

MERRIMACK COLLEGE

WELCOMES YOU TO THE 79TH ANNUAL



Saturday, April 20, 2024

Hosted by the **Department of Biology** R

The Center for Health Inclusion, 불 **Research and Practice**



SCHEDULE OF THE DAY

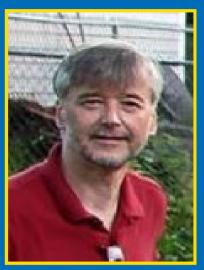


8:00 AM - 9:00 AM	Registration & Breakfast Cascia Hall
9:00 AM - 9:15 AM	Welcoming Remarks Cascia Hall
9:15 AM - 10:15 AM	Keynote Lecture: Dr. Jude T. Deeney, Boston University "Pancreatic beta-cell function and glucolipotoxicity relative to type 2 diabetes" Cascia Hall
10:45 AM - 12:15 PM	Student Oral Presentations Crowe Hall 208, 209, 210, 211
12:15 PM - 1:30PM	Lunch Crowe Hall Executive Room 206
1:00PM - 1:30PM	ENEBC Business Meeting (Faculty) Crowe Hall 208
12:45 PM - 1:15PM	Tour of Shared Instrumentation and Research Facility Palmisano Hall (Tour leaves Crowe Lobby at 12:45PM)
1:30PM - 3:15PM	Poster Session Cascia Hall
3:20 PM	Closing Remarks

MERRIMACK COLLEGE MAP Directions to Campus

KEYNOTE SPEAKER





Jude T. Deeney, PhD Professor, Medicine

Dr. Deeney's research is designed to discern the nutrientderived metabolic signals leading to glucose- and fatty acid (FA)-induced insulin exocytosis from the pancreatic ß-cell. FA acutely stimulates glucose-induced insulin secretion (GSIS) while chronic exposure to elevated FA and glucose can result in glucolipotoxicity (GLT) with basal hypersecretion and inhibition of GSIS. Despite the adverse effects of chronic exposure, FA is known to be required for normal exocytosis from the ß-cell. Deciphering the dual effects of FA on GSIS will lead to the possible development of therapies that would reduce the inhibitory effects while sparing the stimulatory effects of FA on the ß-cell. Studies in his laboratory are aimed at identifying the lipids or lipid classes involved in enhancing and suppressing GSIS and assessing their effects on lipidmodulated or modulating proteins.

ORAL PRESENTATIONS



10:45 AM - 12:15 PM

Molecular Genetics & Development 10:45AM-11:00AM Characterizing the morphology of Aδ high-threshold mechanoreceptors that innervate glabrous skin in mice Analyzing Selective Pressures on Mutations in Barrett's Esophagus and Esophageal Adenocarcinoma Using Computational Methods Kira A. Glasmacher By Way of the Proteasome: Investigating the Regulation of SKN-1A by the KLF Transcription Factors in C. elegans Ugnė Kurdeikaitė Assigning the 'lightning bolt tail' (Bolt) mutation to the Axin2 gene in mice. Gabriel B.-D. Kwarteng and Emily K. Moreau Tag, Turbo Is It: Using Proximity Labeling with TurboID to Study Polycomb **Repressive Complexes in Drosophila** Enva Selders Microbiology CA mutational approach to determine the main protein filament used for Fe(III) oxide reduction by Geobacter sulfurreducens Baha Alsagri The levels of the ConB bacterial conjugation protein modestly rely on the presence of other conjugation proteins Amyloid Aggregation in Bacteria Mapping HCMV gene regulatory elements within a locus that encodes determinants of viral latency and reactivation **Rofail Wassef** Developmental Biology 10:45AM- 11:00AM The nervous system is important for cell division during regeneration of rhinophores in the nudibranch Berghia stephanieae Haleigh Bilodeau Insights into the mechanism of hind limb initiation in Xenopus laevis Imprinted gene expression in cloned cow embryos **Rachel Garv** Investigating the role of Aurora A kinase on localization and expression of postsynaptic dTACC at the neuromuscular junction in Drosophila melanogaster

ORAL PRESENTATIONS CONT.



Impacts of Pollutants on Biological Systems

Crowe 211

10:45AM- 11:00AM	Interaction between perfluoro-octanoic sulfonate and common antibiotics induce developmental anomalies and lethality in Xenopus laevis Shreya Chattapadhyay
11:03AM-11:18AM	Flowing Hazards: Understanding Where Microplastics are Located and their Concentration Alyssa Cugno
11:21AM-11:36AM	Rivers of Resistance: Unveiling Threat of Antibiotic Resistance in the Merrimack River Valley Colby Currier, Aidan Gibbs
11:39AM-11:54AM	Investigating the Effects of Environmentally Realistic Herbicide and Pharmaceutical Exposure on Aggressive Behaviors in the Siamese fighting fish, Betta splendens. Katelyn Smalley

(IN ORDER)



Molecular Genetics & Development

Characterizing the morphology of A $m{\delta}$ high-threshold mechanoreceptors that innervate glabrous skin in mice

Karen Nguyen (1), Karina Lezgiyeva (2), Alan Emanuel (2), Lijun Qi (2), Nikhil Sharma (3), David Ginty (2)

(1) Department of Neuroscience, Simmons University, Boston, MA 02115, (2) Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, 02115, (3) Departments of Pharmacology and Systems Biology, Columbia University, New York, NY 10032

Our touch sensation is mediated by the activation of sensory neurons residing within the skin that decodes a variety of stimuli. Our understanding of intense touch processing stems from past work focusing on C-fiber high threshold mechanoreceptors (HTMRs), furthering our understanding of the neural mechanisms underlying our sense of touch. However, a lesser-understood group of neurons known as A δ high-threshold mechanoreceptors (exhibiting high mechanical thresholds and fast conduction velocities remains understudied. To understand their distinct characteristics, we developed Smr2Cre and Bmpr1bCre mouse lines which we hypothesize to label distinct classes of Aδ-HTMRs that innervate glabrous (non-hairy) skin. To characterize their morphology, we used AP whole-mount labeling to visualize their anatomical receptive fields and structures on a single cell level. Immunohistochemistry was used to visualize the termination patterns in the spinal cord, depicting the projection from the peripheral nervous system to the central nervous system. Findings suggest that this distinct class of neurons labeled by both genetic lines densely innervate glabrous skin and form exhibit large anatomical receptive fields penetrating the epidermis and form free nerve endings. The central projections of neurons labeled with Smr2Cre and Bmpr1bCre terminate across both superficial and deep layers of the spinal cord dorsal horn. Notably, the deep terminals of Smr2Cre-labeled neurons exhibit a distinctive morphology, forming intricate cage-like structures enveloping one or multiple cell bodies of dorsal horn neurons. Together, these genetic tools targeting A δ -HTMRs offer an opportunity to comprehensively study these neurons as sensory neurons reveal their properties and central connectivity to roles in behavior.

Analyzing Selective Pressures on Mutations in Barrett's Esophagus and Esophageal Adenocarcinoma Using Computational Methods

Kira A. Glasmacher, Vincent L. Cannataro

Department of Biology, Emmanuel College, Boston, MA

Esophageal adenocarcinoma (EAC) is one of the deadliest cancer types globally with a five-year relative survival rate of only around 22%. With Barrett's esophagus (BE) being its only known precursor condition, a better understanding of the differential molecular variants driving BE and EAC is crucial to best inform prevention and early detection strategies, and to avoid the most EAC cases and deaths. We can quantify the selective advantages of mutations in different steps of the evolutionary trajectory by analyzing genomic DNA sequencing data from BE and EAC samples. By quantifying the selective advantages rather than frequency of variants, which would be confounded by the differing underlying mutation rate in the genome, we can discern distinct mechanisms driving BE and EAC pathogenesis. Our analysis reveals a diverse array of variants conferring proliferative advantages specific to BE, distinct from previous findings on normal esophageal epithelium. Variants in FBXW7 are highly selected for in BE but exhibit limited effects on further malignant progression. In contrast, variants in KRAS, TP53, and PIK3CA exhibit heightened selection intensity in EAC, but have lower selective advantages in BE. These insights underscore the dynamic genetic landscape in the esophagus, highlighting key variants driving cell proliferation in different steps of evolution towards tumorigenesis. The differences between the results in esophageal adenocarcinoma and previous results for esophageal squamous-cell carcinoma also emphasize the need for targeted investigations specific to individual cancer types, rather than analyses that group together different diagnoses within the same organ.

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Molecular Genetics & Development

By Way of the Proteasome: Investigating the Regulation of SKN-1A by the KLF Transcription Factors in C. elegans

Ugné Kurdeikaité (1),(2), Maria La Terza (1), Jorge Ivan Castillo-Quan (2), (3), T. Keith Blackwell (2), (3), Natalie Karagodsky1

(1) Department of Biology, Emmanuel College, Boston MA, (2) Section of Islet Cell and Regenerative Biology, Joslin Diabetes Center, Boston MA, (3) Department of Genetics, Harvard Medical School, Boston MA

Aging is an inevitable process experienced by all organisms and characterized by a gradual decline in physical function. Aging increases the risk of age-associated diseases (AAD) such as cardiovascular disease and Alzheimer's disease. It has been hypothesized that an accumulation of reactive oxygen species (ROS) contributes to AAD pathology, though this remains incompletely understood. ROS cause direct structural damage to macromolecules such as DNA and proteins, resulting in cellular stress and deterioration. To counteract this, the proteasome plays a crucial role in removing damaged proteins. The transcription factor SKN-1, specifically the isoform SKN-1A has been found responsible for maintaining proteasomal activity in Caenorhabditis elegans (C. elegans). In a genome-wide screen we found that KLF-1, a Krüppel-like factor (KLF), regulates SKN-1. We were interested to know whether the KLFs, a family of transcription factors with known roles in stress resistance and survival, regulate SKN-1A. To evaluate this we assayed the proteasomal recovery response which is regulated by SKN-1A. Using the proteasomal inhibitory drug Bortezomib, we assayed two proteasomal reporter strains: rpn-12p::GFP and rpn-6::tdtomato, in a wild-type (wt) and glp-1 background, a strain with increased SKN-1 activity. We observed that knocking down klf-1 or klf-2 by RNAi had no effects on rpn-12p::GFP expression, with or without Bortezomib, however in glp-1 animals in the presence of Bortezomib, rpn-6::tdtomato expression was reduced upon klf-1 and increased upon klf-2 RNAi. This suggests that KLF-1 is a positive regulator of SKN-1A and proteins that KLF-1 is a positive regulator of SKN-1A and proteasomal maintenance, while KLF-2 is a negative regulator.

Assigning the 'lightning bolt tail' (Bolt) mutation to the Axin2 gene in mice.

Gabriel B.-D. Kwarteng & Emily K. Moreau

Central Connecticut State University, New Britain CT

Carriers of the "lightning bolt tail" mutation (Bolt) can be recognized by their bent tails, but this phenotype can range from dramatic kinking to undetectable (i.e., non-penetrant). Bolt also controls recessive semi-lethality, such that most—but not all—Bolt homozygotes die before birth. As a foundation for positional cloning, we have genetically mapped Bolt based on 500 progeny from a backcross of hybrid Bolt/+ mice to +/+. These mice were typed for Bolt (based on phenotype and on progeny-testing), and DNA samples isolated from each were characterized for various PCR-scorable DNA markers on distal Chromosome 11. This analysis restricted Bolt to an interval where only 4 protein-coding genes lie, including our primary candidate, Axin2. While primer-extension sequence analysis of individual Axin2 exons showed no Bolt-specific defects, long-read sequence analysis of Bolt/Bolt DNA (by our collaborator, Dr. Laura Reinholdt, at The Jackson Laboratory, Bar Harbor, ME) revealed a tandem duplication and insertion within the Axin2 gene. This anomaly is specific to the Bolt mutation, is predicted to result in a truncated protein product, and is the likely molecular basis of the mutatin phenotype.

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Molecular Genetics & Development

Tag, Turbo Is It: Using Proximity Labeling with TurboID to Study Polycomb Repressive Complexes in Drosophila

Enya Selders (1),(2),(3), Melissa Allen(1),(2),(3), Justin Bosch(4), Mitzi Kuroda(2),(3), Janel Cabrera(1),(2),(3)

(1)Department of Biology, Emmanuel College, Boston MA, (2)Department of Genetics, Harvard Medical School, Boston MA, (3)Division of Genetics, Brigham and Women's Hospital, Boston MA, (4)Department of Human Genetics, University of Utah, Salt Lake City, UT

Polycomb Repressive Complexes (PRCs) play an important role in regulating gene silencing during development and differentiation. There are two major canonical complexes, PRC1 and PRC2, which establish and maintain the silent state by catalyzing repressive histone modifications. Mammalian studies have identified five variant PRC1 (vPRC1) complexes that function in gene repression. These vPRC1 complexes have been implicated in initial Polycomb group protein (PcG) targeting, a step that is currently not well understood. Recent findings from our lab have discovered that Drosophila has vPRC1 complexes that are homologous to the complexes seen in mammals. We propose utilizing the proximity labeling method, TurbolD, to analyze these subunits in early developmental time windows. The promiscuous BirA enzyme of TurboID is able to biotinylate interacting proteins rapidly at a temperature suitable for Drosophila culture, which can be identified through pull down and mass spectrometry. Using this system, we tagged known chromatin modifying proteins, Pc and MSL3, in Drosophila S2 cells in order to identify protein-protein interactors as proof of concept for studying earlier time frames of development. We found TurboID successful in biotinylating both proteins of interest and their known interactors in S2 cells. Additionally, the known interactors pulled down in mass spectrometry were unique to the chromatin modifying complex of each tagged protein without overlap, demonstrating specificity of the tag. Dissecting the composition and function of vPRC1 complexes through TurboID labeling in a simple model organism like Drosophila will provide an important complementary approach to mammalian studies of PRCs in development and disease.

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Microbiology

A mutational approach to determine the main protein filament used for Fe(III) oxide reduction by Geobacter sulfurreducens

Baha Alsaqri

Department of Biomolecular Sciences, Central Connecticut State University, New Britain, CT

Fe(III) oxide reduction is a form of respiration used by some anaerobic microorganisms that involves the transfer of electrons on the outer cell surface via electron carrying proteins. Geobacter sulfurreducens, a model species for studying the mechanism of Fe(III) reduction, uses conductive filaments for long-range direct electron transport outside of the cell. Recently, several hypotheses have emerged as to which protein(s) make up these filaments. Some researchers have speculated these filaments are comprised of c-type cytochromes including OmcS, OmcZ, or OmcE, while other studies suggest that PilA-pilin monomers assemble into conductive nanowires that transport electrons from the cell surface. In this study, several deletion mutant strains were constructed that lack one or a combination of the proposed cytochrome filaments. Culturing of each of these cytochrome-deficient strains in Fe(III) oxide medium revealed that all of the mutants were still capable of reducing Fe(III). However, a strain in which the wild-type PilA-pili was replaced with a non-conductive pili prevented G. sulfurreducens from reducing Fe(III) to Fe(II) to Fe(II) oxide reduction by G. sulfurreducens.

The levels of the ConB bacterial conjugation protein modestly rely on the presence of other conjugation proteins

Victoria E. Arinella, Meri Kalashyan, and Melanie B. Berkmen

Department of Biochemistry, Chemistry, Environment, and Physics, Suffolk University, Boston, MA

Bacteria have the remarkable ability to transfer genes from cell to cell in a process known as conjugation or mating. The DNA is transferred through the cell membrane via a specialized DNA translocation channel, which is made up of many proteins. Mating results in the transmission of genes that play roles in vital processes such as symbiosis, metabolism, antibiotic resistance, and pathogenesis. Our research focuses on the DNA translocation channel of ICEBs1, a conjugative DNA element found in the bacterium Bacillus subtilis. The proteins that make up the ICEBs1 DNA translocation channel are ConB, ConC, ConD, ConE, ConG, ConQ, and CwIT. Since these proteins form a complex that might stabilize ConB or protect it from degradation, we asked whether the levels of ConB change when other conjugative machinery proteins are absent. We measured the levels of ConB is trains deleted for other ICEBs1 genes. We grew up the various bacterial strains and created lysates after normalizing for cell quantity. Using quantitative western blots with anti-ConB antibody, we determined that ConB levels were decreased 1.5-, 1.8-, and 2.2-fold in strains lacking ConD, ConE, and ConQ, respectively (P < 0.05). ConB levels in other deletion strains were not statistically different compared to the wild type. Overall, we conclude that the levels of ConB modestly rely on the presence of ConD, ConE, and ConQ, but not other ICEBs1 DNA translocation channel components (ConC, ConG, and CwIT). Our research sheds insight on the function of ConB, which contributes to a deeper understanding of the conjugation process.

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Microbiology

Amyloid Aggregation in Bacteria

Emily Pike(1), Eleanor Fleming(2), Ann Hochschild(2), Padraig Deighan (1),(2)

(1)Department of Biology, Emmanuel College, Boston MA, (2) Department of Microbiology, Harvard Medical School, Boston MA

Fe(III) oxide reduction is a form of respiration used by some anaerobic microorganisms that involves the transfer of electrons on the outer cell surface via electron carrying proteins. Geobacter sulfurreducens, a model species for studying the mechanism of Fe(III) reduction, uses conductive filaments for long-range direct electron transport outside of the cell. Recently, several hypotheses have emerged as to which protein(s) make up these filaments. Some researchers have speculated these filaments are comprised of c-type cytochromes including OmcS, OmcZ, or OmcE, while other studies suggest that PilA-pilin monomers assemble into conductive nanowires that transport electrons from the cell surface. In this study, several deletion mutant strains were constructed that lack one or a combination of the proposed cytochrome filaments. Culturing of each of these cytochrome-deficient strains in Fe(III) oxide medium revealed that all of the mutants were still capable of reducing Fe(III). However, a strain in which the wild-type PilA-pili was replaced with a non-conductive pili prevented G. sulfurreducens from reducing Fe(III) to Fe(II) oxide reduction by G. sulfurreducens.

Mapping HCMV gene regulatory elements within a locus that encodes determinants of viral latency and reactivation

Rofail Wassef(1) and Tracy Rosebrock(1),(2)

Biology(1) and Health Science(2) Departments, Stonehill College.

Human cytomegalovirus (HCMV) is a beta-herpesvirus with a global prevalence estimated between 45% and 100%. Primary infection with HCMV is mild or asymptomatic in immunocompetent individuals. Following a period of initial viral replication, the HCMV genome is maintained in hematopoietic progenitor cells with limited production of viral progeny but with the potential to accelerate viral production in response to changes in host homeostasis. The ULb' region of the HCMV genome includes the protein coding genes (UL135, UL136, and UL138) that help to direct the latency/reactivation switch of HCMV. The functions of the resultant proteins are characterized; UL135 and larger forms of UL136 support viral replication, whereas shorter forms of UL136 and UL138 are suppressive. Yet, the regulatory elements that govern the expression of these genes are not understood. The goal of this study is to map regulatory activity to specific regions of the HCMV genome fragments (TB40/E) for the ability to direct luciferase assay, we tested a tiled library of -500bp HCMV genome fragments (TB40/E) for the ability to direct luciferase expression in the human cell line, HEK293. We have identified two putative promoter regions, one upstream of UL136 and a second upstream of UL135, and a region of repressor activity that spans from UL136 linto UL138. Our results suggest that in cells permissive for viral replication, human transcription factors may bind promoters that activate transcription of UL136 and long forms of UL136.

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Developmental Biology

The nervous system is important for cell division during regeneration of rhinophores in the nudibranch Berghia stephanicae

Haleigh Bilodeau, William H. Scala, James M. Newcomb

Department of Science Health and Education, New England College, Henniker, NH

Recent evidence suggests that the nervous system may be important during early stages of regeneration in the axolotl (Ambystoma mexicanum) and other animals. At these early timepoints, accelerated stem cell proliferation is important for regrowth of limbs and appendages. The nudibranch, Berghia stephanieae, can regenerate its chemosensory rhinophores. With this study, the aim was to determine if inhibition of the nervous system via anesthetic would impact this cell division during early stages of regeneration. One rhinophore was amputated in 28 Berghia. Half of these animals were not exposed to anesthetic at any point during the experiment (i.e., negative controls). The other 14 animals were anesthetized with 0.1M magnesium chloride during amputation of the rhinophore and for the remaining duration of the experiment. Select animals in each group were sacrificed at 4, 24 or 48 hours. Immunohistochemistry was then performed on each animal with an antibody to phosphorylated histone 3 (H3P), a marker for cell division. It was found that anesthetic did not influence the baseline level of cell division occurring in non-regenerating tissues, at all timepoints tested. Inhibition of the nervous system via anesthetic significantly decreased the level of cell division in regenerating tissues 4 hours after amputation (p = 0.028). At 48 hours, anesthetic suggest that the nervous system may be important in early stages of regeneration in both nudibranchs and amphibians, and thus be an ancestral mechanism related to regeneration.

Insights into the mechanism of hind limb initiation in Xenopus laevis

Milena Chaufan(1), Samantha Royle(2), Olive Lucanish(1), Vibhuti Naik(1), John Young(1)

(1)Simmons University, (2)Harvard Medical School Department of Genetics

The vertebrate limb has provided a deep understanding of the cellular, genetic, and molecular mechanisms that generate an appendage. The majority of limb experimentation has been in animals where the limb forms early in embryogenesis. Yet, several tetrapods, most notably frogs, form their limbs well after embryonic patterning and differentiation have occurred. Surprisingly, we know very little about the processes of indirect limb formation in amphibian tadpoles since most limb research in these animals has focused on regeneration. This work investigates the earliest steps in Xenopus hindlimb initiation. We used both molecular methods such as CRISPR/Cas9 and morpholino microinjections, and classical transplant experiments to determine the roles, timing, and origin of the hindlimb forming genes, which were analyzed via histology and whole mounts. Fluorescent transgenic transplant assays revealed lateral plate mesoderm cells of the neurula generate the hindlimb. Histological analyses revealed these cells express Pix1 and Tbx4 in the early tadpole, both key genes involved in limb initiation. These cells are mesenchymal at stage 40 and appear to condense into a bud by stage 46. Surprisingly, Fgfl0, a major contributor to limb formation in amniotes, is not expressed until well after bud formation. These results suggest that, unlike amniotes, Fgfl0 is dispensable for bud formation. Together, these data suggest a model that resembles zebrafish fin formation and condensation. Current efforts are underway to manipulate Tbx4, Fgfl0, and Pitx1 in developing hindlimb.

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Developmental Biology

Imprinted gene expression in cloned cow embryos

Rachel Gary, Audra Antonucci, Leanne Bilo, Jeffrey Nicholas, De'ja Salahuddin and Sadie L. Marjani

Department of Biology, Central Connecticut State University, New Britain, CT

Many people have heard about Dolly the sheep, the first cloned animal. Since then, many animals have been cloned by somatic cell nuclear transfer (SCNT), but cloning suffers from low efficiency and can sometimes produce animals with developmental abnormalities. KCNQIOTI is an imprinted gene in cows and humans that is associated with Beckwith-Wiedemann Syndrome (BWS) in humans and large offspring syndrome in cattle. Our objective was to test the expression of KCNQIOTI in cloned cow embryos and compare it to the expression in the donor cells used for cloning and to control in vivo embryos by reverse transcription quantitative PCR (RT-qPCR). We compared the cycle threshold of cloned embryos (n=10) to in vivo control embryos (n=8) and donor cells and used H3F3B as the reference gene. Data were analyzed using the comparative cycle threshold method. Mann-Whitney U tests showed a statistically significant (p= 0.02) difference in expression of KCNQIOTI between SCNT embryos and the donor cells (p=0.24). These findings suggest that the KCNQIOTI gene in SCNT embryos may not have undergone the normal imprinting mechanisms during development, indicating abnormal nuclear reprogramming of this gene during the cloning process. Due to high rates of pregnancy loss and neonatal death of clones, it is important to identify abnormal gene expression, especially of imprinted genes, which are known to be involved in growth and development.

Investigating the role of Aurora A kinase on localization and expression of postsynaptic dTACC at the neuromuscular junction in Drosophila melanogaster

Sarina Lau, Seth Johnson

Department of Biology, Simmons University, Boston, MA

Dysregulation of chemical synapses have shown implications in neurodevelopmental and neurodegenerative disorders, including Autism Spectrum Disorder (ASD) and intellectual disability (ID). The neuromuscular junction (NMJ) of the common fruit fly, Drosophila melanogaster, can be utilized as a model to examine the molecular and subcellular mechanisms underlying synapse development and growth. Synapse proteins regulate proper growth and development, including factors that alter microtubule dynamics. dTACC, a microtubule-associated protein, has been demonstrated to restrict NMJ growth, and is localized pre- and postsynaptically. However, the mechanism by which postsynaptic dTACC localization and expression is regulated at the NMJ has not been determined. Centrosomal localization of dTACC on microtubules is regulated through phosphorylation on S863 by Aurora A kinase. To evaluate how dTACC localization and expression is regulated by Aurora A kinase, we expressed RNAi against Aurora A kinase using the GAL4/UAS system in the postsynaptic muscle cell and observed a significant decrease in maximum dTACC fluorescence intensity (p = 0.03078) through quantification of the NMJ at the A2 segment in muscles 6/7. In addition, mutant flies containing a GFP-tagged dTACC mutant at the S863 phosphorylation site (UBI-TACCS863E-GFP and UAS-TACCS863L-GFP) were characterized in terms of NMJ morphology, dTACC fluorescence intensity, and fluorescence distribution. Preliminary results using the S863E dTACC mutation show a reduction in postsynaptic localization of dTACC. This data suggests that Aurora A kinase is required for proper localization of dTACC, and also suggests possible requirement of phosphorylation at S863 for proper localization of dTACC.

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Impact of Pollutants on Biological Systems

Interaction between perfluoro-octanoic sulfonate and common antibiotics induce developmental anomalies and lethality in Xenopus laevis

Shreya Chattapadhyay(1), Emma Harrison(1),(2), Ganad Neka(1),(3), Maya Baskin(1), Nora Richmond(1), Quynh Nguyen(1), Isabel Wade(4), Arya Anekal(5), John Young(1)

(1)Simmons University, (2)Center for Childhood Cancer Research, Nationwide Children's Hospital, (3)Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, (4)University of California, Davis, (5)Newton High School

Perfluorooctanesulfonic acid (PFOS) is a synthetic fluorinated organic compound characterized by a unique combination of properties that arise from its intricate molecular structure. PFOS is one of a broader family of perand polyfluoroalkyl substances (PFAS), many of which have been produced and utilized in various industrial applications and consumer products over the past several decades due to their resistant properties. The different PFAS molecules have varying levels of bioaccumulation, and their effects on the physiology of humans and other organisms have been studied extensively in the past. Our study investigates the developmental impacts of being exposed to PFOS in our model organism Xenopus laevis. Upon dosing the embryos with varying concentrations of PFOS, we found that it causes a shortening in total length of the tadpoles. Additionally, we also found the formation of a cellular mass within the dorsal fin which is highly dose dependent. Antibody staining revealed that the cell mass is neither an extension of the fin epithelium nor an extension of the neural tube, rather it is composed of living mesenchymal cells. We found that co-exposure to PFOS with antibiotics caused embryonic cells to lose integrity and increased lethality presumably due to an observed increase in apoptosis. The interaction is exacerbated with antibiotics that target bacterial ribosomes such as gentamicin and tetracycline. Our results suggest a potential mechanism wherein PFOS reached toxic levels when present alongside aforementioned antibiotics.

Flowing Hazards: Understanding Where Microplastics are Located and their Concentration

Alyssa Cugno, Alexis Byrne, Daniel Ayanian, Brian Reilly, Jaidyn Gross, W.G. McDowell Department of Biology, Merrimack College, North Andover, MA

Microplastics (MPs) are a novel class of environmental pollutants that are increasingly affecting bodies of water worldwide, including drinking water reservoirs, surface waters, and oceans, with potential repercussions on ecosystems and human health. This study focuses on samples collected from the Lamprey River in New Hampshire, aiming to identify areas with heightened microplastic contamination and quantify their concentrations. Additionally, we seek to establish potential correlations between watershed characteristics and the abundance and types of microplastics present. To minimize external contamination, water samples were collected in 500 mL glass media bottles on a monthly basis. Back in the lab, we took five milliliters of each sample to undergo digestion in twenty-five milliliters of hydrogen peroxide, followed by each sample being incubated at twenty-seven degrees Celsius for forty-eight hours under a tin foil cover. Once fully digested, samples were filtered using a vacuum filtration system onto 0.45um gridded Millipore filters for quantification. Preliminary findings reveal fluctuating microplastic morphologies and sizes across different sites, likely influenced by environmental variables like rainfall, temperature fluctuations, drought, or sediment disturbance. Initial data suggests a discernible rise in freshwater microplastic levels during specific periods, such as post-heavy rainfall or significant temperature shifts. However, further research is warranted to solidify these findings. In conclusion, microplastic contamination is evident in New Hampshire's freshwater bodies, underscoring the necessity for continued investigation into its impacts.

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Impact of Pollutants on Biological Systems

Rivers of Resistance: Unveiling Threat of Antibiotic Resistance in the Merrimack River Valley

Colby Currier, Aidan Gibbs, and Charlotte Berkes

Department of Biology, Merrimack College, North Andover, MA

Antibiotic resistance refers to the ability of bacteria to resist the effects of antibiotics that normally would slow down their growth and/or kill them. Antibiotic resistance genes (ARG) in bacteria evolve quickly and are transmitted through the environment even faster. The build up of these genes leads to the creation of superbugs, pathogenic bacteria that are highly resistant to a variety of antibiotics. The appearance of these superbugs is a great risk to the public and monitoring the presence of ARG is important for public health. To evaluate the presence of some of these genes in our local environment we took freshwater samples from the Salmon, Spicket, Concord, Nashua, and Merrimack Rivers as well as a combined sewage overflow (CSO) site into the Merrimack river. DNA was isolated from each sample and used as a template in quantitative polymerase chain reactions (qPCR) to measure the amount of two different ARGs (TetA and Sul2) relative to a "total bacteria" gene (I6S). Overall, a higher bacterial population was found in the Merrimack river when compared to other rivers as well as higher rates of TetA and Sul2 presence. TetA and Sul2 were detected in the majority of samples from all rivers, and samples collected at the Merrimack River CSO site displayed ARG levels 3-70 fold higher compared to a nearby upstream site. These data show a robust relationship between the increase of ARG and the bacteria introduced from CSO in freshwater systems as a tangible human impact on the environment.

Investigating the Effects of Environmentally Realistic Herbicide and Pharmaceutical Exposure on Aggressive Behaviors in the Siamese fighting fish, Betta solendens.

Katelyn Smalley*, Yasmin Davis*, Ben McDonnell*, Carlos Viteri* Faculty Mentor: Dr. R. David MacLaren*

Department of Biology, Merrimack College, North Andover

Endocrine-disrupting compounds (EDCs) are pollutants that disrupt the endocrine system and are derived from different anthropogenic sources. These chemicals remain persistent in various aquatic environments. Fish are especially vulnerable to these pollutants as they absorb such xenobiotics like herbicides and pharmaceuticals from the water, sediments, and food they ingest. The Siamese fighting fish, Betta splendens, among other aquatic organisms, are greatly affected by these human-induced chemicals due to having both direct and indirect exposure in their natural environments. The consequences of having this contact has been known to be detrimental to not only their reproductive system but also in altering their natural behaviors. Aggressiveness is a vital component in B. splendens survival, especially for males that are responsible for nest building, defending territories and courting females. The purpose of this study was to observe the combined and individual behavioral effects of exposure to the compounds fluoxetine (pharmaceutical) and atrazine (herbicide) on B. splendens after acute waterborne exposure at two different concentrations (low- and high-dose), expecting to observe reduced aggression as a consequence of exposure. This was accomplished by recording the latency to respond, frontal display, and lateral display behaviors (5 high and 6 low concentration experiments) over 3 days of male conspecific interactions. Preliminary results indicated that exposure to these compounds reduced aggression, but our current findings remain indefinite. The potential consequences of diminished aggression on these key behaviors not only disrupt their natural ecological roles but also threaten their overall fitness and survival.



1:30 PM - 3:15 PM

Cascia Hall

P1

Let's Get CERTIfied: Bringing Micro-Credentialling to the Emmanuel College Biology Department Chris Akut, Padraig Deighan

Department of Biology, Emmanuel College, Boston MA

A micro-credential (MC) recognizes focused learning, training, and corresponding competency in a skill or set of skills. MCs are an emerging trend in higher education and may be additional, or complementary, to a formal qualification such as an associate or bachelor's degree. MCs can be shared as a digital badge on a transcript or LinkedIn page. We explored integrating micro-credentials into the curriculum for Biology majors at Emmanuel College under Project Certified Employment Ready as Trained Instrumentalists-biotechnology (CERTI-biotech). We first researched bachelor-level biotechnology job postings in the Boston area to understand industry-needed skills and experiences to align those needs with the current training offered within the biology and biotechnology programs at Emmanuel College. As a result, we initially focused on exploring CERTIs in DNA transactions, polymerase chain reaction (CERTI-PCR) and next-generation sequencing (CERTI-seq). A CERTI-PCR was developed and includes a background and history of the technique, detailed protocols, explanations, and guided visual trainings, along with an assessment module. As a first step to introducing CERTI-seq, we developed protocols suitable for an undergraduate audience to learn nanopore sequencing and have successfully sequenced and analyzed both bacteriophage and bacterial genomes. Further, we employ the CERTI-seg technique to investigate genomic suppressors in Mycobacterium smegmatis, which are resistant to toxicity from an over-expressed bacteriophage protein. CERTI-PCR and CERTI-seq will be trialed in laboratory-based courses and student-scientists feedback will influence additional CERTI development.

P2

The Evolution of Floral Scent in Honeysuckles Brooke Bailey(1), Nina Theis, Ph.D(1)

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Honeysuckles (Lonicera spp.) are a diverse group of flowering plants distributed throughout the globe. Selection by pollinators drives the evolution of floral scent in some species. Known for its sweet floral odor, the genus Lonicera contains over 180 species, 40 of which grow at the Arnold Arboretum in Jamaica Plain, MA. We predicted that the pattern of scent production in Lonicera species would reflect a strong phylogenetic signal; divergences from that expectation may be explained through selection pressures from pollinators. To test this hypothesis, we collected floral scent using dynamic headspace sampling from several species of honeysuckle at the arboretum. Samples were run on a GC-MS and analyzed using Agilent's Chemstation software. Published phylogenies were used to identify several clades to determine whether floral scent mirrors phylogenetic relatedness. Our results demonstrated that in most <u>cases, sister taxa are more</u> similar in their scent than a closely related outgroup. However, floral scent is so labile that at higher taxonomic groupings phylogenetic patterns are less apparent. Selection for divergent scent was also apparent in the data. When closely related species overlap geographically, there is an expectation that selection will drive traits apart to ensure reproductive isolation. Lonicera sempervirens and Lonicera dioica are sister taxa, native to the eastern United States. These two species may have been subjected to selection for reproductive isolation driving differences in their floral scent. The complexity of scent evolution within the genus Lonicera demonstrates the interaction between phylogenetic relatedness and ecological selection forces.



P3

Anti-Inflammatory and Antioxidant Effects of Kaempferol on Breast Cancer Cells MCF-7 and MDA-MB-231

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Breast cancer is one of the most common forms of cancer. CDC reported that 2.3 million people were diagnosed worldwide in 2022. Breast cancer is a heterogeneous disease that involves both genetic and environmental factors as potential causes. An increased amount of treatment regimens have become available for Breast Cancer. These include chemotherapy, radiation, immunotherapy, surgery, and targeted therapy. With these harsh treatments, many negative side effects occur. Natural medicine which is derived from plants has little to no side effects and exhibits many health properties. The purpose of this study was to investigate the anti-inflammatory and antioxidant effects of Kaempferol on human adenocarcinoma breast cancer cell lines. Kaempferol is a secondary plant metabolite that is known as a flavonoid. It is derived from greens such as broccoli, kale, and spinach. The compound is known for its anticancer, antioxidant, antiinflammatory, neuroprotective, and antimicrobial properties. This was accomplished by performing cell viability, cell integrity, molecular analysis and oxidative stress indicator assays on breast cancer cell lines MDA-MB-231 and MCF-7. These were done by a 60µM exposure concentration of Kaempferol for up to 24 hours. The data showed that Kaempferol significantly impacted cellular viability by inducing apoptosis and high ROS for both cell lines. Results of this study provides better insight on how plant secondary metabolite's antioxidant and anti-inflammatory properties and their mechanisms provide potential breast cancer treatments and prevention from in vitro studies. Furthermore this data provides evidence that Kaempferol may have anticancer properties.

P4

Abnormal Gene Expression of Sirtuin 1 in Cloned Bovine Embryos

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Cells in an adult organism, with a few exceptions, undergo a differentiation process which limits the genes that cell expresses; this process enables different cell types to perform different functions. Using somatic cell nuclear transfer (SCNT), also known as cloning, genetic material of a differentiated cell is removed and placed into an enucleated oocyte. After SCNT is performed, epigenetic mechanisms enable the cell to express genes that were previously shut off by differentiation. If a cloned embryo does not undergo epigenetic reprogramming correctly, that clone will likely fail to thrive. The sirtuins are a family of histone deacetylases involved in the epigenetic control of gene expression. This study tested the relative gene expression of SIRT1 in cloned bovine embryos compared to in vivo embryos using RT-qPCR. RNA from the samples was reverse transcribed into cDNA; using specific primers the samples were tested for the expression of SIRT1 and the expression of H3F3B. This relative comparative CT analysis used H3F3B as a reference gene to standardize and compare the PCR cycle thresholds between each treatment group. The gene expression of SIRT1 was statistically different (p=0.003) between the cloned and the in vivo embryos. Additionally, expression differed significantly between donor cells and clones (p=0.03). SIRT1 has many roles in an embryo that are crucial for healthy development. These roles include facilitating zygotic genome activation, meiotic spindle assembly, and protection against oxidative stress. Abnormal expression of SIRT1 in cloned embryos compared to in vivo controls may contribute to the low viability of clones.



P5

Beneath the SKN: The Regulation of Aging by SKN-1 and KLF-1 in C. elegans

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Aging increases the risk of age-associated diseases (AAD) such as diabetes and Alzheimer's. Meanwhile, increased amounts of oxidative stress are associated with cellular aging and AAD. Thus, targeting oxidative stress on a molecular level may provide new therapeutic interventions for AAD. The conserved SKN-1C transcription factor mediates oxidative stress by regulating Phase II detoxification genes in Caenorhabditis elegans (C. elegans). klf-1, a Krüppel-like (KLF) transcription factor, regulates oxidative stress by regulating Phase I detoxification genes, preceding the Phase II response. The possible regulation of SKN-1C by KLF-1 remains unknown. We hypothesized that klf-1 plays a crucial role in regulating skn-1C under oxidative stress. To evaluate this relationship, we utilized RNA interference (RNAi) to suppress klf-1 and performed an oxidative stress assay using sodium arsenite (NaAsO2). We documented the survivability of wild-type (wt) and germline deficient (glp-1) worms, which exhibit higher SKN-1 activity. Next, we performed a 4-hour NaAsO2 treatment on RNAi-treated wt and glp-1 worms expressing a SKN-1 target reporter, gst-4::GFP. We visually scored each condition for low, medium, or high GFP fluorescence. Through the survival assay, we found that klf-1 knockdown severely reduced the enhanced survivability of glp-1 worms to wt levels under oxidative stress. In the 4-hour NaAsO2 treatment, GFP scoring revealed that glp-1 worms treated with klf-1 RNAi had significantly lower levels of gst-4::GFP compared to controls, whether they were treated with NaAsO2 or not. These findings suggest that KLF-1 is a positive regulator of glp-1-mediated stress resistance and SKN-1C activity under normal and oxidative stress conditions.

P6

Validation of Erythropoietin Receptor – Specific Agonists

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More than 5 million patients in the U.S receive erythropoietin (EPO) stimulating agents to rectify anemia annually. EPO promotes erythropoiesis by enhancing the survival and proliferation of erythroid-committed progenitors in the bone marrow. However, EPO leads to thrombocytosis and increased risk of stroke, suggesting it has pleiotropic roles in disparate cell types and highlighting the need for additional research on the cell-type specific effects of EPO. To examine cell type- and receptor-specific effects of EPO on defined populations of hematopoietic progenitor cells, we plan to utilize previously published, exogenously expressed small polypeptides that activate the canonical homodimer EPO receptor (homoEPOR) or the heterodimer of EPO receptor and CD131 (IRR) by binding their transmembrane domains. To validate the function of the receptor-specific polypeptides, we transduced them into a murine lymphoblastic cell line stably expressing human EPOR that is dependent on IL-3 or EPO for survival and proliferation. We cultured transduced cell lines in the absence of IL-3 or EPO to validate that activation of homoEPOR or IRR is sufficient to promote survival and growth. Indeed, cells expressing the receptor-specific agonists persisted in culture in the absence of IL-3 or EPO, whereas the control cell lines died within 5 days of withdrawing IL-3 and EPO. Confirmation of the function of receptor-specific agonists enables us to define the role of EPO signaling in isolated primary human hematopoietic progenitors, which will improve our understanding of the mechanism of action of EPO and aid in the development of targeted therapies for patients suffering from anemia.



P7

Shading affects settlement of invertebrates to panels in Gosport Harbor, Isles of Shoals

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Many abiotic (water current, surface topography, substrate composition, light intensity) and biotic (biofilms, conspecifics, adult food) factors influence the settlement of marine invertebrate larvae. In particular, shading or the amount of light affects the species composition of communities beneath piers. To gain a better understanding of the relationship between light and settlement of shallow, benthic communities I examined the settlement of invertebrates to the undersides of clear and black Plexiglas panels (n = 3 each). Panels (15 x 15 cm) were deployed beneath the pier at Star Island in Gosport Harbor, Isles of Shoals during July (3 weeks) and August (5 weeks), 2023. All sessile organisms present on the panels were counted and colonial species were traced onto transparencies to estimate area occupied (cm2). Settlement was dominated by non-native species in each month: July: Botrylloides violaceus, Diplosoma listerianum, Membranipora membranacea; August: Didemnum vexillum, D. listerianum, Tricellaria inopinata and M. membranacea. In addition, the blue mussel, Mytilus edulis was abundant in each month. In both July and August, settlement of colonial ascidians, bryozoans, and barnacles was greater on black surfaces than clear panels. In contrast, B. violaceus was more abundant on the undersides of clear panels in each month. These data suggest that shading is an important factor influencing the settlement of epifauna in subtidal marine communities, but the timing of deployment of settlement surfaces and data collection can impact results.

P8

Fostering Belonging: A Research Journey into Undergraduate Student Experiences in STEM at Emmanuel College

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Diversity, equity, inclusion, and belonging (DEIB) efforts in STEM ensure that all individuals, regardless of background, feel valued and empowered within the community. Sense of belonging is crucial for student retention and fosters a supportive environment where every individual feels accepted within STEM communities. Individuals gain the ability to enhance their future success as STEM individuals. Despite past research on DEIB initiatives in STEM, there is an absence of studies evaluating their long-term impacts and sustainability, crucial for refining strategies and ensuring sustained positive outcomes. Our study aims to assess the effectiveness of DEIB initiatives at Emmanuel College in enhancing students' sense of belonging within STEM fields. Through surveys and interviews with participants from minority groups, we investigate factors influencing their sense of belonging before and after engaging in inclusive STEM programing. Our results revealed a higher representation of students of color in the program participants compared to enrollment demographics.

Our findings revealed that 70% of participants reported an enhanced sense of belonging following their engagement in STEM initiatives. Additionally, surveys indicated that while participants felt supported by the STEM faculty, they did not perceive the same level of support from the broader STEM community. Our analysis demonstrates that having target DEIB initiatives at Emmanuel College was significant at increasing minoritized students see of belonging as STEM students while identifying areas of improvements within the STEM community.



P9

Assessing Functional Protocell Compositions Using Bacteriorhodopsin

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Compartmentalization is considered a requirement for life, and primitive membranes are proposed to be compositionally different than modern membranes. Techniques for assessing the functionality of membrane composition beyond permeability have not been developed. We used bacteriorhodopsin, a light-driven proton pump and integral membrane protein, to assess membrane functionality. The bacteriorhodopsin was observed for visible absorbance (560 nm) and a proton pump activity assay where 1.2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was used as the positive control. The other lipids tested were: oleic acid, decanoic acid, and DPPC. Liposome/protein constructs were prepared in 50 mM HEPES at pH 7.2 or water. To evaluate proton pumping, pyranine was used as an encapsulated fluorescent pH probe. Preliminary results suggest DOPC membranes support bacteriorhodopsin insertion demonstrating that membrane protein function is associated with lipid composition.

P10

Antioxidant Effects of (-)-Epigallocatechin Gallate Tested on Breast Cancer Cell Lines

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Cancer impacts millions of people every year. Cancer treatments such as chemotherapy and radiation harm both cancer and healthy cells. As an alternative method, natural medicine can be used for cancer treatment. Natural medicine has been used in different cultures for thousands of years to improve health and treat various diseases. Alternative medicines hold a variety of different properties such as antioxidant and antiinflammatory effects. Camellia sinensis (Green tea) is an example of a plant with antioxidant properties. One of the key extracts of green tea is (-)-Epigallocatechin gallate (EGCG). This study aims to investigate the effects EGCG has on two human breast adenocarcinoma cell lines. EGCG has shown to possess chemopreventive and antioxidant properties. The two breast adenocarcinoma cell lines used in the studies were MDA-MB-231 and MCF-7. The viability of cells was tested using 100uM of the drug concentration at different time points to test the viability and integrity of the cells. This determined the effect the selected metabolite had on the breast cancer cells. The observations throughout the study showed that the selected metabolite had a positive impact on both cell lines. Through testing, the drug showed a significant impact on reducing the growth and development of MDA-MB-231 and MCF-7. EGCG is an example of a natural medicine that holds the potential for pharmaceutical integration. Science is rapidly evolving to combat cancer using innovative approaches and advancing technologies worldwide. These findings provide a new realm of integrating natural medicines into pharmacology.

Key words: Antioxidants, Breast cancer, Epigallocatechin gallate



P11

THO Complex 4 Gene Expression in Cloned Embryos: Uncovering Patterns and Potential Significance

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Somatic cell nuclear transfer (SCNT) holds great promise for regenerative medicine and cloning animals, yet its efficiency in producing viable offspring remains a significant challenge. This study aims to investigate the expression patterns of THOC4, an mRNA-binding protein involved in nuclear transport, in bovine embryos produced through SCNT compared to control in vivo embryos and original donor cells. To analyze expression patterns, real-time quantitative polymerase chain reaction (RT-qPCR) was utilized to analyze THOC4 expression levels in cloned embryos, in vivo embryos, and donor cells, with H3F3B serving as the control gene. Statistical analyses, including the Mann-Whitney U test, were conducted to determine significant differences in THOC4 expression between these groups using the comparative CT method. The findings revealed significant differences in THOC4 expression levels between SCNT embryos and both in vivo embryos (P= 0.02) and donor cells (P=0.04), with cloned embryos exhibiting a higher level of expression. While THOC4 is present in SCNT embryos, its precise function during embryonic development remains unclear. The observed differential expression suggests potential abnormalities during development in SCNT embryos. The discovery identifies THOC4 as a potential target for enhancing the efficiency of SCNT technology. Further research is crucial to better understand THOC4's role in embryonic development, which may contribute to advancements in SCNT.

P12

Everolimus Alleviates CD4+ T Cell Inflammation by Restoring Redox Homeostasis

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Aging is a process characterized by the gradual decline in physiological functions and is associated with the onset and progression of multiple diseases, thereby limiting the human health span. Chronic low-grade inflammation in the absence of overt infection is considered the underlying source that triggers ageassociated diseases. Failure of many cellular processes during aging is mechanistically linked to inflammation. However, the overall decline in cellular redox homeostasis emerges as a significant inducer of inflammation during aging, frequently known as inflammaging. Thus, physiological or pharmacological interventions aimed at improving redox homeostasis are considered geroprotective. Rapamycin analogs (rapalogs) are known for their ability to inhibit mTOR and regulate redox balance in many cell types. This study assessed the efficacy of Everolimus, a rapalog, in regulating proinflammatory cytokine production in T cells from older adults. CD4+T cells from older adults were treated with a physiological dose of Everolimus (0.01µM), and indices of redox regulation and inflammation were assessed to gain a mechanistic understanding of the effect of Everolimus on inflammation. Everolimus (Ever) broadly alleviated inflammatory cytokines produced by multiple T cell subsets. Everolimus's ability to alleviate the cytokines produced by Th17 subsets of T cells, such as IL-17A IL-17F, was dependent on the restoration of redox balance and upregulation of antioxidant enzymes. Repurposing the antineoplastic drug Everolimus for curbing inflammaging is promising, given the drug's ability to restore cellular homeostasis mechanisms.

Keywords: CD4+T cells, Everolimus, Inflammaging, NRF2, ROS, Th17 cytokines.



P13

Does Eliminating B cells cause a Vaginal Complaint? Correlation between Vulvovaginal Symptoms and Endocervical Cell populations in women treated with Rituximab

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The monoclonal antibody Rituximab treats autoimmune diseases by depleting circulating B cells. A case series described inflammatory vaginitis as a possible complication in patients treated with Rituximab. We hypothesized that women on Rituximab would have more severe symptoms compared to those not on the drug therapy and symptoms would be linked to immune cell population. We enrolled people receiving Rituximab and healthy controls. Participants first completed questionnaires describing their vulvovaginal symptoms. Blood and endocervical brush samples were collected. Both samples underwent flow cytometric analysis of the following cell populations: CD45+ CD3-CD19+ B cells, CD45+CD3+CD4+ T cells, CD45+CD3+CD8+T cells, CD45+CD3-CD19-CD56-HLADR+CD14+ monocytes and CD66b+ neutrophils. We used logistic regression to assess the associations between symptoms and immune cell population, adjusting for rituximab treatment and menopause. We enrolled 27 women on Rituximab and 28 healthy controls. Among the whole group, about half reported no -mild symptoms of vulvovaginal itching, vulvovaginal irritation or pain, or vaginal discharge or dryness, while the remainder reported at least one of those symptoms as moderate-severe. Logistic regression found increased odds of moderate-severe vaginal discharge in people with higher neutrophil proportions, but other cell populations were associated with no other symptom severity. Our analysis demonstrated that immune cells predict the severity of symptoms. No individual cell type was associated with worse symptoms after- adjusting for Rituximab treatment and menopause except for an association between neutrophils and vaginal discharge.

P14

Does the spontaneous Bolt mutation complement the Axin2M1J mutation in mice?

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Genetic mapping has identified Axin2 as the likely genetic basis of the spontaneous "lightning bolt tail" (Bolt) mutation in mice, and DNA sequence analysis of Axin2 has revealed an intragenic tandem duplication and insertion that appears to be specific to Bolt (see abstract by Kwarteng & Moreau). Here, I propose to directly determine—by classic complementation testing—whether Bolt is a mutant allele of Axin2. For this test, I have imported the Axin2MIJ mutation that, like Bolt, controls partially penetrant tail kinking and recessive embryonic lethality. Bolt/+ mice have been crossed with Axin2MIJ/+ carriers, and the Bolt and Axin2MIJ genotypes of the resulting offspring will be determined by PCR-based DNA analysis. If these recessive lethal defects can complement one another (expected if their causative bases are in different genes), then about 25% Bolt/+, Axin2MIJ/+ offspring will be recovered. Instead, if Bolt and Axin2MIJ are both defects in Axin2, then the Bolt/Axin2MIJ genotype will be rare (since at least Bolt/Bolt is known to be only semi-lethal) or missing entirely. Such a "failure to complement" would indicate that the Bolt phenotype results from the Axin2 defect it is currently only associated with.



P15

Effect of benzyl butyl phthalate (BBP) and ethanol on reproduction, mortality, microhabitat location, and distribution of Hyalella azteca.

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Amphipods are an ideal model organism to test the impact of benzyl butyl phthalate (BBP) and chemical vehicles such as ethanol on the aquatic environment. We hypothesize when amphipods are exposed to BBP (delivered via ethanol), reproductive rates will decline, microhabitat preference and organism distribution will be altered, and mortality will increase. Aquaria identified as unexposed control (n=3), 2.5 ppm EtOH-exposed (n=3), or 2.5 ppm BBP-exposed (n=3) each housed 10 amphipods in 750mL water. Two sets of aquaria (control/ethanol/BBP) contained an enclosure that had either three or four walls and the third set had no enclosure. Amphipods were dosed once a week for eight weeks; amphipod activity was recorded 3X per week. While mean progeny number was unimpacted by ethanol (E=25.0, C=30.4), it was significantly (p<0.00001) reduced by BBP exposure (BBP=0.8). Amphipods exposed to ethanol preferred to reside within an enclosure regardless of number of walls 1.3X more than unexposed control (p=0.01). Amphipods exposed to BBP were 1.2X more likely to be under the enclosure (p=0.0001). Distribution was significantly impacted by chemical exposure (p=0.0001). Unexposed exhibited clumped distribution (all individuals in one area), ethanol-exposed aggregated in clusters (multiple amphipods in different areas), and BBP-exposed were evenly distributed. Ethanol exposure increased mortality 1.7X as compared to control (p=0.0004), and BBP exposure increased mortality 1.5X (p=0.024) as compared to the ethanol group. Data supports that both BBP and the chemical vehicle ethanol significantly impact amphipods. Therefore, amphipods are a suitable model for testing impacts of BBP on the aquatic environment.

P16

A temporal variation in the distribution of Megaptera novaeangliae on the Gulf of Maine and its possible correlation to warming waters

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Climate change is occurring at an unprecedented rate with unpredictable consequences, from weather with increased severity to the warming of the air and the ocean, it all leads back to climate change. The Gulf of Maine (GoM) is currently experiencing one of the fastest rates of warming of any ocean ecosystem Over the past decade, sea surface temperatures in the GoM increased faster than 99% of the global ocean (Pershing et al., 2015). The sand lance is the preferred diet of M. novaeangliae (Hain et al.) and the sand lance's preferred diet is predominantly the copepod Calanus finmarchicus (Suca et al.) Due to the rapid warming of these waters, there has been a significant impact on the signature subarctic zooplankton species, C. finmarchicus. (Pershing et al., 2021.) We can conclude that with the warming waters and the effects on C. finmarchicus, we may see them follow the cold more oxygenated water further north. Here, we use an ensemble of numerical ocean models to characterize the latitude and longitude of M. novaeangliae from 1985-2023 using data points from the Global Biodiversity Information Facility. Through different computer software platforms, we were able to compare the data from different years to current data to track the movements of M. novaeangliae and compare them to the surface temperature. Initial results from this ongoing study show promise to support the hypothesis, this study will continue to examine the effects of climate change on the behavior of M.



P17

Combatting Aggregation of Pseudomonas aeruginosa to Support a Screen for Potential anti-Pseudomonas Antibiotics

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Pseudomonas aeruginosa (P. aeruginosa) infections are difficult to treat. The bacteria are innately resistant to most commonly used antibiotics, and resistance acquired by gene transfer is frequent. It is a difficult-to-treat bacterium due to its Gram-negative membrane and antibiotic resistance. It causes severe infections in humans with weakened immune systems. P. aeruginosa has significance in nosocomial infections, making it difficult to treat due to its development of resistance to antibiotics. Some antibiotics are natural chemicals secreted by soil bacteria, and we are developing a low-tech, high-throughput screen for soil bacteria that secrete antipseudomonas compounds. Our method employs well-dispersed broth cultures of P. aeruginosa, but these bacteria grow as clumped aggregates in broth.

To inhibit the aggregation without affecting bacterial growth, we have used a surfactant, Tween80 (T80), and a polyphenolic catechin, epigallocatechin-3-gallate (EGCG) and incubated them with P. aeruginosa in LB broth. EGCG has been demonstrated in other research to disrupt the biofilm-forming properties of P. aeruginosa, so it is inferred it can disrupt the aggregation. Using a qualitative visual approach and a spectrophotometer to quantitatively measure the change in aggregation, we observed that T80 is promising in its de-aggregation properties. This method will be included in our antibiotic screening protocols.

P18

Sex differences in the behavioral responses to constant light and SKF-38393 (DIR agonist) consumption in C57BL/6J mice

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Disruptions to the circadian rhythm have been shown to lead to alterations in depression-like behaviors and motivation. Additionally, dopamine, the major reward neurotransmitter, is controlled by the biological clock and circadian desynchronization can lead to alterations in dopamine signaling. This investigation aimed to uncover the behavioral effects of SKF-38393 consumption, a Dopamine-1-receptor agonist (DA), on male and female C57BL/6J mice in constant light (LL). As such, there were four groups for each sex: 1) LD/Water, 2) LL/Water, 3) LD/DA, 4) LL/DA. All mice had their fluid consumption measured were subjected to open field, novel object, and sucrose preference tests to assess exploration and depression-like behaviors. Female mice in LL exhibited reduced open field explorative behaviors in LL compared to male mice, although DA consumption prevented this behavioral change. Additionally, after multiple trials in the novel object assay, F/LL mice exhibited increased interactions with the objects compared to F/LD mice. However, male mice in LL exhibited poorer memory via reduced interactions with the new object compared to male mice in LD and female mice. F/LL/DA mice also exhibited increased sucrose preference compared to M/LL/DA mice. Lastly, female mice consumed more DA under LL compared to males. These results indicate that male mice are more sensitive to the negative cognitive effects of LL, while female mice are more sensitive to explorative effects of LL. Furthermore, female mice may have increased sensitivity in their reward pathway as they showed increased DA consumption and sucrose consumption under DA compared to male mice.



P19

The impact of benzyl butyl phthalate (BBP) and its chemical vehicles on Hyalella azteca mortality and swim pattern.

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We investigated the effect of an environmentally relevant exposure (24-hr, 2.5 ppm) to benzyl butyl phthalate (BBP) and its common chemical vehicles (acetone and ethanol) to determine if there is an interaction among the three chemicals by assessing mortality, productive swimming, and minimal movement among amphipods (Hyalella azteca). No mortality was observed among unexposed-controls or those exposed to ethanol or acetone. BBP-induced mortality was observed among amphipods exposed to BBP delivered in ethanol (10%) and delivered in acetone (13%). Mortality more than doubled (27%) following exposure to BBP delivered in a combination of acetone and ethanol. Productive swimming was observed in 100% of unexposed control and acetone-exposed individuals and in 97% of the ethanol group. Productive swimming decreased significantly (p < 0.001) following BBP exposure (BBP/ethanol 54%, BBP/acetone/ethanol 54-57%). While it appears that there is an interaction with BBP and ethanol that exacerbates the loss of this swim pattern, the greatest loss occurred following BBP administration with acetone (BBP/acetone 67%). Among control, ethanol, and acetone groups, no amphipods exhibited movement scored as "minimal". Following BBP exposure, minimal movement was observed among individuals exposed to BBP/ethanol (30%), BBP/acetone (40%), and BBP/acetone/ethanol (13%) indicating that BBP reduced movement and that there was a chemical interaction among BBP, ethanol, and acetone that increased minimal movement occurrences. Based upon these results, while BBP clearly impacts the mortality and the behavior of amphipods, the outcome appears to also be dependent upon the chemical vehicle utilized.

P20

Testing Different Series Of Identified Bacteria For Use in Bioremediation of Gasoline-Contaminated Soil

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Our project centers on the application of bioremediation utilizing previously identified bacteria capable of catabolizing compounds in gasoline as their sole carbon and energy source. Our assay methodology involves inoculating these identified bacterial isolates into small culture tubes containing sterile soil, sterile water, and small amounts of filtered gasoline. These tubes are incubated for varying times to allow for bacterial growth and bioremediation before seeds are sown; seed germination and plant growth are observed over 14 days This semester, our research efforts have been split into two distinct areas of investigation. The first group focuses on exploring the option of transitioning from Brassica rapa (Wisconsin FastPlantsTM) seeds to radish (Raphanus sativa) seeds as a model organism. Radish seeds offer advantages such as ease of handling and quicker manifestation of gasoline-affected phenotypes, thereby potentially enhancing experimental efficiency. The second group is dedicated to investigating strategies to optimize the growth and efficiency of decontaminating bacteria within the soil matrix. This involves exploring the supplementation of contaminated soil with various chemicals or nutrients to augment bacterial activity and accelerate the bioremediation process.



P21

Size matters: the robustness of the Chinese Mystery Snail when subject to direct desiccation across various simulated water drawdowns

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The Chinese Mystery Snail (Bellamya chinensis) is a non-indigenous and extremely invasive species that presents a clear danger to many watersheds in the United States. It is not an overtly aggressive species yet is extremely competent at out-competing endemic species due to its tendency to spawn rapidly and outconsume the food sources shared between the snail and native species. The Chinese Mystery snail is an extremely resilient species and an effective reproducer, able to survive up to ten days out of water and bearing up to one hundred fertilized eggs at a time. For this experiment, fifty-two snails were desiccated for twenty-eight days in four twenty-three liter buckets, with thirteen snails allotted per bucket and different water drawdown rates across each trial. The drawdown rates used were two hours, six hours, twelve hours, and twenty-four hours respectively. After the twenty-eight days, water was reintroduced, and the snails were given a thirty-six hour acclimation period before survivorship was assessed. Overall trends of snail survivorship increased over extended drawdown periods. With survivorship larger than sixty percent across all treatments, the ability of the snails to survive such events illuminates their inherent resilience to surviving extended periods of desiccation. This provides a crucial insight into their prominence as an invasive species across the U.S., as it has proven very difficult to kill them off through many natural means. As a result of this hardiness, it is most likely to be human intervention that mitigates and ultimately controls the spread of this species.

P22

Exploring a novel treatment strategy that simultaneously inhibits both pathways of ATP production in human breast adenocarcinoma cells.

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Cancer can possess metabolic plasticity to survive in microenvironments, proliferate uncontrollably, and progress to advanced stages of disease. This means the cells can shift between glycolysis and oxidative phosphorylation to generate ATP. This can be advantageous for cancer cells as they can survive when treated with a monotherapy that only targets one of the pathways. Prior research in the Modica-Napolitano laboratory has established the effectiveness of an innovative chemotherapeutic strategy that employs the mitochondria-targeted anticancer agent elesclomol in combination with either of two glycolytic inhibitors 2deoxyglucose (2DG) or 3-bromopyruvate (3BP). These previous results showed that this combination treatment caused additive cytotoxic and antiproliferative effects on MDAMB231 and MCF7 human breast adenocarcinoma cell lines. The objective of this current project was to further explore the metabolic effects of these compounds. This was accomplished by exposing the breast cancer cells to various concentrations of elesclomol and monitoring its impact on mitochondrial membrane potential using confocal microscopy. In addition, the effects of 2DG and 3BP on glycolytic capacity were assessed by monitoring lactate production in the breast cancer cells in the presence and absence of these compounds. The results obtained show that elesclomol induces a significant dose-dependent decrease in mitochondrial membrane potential and that 2DG and 3BP inhibit glycolysis in the two human breast adenocarcinoma cell lines tested. These results confirm and extend the previous findings of this study, and provide further evidence that this novel combination treatment may yield a viable treatment option that can overcome the metabolic plasticity exhibited by



P23

Soil & Antibiotics: A novel approach at eliminating the bacterium Bacillus mycoides from diluted soil samples.

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The method of searching for antibiotics in soil samples has been widely used and accepted. Soil samples are serially diluted and plated to select and isolate colonies of interest. Colonies of interest are defined as those that, after an incubation period of three to five days, exhibit a zone of clearing around them. The selected colonies are tested for susceptibility against Pseudomonas aeruginosa, a gram-negative antibiotic-resistant bacterium. Bacillus mycoides hinders the ability to successfully isolate colonies of interest for antibiotic testing due to its colony morphology and ability to grow filamentous hair-like extensions across plates. B. mycoides is a gram-positive bacterium that is part of the Bacillus genus. This bacterium can grow and spread across nutrient agar plates within one-day post-incubation. An approach was taken to find a bacteriophage that could target B. mycoides and thus reduce or eliminate the bacterium from the samples, allowing for the isolation of colonies that could otherwise not be selected and evaluated. The approach was to filtrates soil samples, isolate filtrates that are presumed to contain bacteriophage, and test them against various samples of B. mycoides, to evaluate their host range and effectiveness. Preliminary data showed filtrates of interest that may contain bacteriophage that could target B. mycoides. However, subsequent experiments did not demonstrate the same level of bacteriophage activity. Therefore, a different approach may be necessary to find bacteriophage in the soil samples.

P24

Effects of Benzyl butyl phthalate (BBP) and ethanol on Fundulus heteroclitus behavior, brain neurotransmitters, and gross internal anatomy.

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Benzyl butyl phthalate (BBP), a plasticizer that increases plastic flexibility, has been shown to alter shoaling in Fundulus heteroclitus. Unexposed control, ethanol-vehicle (0.1 ppm), and BBP (0.1 ppm) exposed fish were assessed for somatic index, swimming agitation, feeding aggression, food consumption, brain/gut/liver gross appearance, and brain neurotransmitters on day 31 and 59 of exposure. Somatic index and food consumption did not differ among treatment groups on either day. On day 31 and 59, ethanol increased feeding aggression 1.6X (p=0.0001) compared to unexposed control; BBP did not, however, alter aggression compared to ethanol. Agitated swimming was 1.3X higher among ethanol-exposed fish (p=0.0006) at 31 day only. BBPexposed fish were more agitated than ethanol-exposed on day 31 (1.3X; p=0.016) and 59 (1.4X; p=0.002). Liver appearance did not differ among treatments or exposure periods, but gut content volume differed between controls on days 31 and 59 (2X; p=0.007). While ethanol and BBP-exposed fish had profoundly empty guts (p=0.0005) on day 31, all treatment groups had reduced gut content by day 59. Mean brain mass did not differ among treatment groups, but serotonin level was higher following BBP exposure (1.9X; p<0.0001) compared to ethanol-exposed fish on day 31 with no difference on day 59. Dopamine levels were reduced among ethanol-exposed fish (4.1X; p=0.012) on day 59. These results support F. heteroclitus as an appropriate model, a 31-day treatment regimen to determine behavioral and serotonin changes, a 59-day regimen to detect changes in dopamine, and the need to explore other chemical vehicles to study the impact of BBP



P25

Decoding the "FUN" of Bacterial Transcription Factors

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Transcription factor (TF) proteins can activate or repress gene expression. An understanding of how TFs modulate RNA Polymerase (RNAP) activity is significant for decoding gene expression circuits in bacteria. Escherichia coli has around 300 TFs, and remarkably, many of them are still function unknown (FUN). In this project we sought to further characterize if 80 selected TFs interacted with RNAP, and to determine the functional consequence on transcription for an exemplary TF-RNAP interaction. As a first step, we utilized a bacterial two-hybrid assay (B2H) resource from our lab to characterize several TF-RNAP interactions. In this resource, 59 sub-domain fragments from RNAP (28 derived from the 7 E. coli σ factors, and 31 derived from the RNAP subunits ($\alpha 2\beta\beta'\omega$)) and 80 TFs were inputted into the B2H assay for systematic testing of more than 4,500 individual TF-RNAP interactions. The TF CedA- β subunit subdomain (β residues 151-451) was selected for further study, as this region encompasses an insertion sequence motif (Bi4) uncommon in other RNAPs, and the function of CedA is not fully understood. We verified the CedA- β 151-451 interaction by validating mutations in both CedA and β 151-451 that disrupted the interaction. Using purified RNAP and wild-type or mutated CedA proteins in a fluorescent-based in vitro transcription (IVT) assay, we reveal that CedA functions as a transcription repressor via interaction with β i4. Our approach of using the B2H assay for initial protein-protein analyses, coupled with IVT, will facilitate the convenient decoding of other TF-RNAP interactions.

P26

Pseudomonas Biofilms and Motility

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Biofilms are communities of microorganisms attached to a surface. Motile bacteria such as Pseudomonads alternate between free swimming and biofilm communities. Pseudomonads are bacteria that colonize rhizospheres, stimulate plant growth, and protect plants against pathogens. These bacteria also cause human infections. It is hypothesized that chemicals in the environment are sensed by bacteria and control both movement and attachment to surfaces. This study aimed to determine what environmental cues, such as amino acids trigger swimming and biofilm phenotypes. The results of this study show that the amino acid arginine enhanced the biofilm formation of most Pseudomonas species whereas cysteine inhibited biofilms. Tryptophan enhanced swimming motility in every Pseudomonas species that was tested in this study but did not consistently enhance biofilms. This study was the first thorough explanation of the effects of amino acids on biofilm and swimming in diverse environments of pseudomonads.



P27

Detecting tau levels in the liver of traumatic brain blast mice

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The misprocessing of microtubule-associated tau (MAPT) in the brain, resulting in the formation of aggregates and other toxic species, has become a significant aspect of our evolving comprehension of the causes and development of traumatic brain injuries. Recent research has shown the importance of tau misprocessing and its propagation into brain cells, which can explain the spread of neuro destruction of the brain. Moreover, how tau is released from neurons by exosomes may play a crucial role in developing neurofibrillary lesions in the brain. Tau secretion is most easily observed in cellular models during disease-related situations. Tau presence in the liver indicates how tau can be transported via the blood passing from the cerebrospinal fluid ending in the liver. Using the ELISA technique and AT270 antibody, we detected tau levels in blast mice, and compared with the control liver, we noted that tau levels increased more than tenfold in the liver. Our study shows that tau levels in the liver can be used as an early indicator of brain neurof brain the liver of blast mice. Our study shows that tau levels in the liver can be used as an early indicator of brain neurodegeneration.

P28

Competition between two acetotrophic methanogens isolated from an anaerobic digester treating sewage sludge

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Methane production from anaerobic digesters treating wastewater is a sustainable method for energy production. Methanogens, archaea responsible for methane production, play a crucial role in this process. Two distinct acetotrophic methanogens, Methanothrix sp E1 and Methanosarcina sp DH1, were isolated from an anaerobic digester inoculated with sewage sludge collected from a wastewater treatment plant and fed low concentrations of butyrate (-1 mM) for 360 days. Methanothrix dominated the microbial community within the reactor, accounting for 99% of the archaeal sequences, while Methanosarcina only constituted 1% of the population. These two methanogens were enriched under different conditions. Methanothrix species exhibit a high affinity for acetate and can survive in low-acetate environments. The reactor maintained very low acetate concentrations (0-0.23 mM) which explains why Methanothrix dominated the reactor community and could be enriched using 1 mM acetate as the growth substrate. In contrast, Methanosarcina thrives in environments with high acetate concentrations. Accordingly, Methanosarcina was enriched from the reactor using 40 mM acetate as the substrate. Characterization of these two strains revealed that Methanosarcina DH1 is an r-strategist, growing at rates that are 3 times faster than Methanothrix E1 when acetate concentrations are high at neutral pH. Methanothrix, on the other hand, is a k-strategist capable of slow growth in harsh environments. Competition experiments between both species were established and cell abundances were monitored with quantitative PCR. These results elucidate why Methanothrix is typically the principal methanogen in anaerobic digesters treating waste with low acetate and high salinity, pH and ammonia concentrations.



P29

Characterizing the Neonatal Seizure Population at Boston Children's Hospital and Beth Israel Deaconess Medical Center

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Neonatal Seizures are sudden, uncontrollable, abnormal changes of electrographic activity during the neonatal period. The prolonged impact of neonatal seizures remains unclear, but due to the immature state of the brain it is likely they can have deleterious effects. It has been hypothesized that if patterns are present within the demographic, clinical, and birthing data of the neonatal seizure population, then long-term health impacts and neonatal correlations can be identified. This cohort study included 167 infants diagnosed with neonatal seizures from Boston Children's Hospital and Beth Israel Deaconess Medical Center, born from February 2010 - August 2022. REDCap and Excel software were used to identify trends between birthing characteristics, encephalopathic indicators, seizure etiologies, and other clinical factors. Among 167 neonates (60.47% male), 13.8% were pre-term, 49% were born by Cesarean section and 37% had Apgar scores \leq 5. Neonates born by cesarean section (1.22 ± 1.13) and premature (1.22 ± 1.12) neonates had the equivalent highest mean number of developmental delays, at 18-24 months; language (29.9%) and gross motor (21.0%) delays were most frequent. 69.46% of the population presented with subclinical seizures; the most prevalent seizure etiologies were hypoxic ischemic encephalopathy (HIE, 44.9%), ischemic strokes (25.1%), and intracranial hemorrhages (15.6%). Premature infants (85%) and infants with Apgar scores \leq 5 (78%) had the highest proportion of their population prescribed at least one anti-seizure medication. This data suggests infants with neonatal seizures, born with birthing complications (i.e., Cesarean section, prematurity, Apgar Scores \leq 5) have an increased likelihood for abnormal developmental and neurological outcomes.

P30

Mechanistic Regulation of Age-Associated Th17 Inflammation by Mitochondrial Complex II

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Dysregulation of mitochondrial function during aging is linked to a progressive decline in the overall health of the individuals, resulting in reduced quality of life that limits health span, defined as the number of years spent in good health. Our data indicates that the activation of STAT3 and its localization in the mitochondria upregulates mitochondrial complex II expression and activity and induced changes that promote Th17 inflammation in T cells from older adults. Th17 cytokines (IL-17A, IL-17F, IL-21) and Th17 supportive cytokines (IL6, TNFa) are specifically known to promote age-associated pathologies such as diabetes and several forms of autoimmunity.

CD4++ T cells from older adults were treated with inhibitors of STAT3 and Mitochondrial Complex II (succinate dehydrogenase; SDH); and indices of mitochondrial function and inflammation were assessed to gain a mechanistic understanding of the regulation of inflammation. Our results show that aging promotes an increase in the localization of STAT3 to the mitochondria (mitoSTAT3), which induces profound changes in mitochondrial structure, dynamics, and function. We observed that mitoSTAT3 increased the OXPHOS dependence of aging T cells. Limiting mitoSTAT3 prevented Th17 inflammation and restored mitochondrial structure and function similar to T cells from young adults. Interestingly, pharmacological inhibition of SDH by dimethyl malonate prevented age-induced inflammatory cytokine production and phenocopied mitoSTAT3 anhibition, thus pinpointing that mitoSTAT3 effects on Th17 inflammation require the participation of SDH. **Keywords:** Aging, CD4+ T cells, inflammation, mitochondria, STAT3



P31

The effect of social isolation on Madagascar Hissing Cockroach exoskeleton luster and determination of critical number.

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People from any group descriptor can experience isolation-induced impacts on health(1). Isolation during the COVID-19 pandemic, for example, has been associated with adverse psychological and physiological effects(2). While social isolation has been studied in a variety of animal models, no studies have focused on anatomical alteration of exoskeleton among social insects or investigated the existence of a critical group number to prevent these changes. In our laboratory, all Madagascar Hissing Cockroaches (MHC) exhibited loss of exoskeleton luster following 10 weeks in isolation. In our current study, MHCs were isolated in pairs or triplet groups for 10 weeks before being reunified in a communal enclosure for three additional weeks. Initially, all exoskeletons exhibited high luster (shine). After five weeks, MHC singles and pairs exhibited small patches of dull exoskeleton while MHC triplets maintained exoskeleton luster. After 10 weeks, MHC triplets did not experience any loss of exoskeleton luster; thus, MHC critical group number to prevent isolation stress appears to be three. Mean dull exoskeleton surface area (as a percentage of the total) among MHC singles (14.40%), however, was 7.5-fold greater than MHC pairs (1.93%) and greater than MHC triplets who exhibited 0% dullness. Following the reunification period, mean dull exoskeleton surface area was 0% for all MHCs. Our study, therefore, supports the hypothesis that social isolation can lead to anatomical changes in MHC exoskeletons when a critical group number is not maintained. Results also provide evidence that this change in anatomy is reversible.

P32

Flow Cytometry Bead Array Assay for the Identification of miRNA and Cancer Detection

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Extracellular vesicles (EVs) are lipid-bound molecules present in high concentrations in various bodily fluids. Their cargo contains microRNAs (miRs), short, non-coding RNA sequences that serve multiple bodily functions with implications for cancer progression. Isolation of EVs and miRs allows them to be prognostic and diagnostic biomarkers. Identifying miR populations within EVs can be challenging due to varying sizes and functions. Therefore, we hypothesized that an EV/miR detection method using Molecular Beacons (MBs) and Streptavidin Bead (SA Beads) complexes (MBBs) with flow cytometry could be an effective method in identifying miR populations within EVs and quantifying disease status. MBBs for three target miR populations (miR-16-5p, miR-495, and miR-451a) were used to evaluate this technique. These MBBs were incubated with a target oligo sequence for each corresponding miR population and run on a nanoflow cytometry instrument, CytoFLEX S (Beckman Coulter Life Sciences), to identify MBB binding to target miRs. Subsequently, the MBBs were incubated with human plasma and human tumor cell samples at different dilutions to identify the miR populations within biological samples, evaluate binding efficiency, and analyze disease status. Results showed the MBBs bound to target miR population controls. However, the three miR populations in plasma or tumor samples were not consistently detected. Possible explanations include low amounts of isolated miRs varying in EV subpopulations. An RNA Atlas can screen top miR targets. Despite the lack of consistent detection, these results show that MBBs have the potential to identify and quantify populations of miR in both plasma and tumor samples.



P33

Impact of Social Isolation on Exoskeleton Luster and Neurotransmitter Level in Madagascar Hissing Cockroaches (Gromphadorhina portentosa)

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Gromphadorhina portentosa, commonly known as the Madagascar Hissing Cockroach (MHC), is a highly gregarious insect species and as such, can provide insight into how isolation impacts behaviors such mating, aggression, and inter/intra-specific interactions.

MHCs, housed in isolation for toxicological studies in our laboratory, exhibited unexpected morphological changes to their exoskeleton (loss of luster). The goal of this study was to better quantify this morphological change over a ten-week period and determine if serotonin, dopamine, or norepinephrine neurotransmitters are associated with this effect.

Initially, there was no difference in somatic index (mass/length) or exoskeleton luster between the communal control (n=10) or isolated (n=10) MHCs. Mean exoskeleton dullness (as percent of the total exoskeleton surface area) remained 0% among communal MHCs over the 10-week experimental period. However, by week five, mean exoskeleton dullness (3.5%) increased significantly (p=0.0053) among isolated MHCs as compared to control. No further progression of dullness occurred between week five and week 10.

The mean serotonin level did not differ between isolated and communal MHCs. Mean Dopamine, however, decreased 2.4-fold (ANOVA, p<0.0001; Student's T-test, p=0.0075), and mean norepinephrine decreased 1.7-fold among isolated MHCs (ANOVA, p<0.01; Student's T-test, p=0.0014) as compared to communal. These results support our hypothesis that the morphological change in exoskeleton luster can be quantified based on exoskeleton surface area and that the brain neurotransmitters dopamine and norepinephrine are implicated in this phenomenon.

P34

Becoming BUDdies with the Mitotic Exit Network by investigating GEF and GTPase function in mutated yeast cells

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The mitotic exit network, or MEN, in budding yeast, is a Ras-like signaling pathway that governs the cell cycle transition from the end of mitosis into G1. MEN activation is coupled to proper chromosome segregation and more specifically to the entry of the daughter spindle pole body (dSPB) into the bud and prevents aneuploidy. The GTPase Tem1 acts at the top of the pathway and is activated in late anaphase. Tem1 is localized to SPBs and SPB localization is required for its activity. The kinase Kin4 inhibits MEN by preventing Tem1 from localizing. The protein Lte1 inhibits Kin4 and is localized to the daughter cell cortex from S phase to late anaphase. Lte1 prevents Kin4 from binding to the dSPB and in this manner acts as an indirect activator of Tem1. However, Lte1 contains N-terminal and C-terminal guanine nucleotide exchange factor (GEF) domains. These domains are important for Lte1 localization, but we hypothesized that these GEF-homology domains are also important for Lte1 to directly activate Tem1 by serving as its GEF. The study explores Lte1's GEF homology domain. Twenty-one different mutations were analyzed on protein stability, function, and LTE1 localization within Helix B. Two different mutations, Lte1-D691A and Lte1-D691L, revealed opposite effects on localization and function. In addition, a bacterial-two hybrid assay was developed to further study Lte1 and Tem1 possible interactions. Overall, this research underscores budding yeast as a valuable model for studying signaling pathway paradigms and provides insights into the intricate regulatory mechanisms within the MEN pathway.



P35

A MENace to Society – Investigating the Hypothesized Interaction Between the Mitotic Exit Network Activators Ltel and Tem1 Within S. cerevisiae

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Mitotic cell division is prone to errors, causing abnormal chromosomal segregation in daughter cells. The Mitotic Exit Network (MEN) in Saccharomyces cerevisiae (budding yeast) is a Ras-like signaling pathway that guides the cell cycle from the end of mitosis to G1. This pathway prevents aneuploidy in yeast cells and is homologous to mammalian HIPPO pathway, which is mutated in cancers. It is known that Kin4 inhibits Tem1, a small GTPase and Lte1, an activator for MEN, inhibits Kin4. In the C-terminus of Lte1, Helix B shares homology to known Guanine Exchange Factors (GEFs) like SOS and RasGRF1 in mammalian cells. It is unknown how Ite1-GEF allele, that lacks Kin4 binding region, maintains its activity within MEN. It is hypothesized that Helix B is important for Kin4 independent mitotic exit. To determine Ltel's hypothesized secondary role, the Cterminus of Helix B was mutated to residues in known GEFs to determine the Ite1-GEF mutant's stability, localization at the daughter cortex of the bud, and non-functional characteristics. The protein stability was measured via western blot assay, the localization was analyzed via Green Fluorescent Protein (GFP) microscopy assay, and the functionality was analyzed via serial dilution assay. Once we found our desired mutant, Tem1 localization will be assessed. We found that two of the 25 mutants tested, Ite1-GEF-N687T and Ite1-GEF-I694A had the desired characteristics, with Ite1-GEF-I694A having the highest percentage of Tem1 non-localization compared to Ite1-GEF-N687T. Data suggest that Ite1-GEF-I694A is the essential amino acid reside to promote mitotic exit and Tem1 Localization.

P36

Uncovering how extracellular electron transfer is regulated in Geobacter sulfurreducens.

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Geobacter sulfurreducens is a key species used to investigate the mechanisms of extracellular electron transfer (EET) via conductive filaments. Understanding microbial EET mechanisms is crucial due to their significant impact on biogeochemical cycles in both contemporary and ancient environments, the advancement of bioenergy strategies, and their application in bioremediation. Recent research in our laboratory identified two genes, GSU1771 and GSU2507, implicated in regulating EET in G. sulfurreducens. Deleting these genes led to faster Fe(III) oxide reduction and increased expression of EET-related genes such as PilA and OmcS. We are currently developing strains that overexpress GSU1771 and GSU2507 to gain deeper insights into their regulatory roles in EET.



P37

Improving Fertilization Rates Through Timed Exposures and EmbryoLove Mediums

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Mouse in vitro fertilization (IVF) involves many steps, including superovulation of ovaries, extraction of oocytes and sperm, and the fertilization of the oocytes to name a few. Each step is important to the success of the process. Other aspects that can affect the outcome of IVF are the media used, timing, and the environment in which the ooctyes and sperm are kept. Fertilization is a key aspect of IVF without which there would be no embryo. Therefore, low fertilization rates can lead to poor results in embryo development. The objective of this study was to investigate ways to increase poor fertilization rates by changing some of the key aspects involved in the process. In these experiments, two specific aspects of the IVF process were targeted- different fertilization times (time in which oocytes are exposed to the sperm) and different fertilization media. Fertilization was determined by observing the presence of two pronuclei after each of the designated hours of fertilization, and again assessed at cleavage (24 hrs). Embryo development rates were assessed at cleavage (24 hrs), expanded blastocyst (96 hrs), and hatching blastocyst (120 hrs) to determine any detriments to the embryo. The results of this study showed that the 4h exposure of oocyte to sperm had the lowest fertilization rate, the 5h and 6h exposures had the highest fertilization rates, and the 5h exposure had a better development rate to hatching blastocyst. Results also indicated that the EmbryoLove HTF high calcium medium had a better fertilization rate compared to the EmbryoLove HTF medium. It can be concluded that by implementing changes in IVF protocol regarding fertilization time and media used can improve fertilization and embryo development rates.

P38

Exploring the Impact of Colored Noise on Spatial Learning in Mice: Insights from the Barnes Maze Paradigm

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Among the essential factors of survival, navigation is a key aspect in nearly all organisms. For example, navigational learning and memory are crucial in being able to locate homes, find food and mates, and avoid predators. Experiments in learning and memory have been widely researched and have led to breakthroughs in drug development and providing insight into disease models. To further examine learning and memory, we sought to make use of the dry version of the water maze, which is the Barnes maze test. However, it has been shown that a possible pitfall of the Barnes maze protocol is the lack of stressful stimuli, which could lead to decreased learning (Pitts, 2018). Taking this into account, our study sought to examine the difference between mild and aversive sound in the form of comparing noise on a continuum of white, being comfortable noise, to brown, characterized by a deeper version of white, and finally to blue noise, which is a shrill, abrasive sound. The subjects in our present study are regularly exposed to white noise due to the air ventilation within their holding facility, so we did not expect them to be affected by that presence. On the other hand, exposure to blue noise is not common throughout a mouse's environment and its shrill, excessive nature can therefore

We expect that exposure to brown noise will enhance the spatial learning and memory performance of mice in the Barnes maze compared to exposure to white or blue noise. This effect is hypothesized to occur due to the over-stimulating and distracting properties of blue noise, which may decrease cognitive function and attention, ultimately leading to decreased memory retention and maze navigation in mice. By including additional stimuli to trials with the Barnes maze, without making those stimuli particularly distressing or aversive due to the subjects' baseline exposure to white noise, the remaining stimuli of brown noise will encourage depth in learning and memory during trialing trials and extend to more precise outcomes during trials and probe run.



P39

Synthesis of N-Furyl Bisaryl-Amidine for Sequence Specific Binding to Nucleic Acids

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Small molecules can be used to combat pathogenic processes, and in some instances have been shown to alleviate disease symptoms by binding to nucleic acids or proteins within cells. The drug pentamidine, a bisaryl-amidine compound, is approved by the FDA for its anti-infective activities against fungal and microbial infections, but results in cytotoxicity. Further investigation into the mechanism of action of pentamidine has shown its affinity to bind the minor groove of DNA at AT-rich regions through hydrogen bonds between the amidines and the base pairs. To exploit this mode of binding, and to tune sequence specificity, N-substituted groups of varying lengths will be appended to the core pentamidine molecule. The current target appendage features a three to five carbon chain linking a methylfuryl group to the amidine, increasing the potential noncovalent interactions the molecule can participate in. The goal is to modify its specificity for gene sequences and increase its suitability for disease treatment to minimize cytotoxic effects. Once synthesized, the strength and sequence specificity of the noncovalent interactions between this pentamidine derivative and a target DNA oligomer will be assessed and compared to the original drug.

P40

High-fat diet consumption alters behavioral and physiological outcomes in mice exposed to a Mars solar day lighting schema

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Many world governments and one prominent businessman believe that the future of humanity lies in the colonization of Mars. Mars has a daylength of 24 hours and 40 minutes. Previous work has shown that altering daylength can lead to desynchronization of the circadian clock, leading to negative behavioral and physiological outcomes. Additionally, consumption of a high-fat diet is also known to lead to behavioral issues and disrupt the circadian rhythm. The purpose of this study was to investigate whether placement into a Mars-day period leads to alterations in circadian and novelty-induced locomotion, and if consumption of highfat diet modulates these outcomes. Male and female Swiss webster mice were given either regular (RC) or high-fat chow (HF) and placed into circadian activity monitoring cages under either a standard earth-like 24hour, 12:12 light:dark cycle (E) or a mars-like 24.66 hour, 12.33:12.33 light:dark cycle (Mars). All animals circadian rhythms, fasting glucose, and explorative behaviors were assessed. All mice, except two F/Mars/HF, were able to entrain to their respective photoperiods. Additionally, while E/HF mice exhibited increased circadian power and reduced locomotor activity compared to Mars/HF, no differences were observed between the two diets on an Earth photoperiod. Novelty-induced locomotion was reduced in Mars mice compared to Earth mice. Lastly, F/Mars mice exhibited reduced fasting glucose compared to M/Mars, while no differences were observed for Earth mice. These results indicate that consumption of HF may lead to issues when exposed to non-24-hour daylengths and that females maybe more susceptible to those changes.



P41

The impact of temperature on benzyl butyl phthalate (BBP) and ethanol behavioral toxicity in amphipods.

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The focus of this experiment is to determine how temperature might modify the outcome of chemical exposure on amphipod reproduction and swim pattern. Amphipods (n=10) were added to glass containers each containing 1000 mL amphipod water. Containers were identified as unexposed control, ethanol (2.5 ppm), or BBP (2.5 ppm). Control, ethanol and BBP experimental groups were housed at either 14.4 oC, 17.2 oC, or 20.0 oC for the duration of the experiment. Approximately 72 hours after amphipods were exposed to a single dose of the appropriate chemical(s), swim pattern was assessed using a Likert scale. Amphipod number in each container was then assessed 10 weeks after exposure. No difference in swim pattern was observed in any treatment group. Under colder temperatures (14.4oC), control populations increased by 2.4fold while ethanol exposed experienced 80% mortality and BBP 100% mortality. Under room temperature (17.2 oC) conditions, control population increased by 3.13-fold, while ethanol and BBP exposed groups had 2.9 and 4.5-fold higher mortality, respectively. Under warmer temperatures (20.0oC), both control and ethanol populations increased 11-fold and 10-fold, respectively, while the BBP group exhibited 60% mortality. Our results indicate that (1) mortality is a better endpoint than swim pattern, (2) warmer temperature not only helps to increase population numbers, but it also mitigates BBP toxicity, and (3) as temperatures cool, toxicity of both the ethanol chemical vehicle and BBP increases. Therefore, climate appears to significantly alter the toxicity of BBP among amphipods.

P42

Imaging the Cerebrovascular Structure and Function in Transgenic Mouse Models of Alzheimer's Disease

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Alzheimer's Disease (AD), a severe neurodegenerative disease, has increasing diagnoses in people over 65 years old, and no current interventions for a cure. AD progression is often associated with abnormal amyloid beta (Ab) and tau protein accumulations along the cortical vessel walls, triggering vascular degeneration, altering cerebrovascular structure, and reducing cerebral blood flow (CBF). However, a systematic study monitoring cerebrovascular changes prior to and along with AD progression, and correlating cerebrovascular MRI with AD pathology, is lacking. We hypothesize that cerebrovascular alterations can be detected before AD symptoms occur, and the Ab and tau accumulation reduces CBF and alters vasculature as the disease progresses. Using advanced MRI techniques, longitudinal changes in regional CBF (rCBF) and vessel size were non-invasively investigated in four transgenic mouse models (wild-type, APP/PS1, rTg4510, 3xTg) at 4, 8, and 12 months old. T1-weighted MRI was used to obtain high-resolution anatomical images. Perfusion MRI, diffusion MRI, and vessel size imaging were acquired to measure rCBF and vessel size. At 4 months, no significant group differences were observed in the hippocampal CBF, and the vessel size index (VSI) correlated positively with rCBF, as expected at the pre-symptomatic stage. At 8 months, we found a trend of increased amygdala CBF and CBV, and decreased VSI, consistent with prior microscopy findings. These transgenic models typically start to express AD symptoms at 5-6 months of age. At 4 months, these models are likely still at the pre-symptomatic baseline stage, and at 8 months, are likely in the early-onset stage.



P43

Floral Volatiles of a Threatened Thistle and the Damaging Effects of an Introduced Weevil

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Floral scent provides valuable information about the nature and extent of chemical interactions between plants and insects. In 2010, Pitcher's thistle was listed by the U.S. Fish and Wildlife Service (USFWS) as a threatened species, partly due to the loss of its habitats and damages caused by invasive species like weevils. To better understand the complexity of the damages caused by the introduced weevil, Larinus planus on Cirsium pitcheri, the goal of this research was to investigate whether weevil damage changes the composition of the floral scent of thistle, potentially affecting pollinator attraction. Fragrance was collected from the flowerheads of C. pitcheri plants using dynamic headspace sampling, and analyzed using gas chromatography mass spectrometry (GC-MS). It was hypothesized that weevil damage would alter floral volatile emissions by reducing the scent composition of the plant, and therefore, result in a decrease of pollinator visits. However, although there was a lot of variation in the results, overall we did not see that decrease. In fact, one compound alpha copaene showed a significant increase in emission rate with weevil damage, which is a deviation from our expectations. The abundance and pattern of floral volatile signatures of C. pitcheri are used as the basis to discuss the ecological differences across predated and non-predated flowerheads.

P44

Investigating the Cytotoxic and Metabolic Effects of Secondary Plant Metabolites on Two human Breast Adenocarcinoma Cell Lines

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The high production costs and often severe side effects of synthetic medicines has prompted interest in the use of plant-based "natural" medicines as alternatives or complements to these compounds in the treatment and prevention of disease. Curcumin, a polyphenol derived from turmeric, and ursolic acid, a pentacyclic triterpenoid found in certain fruits and herbs such as rosemary, are two secondary plant metabolites that have demonstrated both anti-inflammatory and anti-cancer properties. Previous studies in the Modica-Napolitano laboratory have shown that curcumin and ursolic acid have a significant dose dependent cytotoxic effect on MDAMB231 and MCF7 human breast adenocarcinoma cell lines, and that both compounds inhibit specific electron transport chain enzyme complexes in isolated rat liver mitochondria. The purpose of this study was to further investigate the cytotoxic and metabolic effects of curcumin and ursolic acid on breast cancer cells. This was accomplished by exposing the breast cancer cells to various concentrations of the compounds and monitoring the effects on mitochondrial membrane potential using confocal microscopy and on cancer cell proliferative capacity using the wound healing assay. The results show that both curcumin and ursolic acid cause a significant dose-dependent decrease in mitochondrial membrane potential and inhibition of cellular proliferation in the two breast cancer cell lines. The results obtained in this study are important in that they provide further evidence to support the possibility of using natural plant metabolites as less harmful



P45

Transcriptional Profiling of Dorsal Root Ganglia Sensory Neurons Following Sciatic Nerve Injury

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Chronic pain is a debilitating condition that nociception and is classified as persistent pain lasting for more than 3 months. It is often caused by peripheral neuropathy resulting from injury to the peripheral nerves that extend from the spinal cord to the lower and upper extremities. Sensory transduction and pain signaling are modulated by sensory neurons located along these axons, in the dorsal root ganglia (DRG), transmitting information from the periphery to the central nervous system. Diagnosis and management of peripheral neuropathy remain nuanced and adequate treatment requires a multifactorial approach. The present study focuses on identifying the transcriptomic profile of sensory neurons after peripheral nerve injury. Our results will provide further insight into the generation of novel analgesic therapeutic approaches that selectively target injured peripheral neurons.

P46

Determining the minimal pathway for Fe(III) oxide reduction through the inner membrane in Geobacter metallireducens.

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Geobacter metallireducens is an ideal species for investigating extracellular electron transfer (EET), with applications in bioremediation, bioenergy production, and metal corrosion mitigation. Understanding the individual functions of proteins involved in the EET pathway is required for developing genetically engineered strains suitable for large-scale applications. However, the presence of redundant proteins poses a significant challenge in creating minimal EET pathways. For instance, G. metallireducens has six distinct cytochrome bc complexes (Cbc) within the inner membrane for electron transfer from the interior of the cell to the periplasm. Of the six, genes for Cbc2 and Cbc3 were found to be transcribed at increased rates in cells cultured with Fe(III) oxide as the sole electron acceptor. This study aims to determine the minimal number of Cbc complexes necessary for EET by employing scarless genome editing techniques to eliminate redundant Cbc genes. After the successful creation of mutant strains, Fe(III) is measured through ferrozine assays.

P47

Tau Protein as marker in exosomes for detection of prostate cancer

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Exosomes are multivesicular bodies released by cells into bodily fluids. Exosomes play an essential role as markers of diseases. What they contain, proteins, and microRNA, can be potential markers and are linked to diseases such as prostate cancer; these molecules can be used as a practical approach to exploring the response of cancer cells to therapeutic intervention(s) when monitoring cancer progression and treatment. In our study, we isolated exosomes by the use of exo-quick from PC3 cell lines, and by detecting the expression of antibody PHF-1 by ELISA technique, we found an increase in tau levels, suggesting that phospho-tau isolated from exosomes of prostate cancer cells can be used as a disease marker. In summary, exosomes are prospective tools for developing and diagnosing prostate cancer.



P48

Waste Not, Want Not: Food Waste and Its Environmental Impact in the COF

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Food waste is a significant contributor to greenhouse gas emissions. US universities are among the largest contributors to food waste at the consumer level. The measurement of food waste in the Colleges of the Fenway (COF) is an indicator of the environmental impact of the COF. The environmental impact of the food waste produced in the COF had not been previously evaluated. It had been hypothesized that schools that had dining providers that tracked waste daily would have lower levels of waste relative to the student body. To evaluate how much food waste was produced in the COF, physical food waste was weighed over a three-hour period at each of the largest dining halls in the COF. The waste was categorized, and a food waste calculator was used to convert food waste data into a carbon footprint score. Interviews were conducted with dining hall managers to understand qualitative barriers to reducing food waste per institution. It was found that an educational campaign was the most cost-effective method for waste reduction. Educational deficiencies of students themselves pertaining to food waste was commonly cited in qualitative interviews. This data suggests that the most effective waste reduction strategy is one that educates students to change their food waste, and the carbon footprint of the COF can be significantly reduced when campaigns that address education are used.

P49

Periplasmic and inner membrane redox proteins involved in extracellular electron transport by Geobacter species

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Geobacter species play a pivotal role in biogeochemical cycles by respiring insoluble Fe(III) oxides, minerals that are abundant in many subsurface environments. The utilization of iron as an electron acceptor by *Geobacter* necessitates the transport of electrons from the cytoplasm to extracellular Fe(III) compounds. Consequently, the electron transport chain (ETC) of *Geobacter* spans the cell's inner membrane, periplasmic space, and outer membrane. While research has predominantly focused on outer surface redox proteins that make direct contact with iron, little attention has been given to redox proteins within the inner membrane and periplasmic space. Currently, genetic systems have been developed for two *Geobacter* species, *G. sulfurreducens* and *G. metallireducens*. The genomes from both organisms harbor multiple copies of many redox proteins found within the periplasm and inner membrane. For example, both species have 5 genes coding for periplasmic c-type cytochromes (Ppc), and 6 and 5 genes encoding inner membrane cytochrome bc complexes (Cbc) are found in *G. metallireducens* and *G. sulfurreducens*, respectively. Transcriptomic studies were conducted to identify the most highly expressed *ppc* and *cbc* genes in both *Geobacter* species. Furthermore, the construction of deletion mutant strains targeting several Ppc proteins contribute to the identification of critical components within *Geobacter's* ETC involved in Fe(III) respiration.



P50

Taking the bait: Capturing the interplay of transcription termination protein Hrp1 with multiple RNA targets

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Premature transcription termination (i.e., attenuation), is an ancient and widespread form of gene regulation that inhibits the synthesis of full-length mRNA. Defects in attenuation have been linked to cancer, viral infections, developmental abnormalities, and neurodegeneration. In the yeast, S. cerevisiae, attenuation of RNA polymerase II (Pol II) transcription depends on interactions between the 3'-end transcription termination protein Hrp1 and attenuator RNA. However, it is not clear what specific Hrp1 protein functions and RNA recognition elements are important for Pol II attenuation. We hypothesized that Hrp1 functions in attenuation at upstream gene regions by directly binding 5'-end RNA elements. We used a bacterial three-hybrid (B3H) system to detect interactions between the Hrp1 RNA-recognition motif (RRM) and various RNA terminator candidates (i.e., GAL7, CYC1, DEF1). We also performed a yeast genetic selection to identify mutations in a GAL7-CUP1 reporter plasmid that confer copper resistance via Pol II terminator read-through. Using the B3H system, we confirmed RNA-protein interactions of positive control bacterial complexes, but we only detected weak Hrp1-RNA interactions despite confirming stable Hrp1 RRM protein expression and testing variations in RNA size and assay temperature. For the yeast genetic selection, we confirmed GAL7-CUP1 copper sensitivity at 0.2 mM Cu2+, similar to strong Pol II terminators. We are currently expanding B3H to include full-length Hrp1 and analyzing copper-resistant mutants for Pol II read-though defects. Overall, we conclude that B3H requires further optimization for detecting eukaryotic RNA-protein interactions and that GAL7 exhibits strong termination activity, suitable for use in identifying Pol II termination elements.

P51

Exploring the Impact of Colored Noise on Spatial Learning in Mice: Insights from the Barnes Maze Paradigm

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Among the essential factors of survival, navigation is a key aspect done in nearly all organisms. For example, navigational learning and memory is crucial in being able to locate homes, find food and mates, and avoid predators. To further examine learning and memory, the Morris water maze is commonplace in rodent behavioral paradigms; however the test creates an incredible amount of stress towards the mice. In this present study, we sought to make use of the dry version of the Morris water maze, which is the Barnes maze test, specifically addressing a potential pitfall of the common Barnes maze protocol, which is the lack of stressful stimuli. In our testing apparatus, mice were placed on the maze set in a brightly lit room, with the addition of being exposed to sound on a continuum of white, being comfortable noise, to brown, characterized as a lower frequency rumble, and finally to blue noise, which is a shrill, hissing, abrasive sound. Currently, we are conducting our final experiments and running data analysis. However, because our subjects have baseline exposure to white noise, we expect that the remaining stimuli of brown noise will encourage depth in exploratory behavior and therefore spatial learning and memory performance of mice in the Barnes maze compared to exposure to white or blue noise, this being hypothesized to occur due to the overstimulating and distracting properties of blue noise, which may decrease cognitive function and attention, ultimately leading to decreased memory retention and maze navigation in mice.



P52

Inclusivity of the LGBTQ+ Community in Emmanuel College Biology Courses

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Teaching a diversely inclusive and representative curriculum can result in reduced stigma, higher student outcomes, and a strengthened sense of belonging. However, discrimination and lack of inclusion of the LGBTQ+ community has been growing across the US, and queer representation and education in schools is scarce. This contributes to valuable diversity being reduced in professional areas, particularly in STEM fields. While various studies have been conducted regarding effects of inclusive education and the lack of LGBTQ+ inclusivity in STEM, there is little information available on implementing LGBTQ+-inclusive curricula and incorporating such learning in STEM classes. This research surveyed students and instructors in biology courses at Emmanuel College to examine feelings of classroom and curriculum inclusivity on the basis of sexuality and gender identity. Data analysis focused on comparing responses from LGBTQ+-identifying students to overall responses, and instructor evaluations of personal knowledge and comfort with teaching LGBTQ+ topics. It was found that students generally feel accepted and represented in classrooms and material, but little information relating to the LGBTQ+ community is being taught. Instructors report overall support in implementing inclusive curricula, but they would benefit from focused education and resource distribution for aid in moving forward. Further research must be conducted and resources to fill gaps in instructor knowledge of the LGBTQ+ community and inclusive curricula must be distributed to support implementing curriculum changes. There will be a continued push for increased LGBTQ+ inclusivity in Emmanuel's biology department, as well as a goal to expand to other departments in the future.

P53

The impact of benzyl butyl phthalate (BBP) on gut flora diversity in the common mummichog (Fundulus heteroclitus).

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Changes in environmental conditions may alter gut flora diversity in animal models and potentially impair multiple organ systems1. Captive housing conditions, for example, significantly diminish gut flora diversity in Fundulus heteroclitus2. To determine if chemical exposure under captive conditions can further alter fish gut flora diversity, we hypothesize that short-term (50-day) administration of 0.1 ppm benzyl butyl phthalate (BBP) and/or the chemical vehicles acetone or ethanol will measurably impact fish gut flora diversity. Alpha diversity metrics (Shannon Evenness (EH) and Berger-Parker Dominance (1/d)) indicate that unexposed control fish had significantly altered gut flora (EH[A], 2.0-fold; EH[E], 2.5-fold; 1/dA, 1.2-fold; 1/dE, 3.9-fold), as compared to those exposed to acetone or ethanol. Taxonomic distribution for family, genera, and species also differed (B>C>A>E).

Following BBP exposure, evenness and dominance (EH[A], 2.0-fold; EH[E], 2.4-fold; 1/dA, 1.0-fold; 1/dE, 3.3fold) were altered as compared to the chemical vehicle controls. Taxonomic distribution was also altered for family, genera, and species (B>A>E). Sorenson's Similarity Coefficient (CC) indicates little overlap in gut flora populations between unexposed controls and those exposed to acetone or ethanol (CC: CVA=0.32, CVE=0.211), or between vehicle controls and BBP treated fish (BvA=0.182, BvE=0.182). These alpha and beta diversity metrics indicate chemical vehicle exposure altered the composition of the gut flora as compared to unexposed controls and BBP exposure further altered these metrics. Therefore, even with captive-housing stressors, these results support the use of this animal model for evaluating the impact of chemical exposure



P54

STRETCHing the Boundaries -- is NRP2 Mechanically Sensitive in Smooth Muscle?

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The bladder functions to store urine and release it at a socially acceptable time. The inability to control either storage or voiding leads to urinary incontinence (UI), a condition that affects 200 million people worldwide and greatly diminishes the quality of life. UI can reflect either an overactive or underactive bladder. Although medications exist to treat overactive bladder, none exist for underactive bladder. Our lab showed that deletion of a gene called neuropilin-2 (NRP2) in mice leads to increased contraction of bladder tissues, suggesting inhibition of NRP2 may treat underactive bladder. Whether NRP2 itself is mechanically sensitive remains unknown. Given the association between bladder distension and subsequent contraction, we hypothesized that NRP2 would decrease in response to mechanical stretch. To test this hypothesis, we performed in silico analyses of microarray datasets from cells subjected to stretch to assess its impact on the expression of NRP2 and NRP2-associated genes (NRP2-AGs). For validation, we exposed rat bladder mesenchymal cells (RBMCs) to stretch in vitro and used qPCR and immunoblotting to measure the expression of NRP2 and NRP2-AGs at the mRNA and protein levels, respectively. NRP2 expression was decreased by stretch of RBMCs, along with the NRP2-AGs RYK and CANX, whereas other NRP2-AGs (LY6D, HBEGF, FLT1) showed increased expression. The decrease in NRP2 was confirmed by immunoblot analysis. These data suggest that NRP2 is directly regulated by stretch and is associated with a network of genes that may regulate cell behavior. Future studies will focus on the biological relevance of NRP2-AGs to bladder

P55

Hydrogen peroxide prevents outgrowth of Clostridioides difficile spores after germinant sensing.

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Clostridioides difficile is a gram-positive bacteria that causes an infection of the colon. As C. difficile is an obligate anaerobe, the transmission of C. difficile infections (CDIs) is dependent on their spores, which germinate into toxin-producing cells upon entering the small intestine. Owing to their metabolic dormancy and protective layers, these infectious spores are resistant to many environmental stressors. As a result, CDIs are a common nosocomial infection, and understanding their resistance properties is necessary for developing methods of CDI prevention. The spore coat contains proteins that play roles in survival in the environment, and previous studies have identified proteins that are predicted to express enzymatic activities that inactivate reactive oxygen species (ROS). The roles of two of these proteins, SodA, a superoxide dismutase, and CotD, a manganese catalase, in C. difficile spores are unclear. We are working to produce C. difficile strains deficient in these ROS-inactivating proteins to determine if they provide some protection to spores from ROS. To better understand the effects of ROS on spores, we tested spore viability and germination efficiency when exposed to hydrogen peroxide, a ROS-producing compound. Through optical density and growth-based germination assays, we found that exposure to hydrogen peroxide does not affect the ability of spores to sense germinates but inhibits cell growth after germination. This may suggest that hydrogen peroxide treatment could be an effective method of decontamination for reducing CDI transmission rates. We hope to learn more about the possible roles of SodA and CotD in this protection against ROS.



P56

To bind or not to bind? Characterizing RNA-Protein interactions during transcription termination.

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Premature transcription termination (attenuation) regulates up to 15% of mRNA-producing eukaryotic genes, and attenuation defects are linked to cancer, neurodegeneration, and viral infection. Attenuation involves early cessation of RNA Polymerase II transcription, resulting in non-coding RNA rather than protein-coding mRNA. The S. cerevisiae yeast RNA-binding protein Hrp1 is important for 3'-end processing at polyadenylation (pA) sites as well as 5'-end attenuation. However, it remains unclear what Hrp1 protein functions and RNA recognition elements are necessary for attenuation. We hypothesized that the Hrp1 RNA recognition motif (RRM) would be sufficient to bind attenuator RNA, and mutations in conserved Hrp1 RRM residue F162 would be less detrimental if an aromatic interface was maintained. To measure Hrp1-RNA interactions, we fused Hrp1 full-length protein or Hrp1 RRM alone with an HA tag, incubated with synthetic RNA, and performed an RNA-protein pull-down assay. To evaluate the importance of the Hrp1 RRM for transcription termination, the GAL7 pA site terminator was placed upstream of a lacZ reporter gene and tested in the presence of F162 mutants. We observed that the Hrp1 RRM is insufficient for RNA binding in the pull-down assay, despite stable protein expression. The GAL7 pA site was strongly active (-20-fold lacZ repression) and substitutions in F162 resulted in 20-70 fold terminator read-through. The F162Y substitution was least detrimental, consistent with aromatic preference, but F162H was interestingly most deleterious, even more than non-aromatic substitutions. These data suggest that full-length Hrp1 and RRM F162 are necessary for terminator RNA recognition and transcription termination.

P57

Analyzing the Role of BCOR (BCL6 corepressor) in variant Polycomb Repressive Complex 1.1 in Drosophila

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Polycomb repressive complexes (PRCs) play an important role in regulating gene silencing during development and differentiation. These complexes establish and maintain the silent state by catalyzing repressive histone modifications. There are two major canonical complexes, PRC1 and PRC2, that have been extensively studied from Drosophila to mammals. Mammalian studies have identified several variant PRC1 (vPRC1) complexes that function in gene repression, and recent findings from our lab have discovered that Drosophila also possesses vPRC1 complexes, which are homologous to those observed in mammals. This, study focuses on understanding the role of the novel subunit Corepressor BCOR in vPRC1.1. Mammalian BCOR has been implicated in many human cancers including acute myeloid leukemia and is essential for PRC1.1 function and stem cell pluripotency. In Drosophila, BCOR (CG14073) is a novel gene that has not yet been characterized. We are generating several genetic tools that will allow us to dissect the role BCOR in development. We are generating a BioTAP-tagged BCOR transgenic fly that will allow us to perform BioTAP-XL and Mass Spectrometry analysis. To determine if BCOR is necessary for proper incorporation of PRCspecific histone modifications on polytene chromosomes, we are generating a salivary gland specific BCOR RNAi knockdown line. We are also creating a BCOR knockout line to analyze the effects of BCOR deletion on embryonic development. Dissecting the function of the vPRC1.1 complex by studying BCOR in a simple model organism like Drosophila will provide an important complementary approach to mammalians studies of Polycomb repressive complexes in development and disease.



P58

How Does Liver Cancer Restart Fetal Genes? Comparative Analysis of AFP Gene Locus in Healthy vs Hep 1-6 Mouse Cells.

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Hepatocellular carcinoma (HCC), a prevalent liver cancer, often exhibits reactivation of the Alpha-fetoprotein (AFP) gene which is a significant biomarker in its diagnosis. Despite its critical role, the precise mechanisms behind AFP gene reactivation remain elusive. In this study, we employed the Mouse Hep 1-6 cell line, a model system for HCC research due to its orthologous AFP gene activation resembling human counterparts. By sequencing both RNA and genomic DNA (gDNA) from Hep 1-6 cells, we compared them with healthy liver cells.

Our findings revealed that all 15 exons of the AFP gene were present in Hep 1-6 mRNA, with two distinct AFP protein variants observed: one normal-sized and one shorter. Intriguingly, the regulatory region and the initial portion of the AFP gene sequence in Hep 1-6 gDNA mirrored those of healthy cells, suggesting conservation in certain regions. Additionally, the sequence at the other end of the AFP gene exhibited identical patterns between the two cell types.

These results underscore a potential mutation in the spliceosome machinery, leading to exon skipping and altered protein synthesis. Further investigations into epigenetic modifications and nucleotide-level variations are warranted to elucidate the underlying mechanisms of AFP gene reactivation fully.

Moving forward, our research aims to explore the functionality of the smaller mRNA variant produced in Hep 1-6 cells and to continue sequencing the entire AFP gene to uncover additional disparities. Additionally, we propose examining epigenetic factors and potentially manipulating regulatory sequences surrounding the AFP gene in Hep 1-6 cells to discern their impact on gene expression.

P59

The levels of the ConB bacterial conjugation protein modestly rely on the presence of other conjugation proteins

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Bacteria have the remarkable ability to transfer genes from cell to cell in a process known as conjugation or mating. The DNA is transferred through the cell membrane via a specialized DNA translocation channel, which is made up of many proteins. Mating results in the transmission of genes that play roles in vital processes such as symbiosis, metabolism, antibiotic resistance, and pathogenesis. Our research focuses on the DNA translocation channel of ICEBs1, a conjugative DNA element found in the bacterium Bacillus subtilis. The proteins that make up the ICEBs1 DNA translocation channel are ConB, ConC, ConD, ConE, ConG, ConQ, and CwIT. Since these proteins form a complex that might stabilize ConB or protect it from degradation, we asked whether the levels of ConB change when other conjugative machinery proteins are absent. We measured the levels of ConB in strains deleted for other ICEBs1 genes. We grew up the various bacterial strains and created lysates after normalizing for cell quantity. Using quantitative western blots with anti-ConB antibody, we determined that ConB levels were decreased 1.5-, 1.8-, and 2.2-fold in strains lacking ConD, ConE, and ConQ, respectively (P < 0.05). ConB levels in other deletion strains were not statistically different compared to the wild type. Overall, we conclude that the levels of ConB modestly rely on the presence of ConD, ConE, and ConQ, but not other ICEBs1 DNA translocation channel components (ConC, ConG, and CwIT). Our research sheds insight on the function of ConB, which contributes to a deeper understanding of the conjugation process.



P60

Rivers of Resistance: Unveiling Threat of Antibiotic Resistance in the Merrimack River Valley

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Antibiotic resistance refers to the ability of bacteria to resist the effects of antibiotics that normally would slow down their growth and/or kill them. Antibiotic resistance genes (ARG) in bacteria evolve quickly and are transmitted through the environment even faster. The build up of these genes leads to the creation of superbugs, pathogenic bacteria that are highly resistant to a variety of antibiotics. The appearance of these superbugs is a great risk to the public and monitoring the presence of ARG is important for public health. To evaluate the presence of some of these genes in our local environment we took freshwater samples from the Salmon, Spicket, Concord, Nashua, and Merrimack Rivers as well as a combined sewage overflow (CSO) site into the Merrimack river. DNA was isolated from each sample and used as a template in quantitative polymerase chain reactions (qPCR) to measure the amount of two different ARGs (TetA and Sul2) relative to a "total bacteria" gene (16S). Overall, a higher bacterial population was found in the Merrimack river when compared to other rivers as well as higher rates of TetA and Sul2 presence. TetA and Sul2 were detected in the majority of samples from all rivers, and samples collected at the Merrimack River CSO site displayed ARG levels 3-70 fold higher compared to a nearby upstream site. These data show a robust relationship between the increase of ARG and the bacteria introduced from CSO in freshwater systems as a tangible human impact on the environment.

P61

Imprinted gene expression in cloned cow embryos

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Many people have heard about Dolly the sheep, the first cloned animal. Since then, many animals have been cloned by somatic cell nuclear transfer (SCNT), but cloning suffers from low efficiency and can sometimes produce animals with developmental abnormalities. KCNQIOT1 is an imprinted gene in cows and humans that is associated with Beckwith-Wiedemann Syndrome (BWS) in humans and large offspring syndrome in cattle. Our objective was to test the expression of KCNQIOT1 in cloned cow embryos and compare it to the expression in the donor cells used for cloning and to control in vivo embryos by reverse transcription quantitative PCR (RT-qPCR). We compared the cycle threshold of cloned embryos (n=10) to in vivo control embryos (n=8) and donor cells and used H3F3B as the reference gene. Data were analyzed using the comparative cycle threshold method. Mann-Whitney U tests showed a statistically significant (p= 0.02) difference in expression of KCNQIOT1 between SCNT embryos and the donor cells (p=0.24). These findings suggest that the KCNQIOT1 gene in SCNT embryos may not have undergone the normal imprinting mechanisms during development, indicating abnormal nuclear reprogramming of this gene during the cloning process. Due to high rates of pregnancy loss and neonatal death of clones, it is important to identify abnormal gene expression, especially of imprinted genes, which are known to be involved in growth and development.



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