more anxiety than control. The sugar fed rats showed the best short-term memory with the caffeine alone group doing the worst. The results of this experiment can be used to determine if caffeine can help ameliorate some of the negative health consequences of sugar.

PH-DEPENDENCE OF THE OXIDATIVE CLEAVAGE OF 2,4'-DIHYDROXYACETOPHENONE BY 2,4'-DIHYDROXYACETOPHENONE DIOXYGENASE

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The enzyme, 2,4'-dihydroxyacetophenone dioxygenase (DAD), originally identified in betaproteobacteria, catalyzes the oxidative cleavage of 2,4'-dihydroxyacetophenone (DHA) into benzoic acid and formic acid. The nature of the cleavage has led to the proposal of a mechanism reminiscent of either the intradiol- or extradiol dioxygenases. To better characterize the components that make up the reaction, this study focuses on enzyme activity across a range of buffer pH. To determine the pH-dependence of activity, absorbance assays monitoring the conversion of DHA into benzoic acid were done at pH intervals of 0.5 from 2.5 to 10.0. Assays across the basic pH range showed both a decrease in activity and a shift in the UV spectrum of the DHA substrate. Analysis of the pH-dependence of reaction rate will be compared to the pKa determined for the substrate to help identify the source of the pH-dependence. This information will assist in elucidating the role of pH in the DAD mechanism. The culmination of data from these experiments will be used to interpret the source of the pH-dependence.

INVESTIGATION OF *MORINGA OLEIFERA* LEAF EXTRACT AND ITS CANCER SELECTIVE ANTIPROLIFERATIVE PROPERTIES

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Moringa oleifera is a tree native to a number of Asian, African, and Central American countries and has been used in traditional medicine for an assortment of medicinal uses for centuries. Due to bioactive compounds within *M. oleifera* leaves, it is believed that *M. oleifera* leaf extract may possess cancer-selective antiproliferative properties. Previous research has been conducted in regards to this topic, but poor experimental design due to lack of necessary controls limits the legitimacy of anticancer claims. While previous research has shown that *M. oleifera* leaf extract has the potential to kill cancer cells, the research fails to demonstrate the effects of *M. oleifera* leaf extract on healthy cells. In order for anticancer claims to be sufficient and yield the possibility of a future cancer treatment, *M. oleifera* leaf extract must not harm noncancerous cells. This is essential in order to be considered a cancer-selective killing agent. The current study was designed using tissue type pairs including both cancerous and non-cancerous cell lines. These cell lines were treated with differing concentrations of *M. oleifera* leaf extracts. After 48 hours, cell proliferation was measured, and statistical analyses were completed. Results showed that the *M. oleifera* leaf extract had no significant effect on either of the breast cell lines, cancerous or non-cancerous. However, the results suggest there is a difference in cell proliferation between the lung cell lines. Low concentrations increased cell proliferation in the healthy lung cells while having no significant effect on the cancerous lung cells. The effects reversed at higher concentrations. This could be due to the difference in cell responses between cancerous lung cells and healthy lung cells. This research contradicts previous findings that *M. oleifera* leaf extract is a cancer killing agent; therefore, more research should be completed to understand these new findings.

DETERMINING THE ROLE OF LDLRAP1A IN CRANIOFACIAL DEVELOPMENT IN ZEBRAFISH

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A line of mutant zebrafish containing a jaw mutation named b1187 was discovered during a forward genetics screen. This mutation is characterized by fused joints and abnormal shaping in cartilage and bone in the craniofacial region of zebrafish. To find the gene behind the b1187 mutation multiple genes were sequenced, however there were no differences between mutant and wild-type sibling cDNA. This led to a reverse genetics approach using a CRISPR/Cas9 system to create of a line of zebrafish with a mutated ldlrap1a gene. The ldlrap1a gene (low density lipoprotein receptor adaptor protein 1a) is known to be involved in cholesterol signaling, however we believe it could also have a role in craniofacial development of zebrafish. A F0 generation containing an ldlrap1a mutation was generated using the CRISPR/Cas9 system and was then crossed with wild-type siblings to create 3 separate F1 generations. The F1 generations were screened using PCR and T7 endonuclease digest to identify approximately half of the offspring who were heterozygous mutants for the ldlrap1a gene. Fin clip samples were taken from all three pairs and a wild-type zebrafish and sent for sequencing. Of the three pairs, two appear to have favorable missense mutations. These fish were then crossed to their genotyped siblings to create an F2 generation. Histological stains will be performed on these zebrafish, which will allow us to observe any abnormal phenotypes which resemble those of the b1187 jaw mutation. If we do observe these abnormalities it could conclude that ldlrap1a is the gene underlying the mutation and that it is involved in craniofacial development.

THE USE OF BONE GROWTH STIMULATORS FOR OSTEOARTHRITIS OF THE KNEE

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The aim of the present study was to determine if there is a benefit for the use of Ultrasound Bone Growth Stimulators for osteoarthritis of the knee. There is evidence that osteoarthritis of the knee is primarily a disease of subchondral bone and the joint changes are secondary. Since subchondral bone in osteoarthritis contains fibrous tissue and bone growth stimulators function to change the fibrous tissue in the callus of fracture nonunion into normal bone, there exists the possibility for a treatment of osteoarthritis of the knee with bone growth stimulators.

Ten patients with confirmed osteoarthritis of the knee were included in this pilot study. Each patient's pain and quality of life were assessed on three independent scales before and after treatment with noninvasive bone growth stimulators. Eight participants were treated utilizing ultrasound technology, one was treated using pulsed electromagnetic fields and one was treated with combined magnetic fields.

There was a high level of significance for nine of the eleven statistical tests which were performed on three independent scales for ultrasound. The participants who were treated using pulsed electromagnetic fields and combined magnetic fields experienced greater than 80% improvement for the comprehensive scores on all three measurement scales.

This was the first clinical use of bone growth stimulators for osteoarthritis of the knee. All three technologies of ultrasound, pulsed electromagnetic fields, and combined magnetic fields were shown to be effective. The initial results are encouraging and directions for future research are discussed.

USING CRISPR/CAS9 TO STUDY THE ROLE OF ZMYM2 AND ZMYM3 IN ZEBRAFISH CRANIOFACIAL DEVELOPMENT

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Potocki-Shaffer syndrome (PSS) is a rare contiguous gene-deletion caused by heterozygous interstitial microdeletions of chromosome region 11p11-p12 and is characterized by developmental defects that include intellectual disability and craniofacial anomalies. PSS is associated with mutations in genes encoding factors in the PHF21A protein complex, including KDMA1(Lysine-specific histone demethylase 1A), ZMYM2 (Zinc finger protein 198), and ZMYM3 (Zinc finger protein 261) proteins. It is hypothesized that this protein complex affects craniofacial development of zebrafish in a way that reflects their function in humans. At present, the individual actions between proteins in craniofacial development remain not fully understood. Previously, F0 founder fish carrying mutations in zmym2 and zmym3 were generated by microinjection of CRISPR constructs including a guide RNA (gRNA) and nCas9n mRNA at the 1-cell stage. Founders were screened by PCR and T7 endonuclease digest which identifies mutations in the DNA, and founders were used to generate F1 lines. The F1 generation was screened by using tail fin DNA for PCR and T7 endonuclease digest. F1 zebrafish were sequenced and frameshift mutations were identified. Zebrafish with confirmed frameshifts will be incrossed to produce an F2 generation. The F2 generation, of which 25% are expected to be homozygous mutants, will be studied for anatomical abnormalities in craniofacial development by using Alcian Blue and Alizarin Red histological stains for cartilage and bone. The work in this project will be used to identify the roles of zmym2 and zmym3 in zebrafish development, and how loss of function of these factors may underlie the defects seen in PSS.

PHIDIANIDINE ANALOGUES CONTAINING AN ISOXAZOLE RING STRUCTURE

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The natural world is rich with medicinal compounds that can prove effective in the treatment of pain, disease, and even cancer. Phidianidines A and B are alkaloids that were isolated from the marine mollusk Phidiana militaris in 2011. Studies have shown that the molecule is capable of binding to the μ-opioid receptor, but does not bind to the δ- or κ - opioid receptors. Consequently, the drug can reduce pain without causing a euphoric effect. Phidianidine has also demonstrated cytotoxicity against cancerous cell lines, while somatic cells are unaffected. A potentially strong factor in the drug's activity is its unique 1,2,4-oxadiazole ring structure. Phidianidine is the only naturally occurring molecule to exhibit this particular structure. This study was conducted to substitute the 1,2,4-oxadiazole ring with an isoxazole ring to produce a phidianidine analog that can be investigated for its pharmacological properties. The initial approach relied on a a cyclization reaction between propargyl indole and an oxime substrate to form the isoxazole, but this provide ineffective. A different route was identified: the addition of a functionalized isoxazole to the 3-position of indole using MeMgI as a base. This method proved effective for various substituted indoles though the yields moderate. Work is ongoing to improve the yield of these reactions. Additionally, more complex isoxazoles are being synthesized for use in this reaction so that analogs that contain all of the structural motifs found in the phidianidines can be made.

NUMERICAL SIMULATIONS OF A JET-CLOUD COLLISION AND STARBURST: APPLICATION TO MINKOWSKI'S OBJECT

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We present results of two- and three-dimensional, multi-physics simulations of an AGN jet colliding with an intergalactic cloud. The purpose of these simulations is to assess the degree of "positive feedback," i.e. jet-induced star formation, that results from such a collision. We have specifically tailored our simulation parameters to facilitate comparison with recent observations of Minkowski's Object (M.O.), a stellar nursery located at the termination point of a radio jet coming from galaxy NGC 541. As shown in our simulations, such a collision triggers shocks which propagate around and through the cloud. These shocks condense the gas and trigger cooling instabilities, creating runaway increases in density, to the point that individual clumps can become Jeans unstable. Our simulations provide information about the expected star formation rate, total mass converted to HI, H_2, and stars, and the relative velocity of the stars and gas. Our results confirm the possibility of jet-induced star formation, though fail to match the level observed in M.O. We discuss ways in which the agreement might be improved in future simulations.

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