Keynote Speaker

Clifford J. Rosen, M.D., Director, Clinical and Translational Research and Senior Scientist, Maine Medical Center’s Research Institute

Dr. Rosen is the founder and Former Director of the Maine Center for Osteoporosis Research and Education.

Dr. Clifford J. Rosen, M.D. is the Director of Clinical and Translational Research and a Senior Scientist at Maine Medical Center’s Research Institute. His other current positions include Adjunct Staff Scientist at the Jackson Laboratory, and Professor of Medicine at Tufts University School of Medicine. Dr. Rosen is the founder and Former Director of the Maine Center for Osteoporosis Research and Education. He was the first Editor-in-Chief of the Journal of Clinical Densitometry, is the current Editor-in-Chief of The Primer in Metabolic Bone Diseases, and just began a term as Associate Editor for JCEM. His publications include more than 300 peer-reviewed manuscripts, covering both clinical and basic bone biology.

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Cover image credit: Dustin Updike Lab: P granule mosaic.
42nd MAINE BIOLOGICAL AND MEDICAL SCIENCES SYMPOSIUM

Friday, April 24

11:30 a.m. Symposium Registration & Lunch
MDI Biological Laboratory Maren Auditorium & Dining Hall

12:35 p.m. Symposium welcome and introduction
Chuck Fidler, Ph.D., Director of Education, MDI Biological Laboratory
Maren Auditorium—MDI Biological Laboratory Conference Center

SESSION I: GENETICS/GENOMICS & BIOINFOMATICS
Session Chair: Keith Hutchison, Ph.D., Professor, University of Maine

12:45-1:00 p.m. Karolina Andralojc, MDI Biological Laboratory, post-doctoral researcher
Understanding the role of C. elegans germ granules in maintaining germ-line pluripotency

1:00-1:15 p.m. Craig Lessard, University of Southern Maine, undergraduate student
Computational identification and biological validation of FOXJ1 regulatory regions in strongylocentrotus purpuratus

1:15-1:30 p.m. Clare Bates Congdon, Ph.D., Associate Professor, University of Maine, Maine Medical Center Research Institute
GenomePatternScan: Computational identification of genome-wide candidate binding sites for FOXD1

1:30-1:45 p.m. Katrina Harris, University of Maine, undergraduate student
Computational genomic analysis of Mycobacteriophage ChipMunk

1:45-2:00 p.m. Kathleen Morrill, Bates College, undergraduate student
Genetic diversity and the 2013 decline of Eelgrass (Zostera marina) beds of the Frenchman Bay, ME coastlines

2:00-2:15 p.m. Kyle Beauchemin, University of Maine, graduate student
Characterization of the murine lung transcriptome during pre- and post-natal development in three strains of laboratory mice

2:15-2:30 p.m. Gwen Beacham, University of Maine, undergraduate student
Characterization of lysogeny regulation in the Cluster E. Mycobacteriophage Ukulele

2:30-2:45 p.m. Jarod Rollins, Ph.D., MDI Biological Laboratory, post-doctoral researcher
Post-transcriptional processing is increased upon dietary restriction in C. elegans: a possible role for nonsense mediated decay and alternative splicing in lifespan extension

2:45-3:00 p.m. Break
SESSION II: ENVIRONMENTAL BIOLOGY/STRESS AND TOXICOLOGY
Session Chair: Markus Frederich, Ph.D., Associate Professor, University of New England

3:00-3:15 p.m.  **Julia Middleton**, Colby College, undergraduate student
Functional forms of *Emiliania huxleyi* interactions with a host specific virus

3:15-3:30 p.m.  **Amber Howard**, Ph.D., MDI Biological Laboratory, post-doctoral researcher
Lowering eukaryotic initiation factor 4G improves proteostasis and helps explain the associated longevity phenotype in *Caenorhabditis elegans*

3:30-3:45 p.m.  **Stephanie Nichols**, Pharm.D., BCPS, BCPP, Associate Professor, Husson University
Analysis of the Maine Prescription Monitoring Program, 2010-2014

3:45-4:00 p.m.  Break

KEYNOTE
4:00 p.m.  Keynote Speaker welcome and introduction
**Chuck Fidler**, Ph.D., Director of Education, MDI Biological Laboratory

4:05 p.m.  **Cliff Rosen**, M.D., Director of Clinical and Translational Research and Senior Scientist, Maine Medical Center Research Institute
*How now brown fat: Where we were and where we are going?*

5:00-6:30 p.m.  Dinner
Dining Hall

6:30-7:45 p.m.  Poster Session A and Evening Reception
Dahlgren Hall

Saturday, April 25
8:00-9:00 a.m.  Breakfast
Dining Hall

SESSION III: DEVELOPMENTAL BIOLOGY AND REGENERATION
Session Chair: James Coffman, Ph.D., Associate Professor, MDI Biological Laboratory

9:00-9:15 a.m.  **Tariq Ahmad**, Ph.D., Assistant Professor, Colby College, new INBRE investigator
*Drosophila* model of Frontotemporal Dementia: A view from fly eyes

9:15-9:30 a.m.  **Minh-Tam Pham**, Bates College, undergraduate student
Transcriptional Regulation of the Nuclear factor erythroid 2-related factor (NRF) family by the Aryl hydrocarbon receptor-1b (AHR-1b) during *Danio rerio* development by Chromatin Immunoprecipitation
9:30-9:45 a.m.  Sree Deepthi Muthukrishnan, University of Maine, graduate student  
BMP7-TAK1-JNK-JUN signaling pathway governs the proliferation of nephron progenitor cells

9:45-10:30 a.m.  Poster Session B and Morning Refreshment  
Dahlgren Hall

SESSION IV: NEUROSCIENCE AND PHYSIOLOGY  
Session Chair: Patsy Dickinson, Ph.D., Josiah Little Professor of Natural Sciences, Bowdoin College

10:30-10:45 a.m.  Melissa Maginnis, Ph.D., Assistant Professor, University of Maine, new INBRE investigator  
Molecular determinants of JC Polyomavirus attachment and entry

10:45-11:00 a.m.  Scott Dobrin, Ph.D., Assistant Professor of Biology, University of Maine at Presque Isle, new Maine investigator  
Development of standard lab and field tests of honey bee health

11:00-11:15 a.m.  Kristy Townsend, Ph.D., Assistant Professor of Neurobiology, University of Maine, new Maine investigator  
Regulation of Energy Balance by the Bone Morphogenetic Proteins

11:15-11:30 a.m.  Peter Reifsnyder, Research Assistant, The Jackson Laboratory  
Rapamycin with metformin treatment ameliorates comorbidities in a type 2 diabetic mouse model, NONcNZO10/LtJ

11:30-11:45 a.m.  Taylor Follansbee, University of New England, undergraduate student  
BMP signaling is required for allodynia in Drosophila

11:45-12:00 p.m.  Julia Mitchell, Colby College, undergraduate student  
Investigation of Disc1 gene status and stress exposure in female rats on behavior and hippocampal plasticity

12:00-1:00 p.m.  Lunch  
Dining Hall

1:00 p.m.  Symposium Conclusion

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Maine Biological and Biomedical Sciences Symposium
April 24-25, 2015

MDI Biological Laboratory
Salisbury Cove, ME 04672

Abstracts of Poster Presentations

Abstracts are in alphabetical order by first author, you may open the bookmarks menu in the left navigation column to easily skip to the abstract of interest.
Drosophila model of Frontotemporal Dementia: A view from fly eyes

Frontotemporal dementia (FTD) is associated with a progressive decline in the cognitive functions due to age-dependent loss of neurons in frontal and temporal lobes of the brain. The development of early therapeutic interventions has been hampered by the lack of genetic markers. One genetic marker associated with a hereditary form of FTD is a mutation in CHMP2B - a protein involved in the endosomal-lysosomal pathway that delivers cell surface proteins for recycling and degradation. However, it remains unclear how these mutant proteins lead to neurodegeneration. My lab utilizes fruit fly Drosophila, a well-established model system for studying human neurodegenerative diseases to understand such problems. We use the genetic tools to ectopically express human mutant CHMP2B in Drosophila and analyze the effects at molecular, cellular, and behavioral level. This presentation will provide an update on our efforts to characterize the effects of expression of FTD-associated human mutant protein in Drosophila.

Tariq Ahmad, PhD
Assistant Professor
Department of Biology
Colby College
The regulatory role of the phosphorylated G protein:

Implication of lipid microdomains

Sarah, Alamer\textsuperscript{1,2} and Robert, Gundersen\textsuperscript{1,2}

\textsuperscript{1} Graduate School of Biomedical Sciences and Engineering, University of Maine, ME 04469
\textsuperscript{2} Department of Molecular and Biomedical Sciences, University of Maine, ME 04469

Heterotrimeric G-proteins play a crucial role in various signal transduction pathways, where this protein acts as a molecular switch to transduce the signal from G protein-coupled receptors (GPCRs) to downstream effectors. Post-translation modifications such as phosphorylation and palmitoylation can be important factors in regulating G protein function at the plasma membrane, but the mechanism of their involvement remains poorly defined. To elucidate this mechanism we use \textit{Dictyostelium discoideum} as a cellular model that relies on chemotaxis toward a secreted chemoattractant, cyclic adenosine monophosphate (cAMP) during the development phase of their life cycle. This process is G protein dependent specifically the G\textalpha\textsubscript{2} subunit of \textit{D. discoideum} is used. Our preliminary data demonstrate that the G\textalpha\textsubscript{2} subunit is enriched in a lipid raft fraction (LRs). This localization was palmitoylation-dependent. We further show that activation significantly shifts G\textalpha\textsubscript{2} out of the LRs in a F-actin dependent manner. Once activation occurs, G\textalpha\textsubscript{2} is known to be phosphorylated on serine 113. The function of the G\textalpha\textsubscript{2} phosphorylation in signaling, and how membrane microdomains can be involved remain unknown. This study focus on understanding the regulatory function of activated G proteins and phosphorylated G\textalpha\textsubscript{2} in the localization to LRs.
Downregulation of the cystic fibrosis transmembrane conductance regulator negatively impacts the innate immune response to \textit{Pseudomonas aeruginosa} infection in zebrafish

Lucy D. Algeo, Alexis R. Bowman, Conner R. Lajoie, Kathryn A. Liberman, Spencer E. Traxler

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Cystic Fibrosis (CF) is a life-threatening disease caused by a recessive mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Over 80% of CF patients succumb to respiratory failure due to lung infections primarily caused by \textit{Pseudomonas aeruginosa}. Previous studies have shown that the innate immune system, the first line of host defense, plays a critical role in combating \textit{P. aeruginosa}. The goal of the present study is to characterize the role of \textit{cftr} in the innate immune response to \textit{P. aeruginosa} infection using the zebrafish \textit{Danio rerio} as a model for human infectious diseases. We hypothesize that CFTR morphant zebrafish have a dampened innate immune response upon infection with \textit{P. aeruginosa}. Knockdown of \textit{cftr} with morpholino oligonucleotides and infection with \textit{P. aeruginosa} results in higher bacterial burden and decreased neutrophil migration. This suggests that CFTR is necessary for a proper and efficient innate immune response against \textit{P. aeruginosa}. 
Identification of novel genes regulating germ granule function in C. elegans.  
Andraloje K, Terrey M, Senter-Zapata M, King B, Campbell AC, Updike DL

Kathryn W. Davis Center for Regenerative Biology & Medicine,  
Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

Germ-granule components in C. elegans, are associated with RNA metabolism, and function to maintain the totipotent and immortal properties of the germ cells. The Argonaute protein CSR-1 is a core component of germ-granules and acts to suppress sperm-gene expression in the hermaphrodite germline. To identify additional CSR-1 pathway components we performed an EMS mutagenesis in a PGL-1::GFP strain and analyzed 4000 haploid genomes for large/missshaped germ-granules. From this screen we isolated ten alleles that fall into six complementation groups. We have shown that multiple endogenous germ-granule components are enlarged and overexpressed in all our mutants. Using Hawaiian Variant Mapping combined with CloudMap we found that two alleles, sam15 and sam18, contain mutations in the PAZ domain of csr-1 itself, validating our approach. We focus on a novel gene mutated in sam3 and sam6 that alters germ-granule appearance and expression, but is not required for fertility. Our results contribute to further understanding of epigenetic regulation and germ-granule function in germ cells.
**Characterization of lysogeny regulation in the Cluster E mycobacteriophage Ukulele**

Beacham, GM, Whitacker, EE, Harris, KB, Hutchison, KW, and Molloy, SD

Department of Molecular and Biomedical Sciences
The University of Maine, Orono, ME 04469

Mycobacteriophage (phage) are viruses that infect species of the genus *Mycobacterium*. The temperate Cluster E phage Ukulele was isolated at the University of Maine in 2011. Temperate phage follow the lysogenic lifecycle. After the phage genome is injected into the host cell it integrates into the host genome, forming a lysogen. Though Ukulele is temperate, its genome does not obviously encode repressor or excise genes. The repressor protein is required to maintain the lysogenic state. Under certain conditions, the excise promotes excision of the phage genome and the phage enters the replicative lytic cycle. I identified putative Ukulele excise (gp52) and repressor (gp88) genes. Ukulele gp52 also has a similar predicted fold as a cro-like protein found in several other phage. I deleted gp52 and gp88 independently using the Bacteriophage Recombineering of Electroporated DNA system. Gp88Δ mutants are not viable and therefore I am using complementation to isolate them and to determine whether over or under expression of gp88 has an effect on plaque morphology. Gp52Δ mutants have been detected in plaques containing a mix of wild type and recombinant phage. Gp52Δ mutants must be isolated before performing additional experiments to determine whether gp52 may or may not encode the excise or a cro-like protein.
Lung development requires the precise spatiotemporal coordination of a suite of genomic programs to direct the formation of a highly branched airway that maximizes gas-exchange surface area. To capture changes in gene expression correlated with lung development, and compare strain-dependent differences in expression between common strains of laboratory mice, we performed genome wide transcriptional profiling of A/J, C57BL/6J, and C3H/HeJ mice at 26 timepoints ranging between embryonic day 9.5 to postnatal day 56. Using principal components analysis (PCA) we identified 10 factors (principal components) that define the majority of sample variation within the dataset. Mining the genes contributing to these components yielded patterns of gene expression in common across developmental time. Ontological term enrichment analysis using the Visual Annotation Display (VLAD) hosted by the Mouse Genome Informatics (MGI) database was used to identify processes and phenotypes enriched in each principal component. Genes associated with lung development (GO:0030324) included Fgf1, Pdgfa, and Gli1/2/3, as well as a host of genes previously unassociated with lung development.
MicroRNA-101 controls cardiomyocyte proliferation and scar tissue resolution during heart regeneration

Megan Beauchemin 1,2, Ashley Smith 2 and Viravuth P. Yin 2

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2 Mount Desert Island Biological Laboratory, Davis Center for Regenerative Biology and Medicine, Salisbury Cove, ME 04672
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Cardiovascular disease is the leading cause of death in the United States due to limited cardiac regenerative capacity in the adult. In response to injury, necrotic tissue is replaced with non-contractile scar tissue. In contrast, zebrafish display a robust regenerative capacity following cardiac injury. In these studies, we examined the contributions of microRNA-101 in cardiomyocyte proliferation and scar tissue resolution during heart regeneration. We demonstrate that miR-101 expression is rapidly depleted within 3 days following ventricular resection but is upregulated by 7-14 days post-amputation. We demonstrate that decreases in miR-101 levels at the onset of cardiac injury enhanced cardiomyocyte proliferation. Interestingly, prolonged inhibition of miR-101 activity resulted in defects in scar tissue resolution. We have identified that combinatorial depletion of miR-101 and its target gene, c-fos, rescued defects in scar tissue resolution mediated by miR-101 inhibition alone. Our studies indicate that the temporal modulation in the miR-101/c-fos genetic axis is critical for coordinating two essential cellular programs during heart regeneration.
The role of RAD51 in chemical carcinogenesis: prolonged exposure to particulate chromate inhibits filament formation and induces cytoplasmic accumulation

Browning, CL\textsuperscript{1,2}, Xie, H\textsuperscript{1,2}, Jasin, M\textsuperscript{3}, Prakash, R\textsuperscript{3}, Kelly, DF\textsuperscript{4} and Wise Sr, JP\textsuperscript{1,2}

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Particulate hexavalent chromium (Cr(VI)) is a human lung carcinogen that produces several forms of DNA damage, including DNA double strand breaks (DSBs). Homologous recombination (HR) repair is a crucial mechanism that repairs DSBs in an error-free manner. Previous data suggest that prolonged Cr(VI) exposure inhibits HR repair by misregulating RAD51 and that loss of HR leads to Cr(VI)-induced chromosome instability. RAD51 nuclear transport must be tightly regulated to protect against genomic instability. However, no studies have investigated how a chemical carcinogen affects RAD51 transport or its transport partners, RAD51C and BRCA2. In this study, we investigated the effect of particulate Cr(VI) exposure on the localization of RAD51, RAD51C and BRCA2. We exposed human lung fibroblasts to zinc chromate for 24-120h. Using affinity grid capture and TEM, we show that prolonged Cr(VI) exposure inhibits RAD51 filament formation. Specifically, after 24h and 120h exposure to 0.2 ug/cm\textsuperscript{2} zinc chromate, the number of RAD51 filaments decreased from 104 to 7, respectively. In addition, RAD51C and BRCA2 foci formation increased after 24h Cr(VI) exposure, which corresponds with no increase in RAD51 cytoplasmic accumulation. However, prolonged Cr(VI) exposure inhibits RAD51C foci formation, corresponding with an increase in RAD51 cytoplasmic accumulation. BRCA2 foci formation was not inhibited after prolonged Cr(VI) exposure. Using RAD51C deficient cells, we demonstrate that RAD51C depletion induces the cytoplasmic accumulation of RAD51. These results suggest that prolonged Cr(VI) exposure inhibits RAD51 by inhibiting its transport partner, RAD51C. This work was supported by NIEHS grant ES016893 (J.P.W.).
CSR-1 and P granules suppress sperm-specific transcription in the Caenorhabditis elegans germline

Campbell, AC, Updike, DL

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Germ granules, called P granules in C. elegans, are conserved ribonucleoprotein aggregates that are required to maintain germline pluripotency. Though it is unknown how P granules maintain pluripotency, one model is that they act as a safety net, selectively silencing transcripts not licensed for germline expression. Such germline-licensing has recently been ascribed to the Argonaute protein CSR-1, a core component of P granules. In order to determine how P granules regulate RNA, we performed RNA-seq on dissected germlines and compared RNA expression on CSR-1 RNAi and after severely impairing P granule assembly. Our results suggest that P granules function, in part, with CSR-1 to prevent the transcription of sperm-specific mRNAs in the germline until needed during gametogenesis, while also acting as part of a larger mechanism to maintain germ cell totipotency.

The Effects of NAD+ on a Secondary Dystroglycanopathy in Zebrafish

Carter, EV\textsuperscript{1,2}, Belanger, JJ\textsuperscript{2}, Pasquarella, ME\textsuperscript{2}, Archambault, LS\textsuperscript{3}, Henry CA\textsuperscript{1,2}

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Congenital muscular dystrophies are incurable genetic diseases that lead to muscle wasting and compromised locomotion. Defects in components of the extracellular matrix (ECM) lead to a vast array of congenital muscular dystrophies. Among these are dystroglycanopathies, which occur when dystroglycan is either completely absent or when dystroglycan is improperly glycosylated. Our lab previously determined that supplementing dystroglycan-deficient fish with exogenous NAD+ or B vitamin precursors of NAD+ ameliorates dystrophy symptoms. This project aims to identify if supplementary NAD+ can rescue a broader range of muscular dystrophies in zebrafish, as the absence of dystroglycan in humans is incredibly rare and secondary dystroglycanopathies are more common. Here, our goal was to determine whether or not supplementary NAD+ can rescue secondary dystroglycanopathies, in which mutations in glycosylation proteins lead to defective dystroglycan glycosylation. We examined an established zebrafish model of a fukutin-related protein (FKRP) morphant. FKRP morphants exhibited thinner and disorganized muscle fibers, fibers that crossed the myotendinous junction (MTJ), and reduced locomotion. At 72 hpf, FKRP morphants also displayed muscle fiber detachment. Supplementation with exogenous B vitamin precursors to NAD+ thickened muscle fibers, improved fiber organization, increased overall mobility, and reduced fiber detachment. Overall, our results indicate that NAD+ supplementation may have therapeutic implications for a broad spectrum of muscular dystrophies.
**GenomePatternScan: Computational identification of genome-wide candidate binding sites for FOXD1**

Clare Bates Congdon\(^1\), Samuel McFarland\(^2\), Jennifer Fetting\(^1\), Craig R. Lessard\(^{1,2}\), Jeffrey A. Thompson\(^3\), Christine W. Duarte\(^1\), Leif Oxburgh\(^1\)

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We have developed GenomePatternScan (GPS), a computational tool that identifies the locations of a DNA pattern, such as a transcription factor binding site (TFBS), throughout a genome or other long genetic sequence. In this work, we use GPS to identify putative genes regulated by FOXD1, an important transcription factor in kidney development.

The program searches a genome for a TFBS represented using the standard A, C, G, T alphabet or the extended IUPAC notation. Output includes the genetic context of each candidate hit as well as a link into the UCSC Genome Browser to simplify further investigation of the genomic context. Cross-referencing is also provided, e.g., to look for hits in the same gene across different species and the ability to input a listing of genes of interest, such as the results from microarray experiments.

Using GPS, we identified 512 candidate locations of the FOXD1 binding site in the noncoding regions for the same genes in human, rat, and mouse. We further reduced this listing by cross-referencing with literature searches and are confirming the resulting short list of genes at the bench using qRT-PCR and chromatin immunoprecipitation (ChIP).
Genetic screening of CYP2C19 can determine drug efficacy

Curtis CD\textsuperscript{1}, Foster A\textsuperscript{2}, Goodney C\textsuperscript{1}, Sewell E\textsuperscript{3}, Soohey S\textsuperscript{3}

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Heart disease is the leading cause of death for both men and women in the United States. Genetic screening allows for tailored treatment plans for patients after a heart attack. Plavix is a drug that is commonly used after a heart attack to prevent platelets from adhering to blood vessels, reducing the risk of clots, and therefore another heart attack. Plavix is not always effective due to mutations in exons 4 or 5 of the CYP2C19 gene, which encodes the enzyme that activates Plavix. If homozygous mutations are present on one of the two exons, the functional enzyme will not be synthesized. The goal of this experiment was to identify mutations in CYP2C19 using PCR, followed by restriction digest analysis and Sanger sequencing. Of fifteen samples, three were heterozygous, and twelve were homozygous wild-type. All of these individuals can be prescribed Plavix with confidence by their physicians.
Microengineering the Neural Tube

Demers, C\textsuperscript{1}, Soundararajan, P\textsuperscript{2}, Cox, G\textsuperscript{2}, Smith, R\textsuperscript{1}, Collins, S\textsuperscript{1}

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\textsuperscript{2}The Jackson Laboratory, Bar Harbor, ME 04609

The differentiation of stem cells to motor neurons \textit{in vitro} is a powerful tool for both developmental and disease biology. While select motor neuron subtypes have been generated \textit{in vitro}, yields are often low. Furthermore, some subtypes have been impossible to generate with fixed concentrations of morphogens. In the developing neural tube, subtypes of neurons are directed to differentiate by soluble molecules that are released from floor plate cells and the paraxial mesoderm. We have engineered a microfluidic instrument that can mimic the spatially and temporally varying chemical environments found \textit{in vivo} during neurogenesis. Using our instrument, we were able to subject embryonic stem cells to a concentration gradient of sonic hedgehog for up to one week in culture. As a result, the motor neuron “band” that forms within the ventral neural tube has been recreated for the first time \textit{in vitro}. We were also able to track in real time the differentiation of naïve stem cells into motor neurons using the \textit{Hb9::GFP} mouse stem cell line. Our device design additionally provides easy access to motor neurons for post-differentiation analysis such as immunocytochemistry. Using the new capabilities afforded by our microfluidic instrument, an \textit{in vitro} model of neural differentiation can be developed to help answer some of the lingering questions in developmental neurobiology.
Anecdata: A Platform for Crowdsourced Public Health Data and Health Research Studies

Duncan Bailey and Jane Disney

Mt. Desert Island Biological Laboratory, PO Box 35, Salisbury Cove, ME

The Internet and mobile devices have opened new opportunities for citizen science, but existing citizen science websites are limited in the scope of the data they can collect. Anecdata.org, developed at MDI Biological Laboratory's Community Environmental Health Laboratory, is a new mobile-friendly web app that was developed to address data collection limitations, thus expanding the potential for environmental citizen science initiatives to achieve their goals.

Anecdata.org allows students, teachers, researchers, regulatory agencies and community organizations to crowdsource a wide range of information. Project creators can make custom datasheets with lists of locations where users can add data. Users can include their device's GPS coordinates, upload photos and videos, and add additional fields on the fly while posting. They can also run advanced searches and download custom reports in spreadsheet or Google Earth format as well as ArcGIS shapefiles for use with ArcMap and QGIS.

Anecdata.org will enable many groups from around the globe to get started on data collection projects without having to invent data management or mapping tools. In addition to collection of environmental data by citizen groups, municipalities, or teachers and students, we anticipate other interest in the use of the Anecdata platform. This is why we are developing Anecdata.io which will expand the data realm from environmental to all forms of observations and will offer options for the collection of confidential data. These uses may include: regulators collecting input from citizens who may or may not have been able to attend or speak at public hearings; researchers collecting data for health research studies, and health interest communities collecting information related to health concerns. These could include demographic information and tracking of exposures, self-reporting of symptoms, follow-up treatments and exposure outcomes.
Development of standard lab and field tests of honey bee health
Scott E. Dobrin

University of Maine at Presque Isle, Biology Department, Presque Isle, ME, 04769
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Despite the importance of insect pollinators, honey bees are being threatened by commercial agricultural practices, such as pesticide treatment and migratory bee keeping. Traditional pharmacological tests, such as LD$_{50}$ which measures death rates, or measures of learning, such as the classic olfactory classical conditioning paradigm (PER), may not be sensitive enough to identify subtle changes in bees as they become vulnerable. The Dobrin lab is working to develop analytical methods for use in a research laboratory to measure subtle changes in the health of honey bees as well as a field test for commercial bee keepers to easily identify weak colonies while an intervention might still be beneficial.
In the United States alone over 100 million people suffer from chronic pain and unfortunately, even still, there is a lack in scientific understanding for the mechanisms of abnormal pain sensitivity. The present study utilized a candidate gene approach to identify novel components required for modulation of the tissue damage induced pain sensitization pathway in *Drosophila melanogaster*. We have shown that nociceptor specific RNAi silencing of several members of the Bone Morphogenetic Protein (BMP) signaling pathway significantly attenuated sensitization. Furthermore, overexpression of BMP 2/4 in nociceptor neurons was sufficient to induce sensitization. We then showed that the effects of BMP signaling were largely specific to the sensitization pathway and not to normal nociception or dendritic morphology. Thus, we have shown that the BMP family has a crucial and novel role in sensitization. Because the BMP family is so strongly conserved between vertebrates and invertebrates we are confident that the genes we discover will represent potential therapeutic targets applicable to humans.
The Effects of Arsenic on the Development and Behavior of *Fundulus heteroclitus*

Marissa Giroux¹, Diane Nacci², Rebecca Van Beneden¹, Bryan Clark², Ashley Bertrand², Denise Champlin²

¹School of Marine Sciences, University of Maine, Orono
²EPA Atlantic Ecology Division, Narragansett, RI

Arsenic (As) is a known carcinogen and neurotoxin; however, little research has been done regarding the mechanisms of effects on development. In large areas of the US including Maine, arsenic is present in marine systems through natural deposits in the ground. This often raises the concentration of arsenic in water above 0.010 µg As/mL, which is the highest level of As that is allowed in drinking water. *Fundulus heteroclitus* (Atlantic killifish) is a coastal fish species whose early development provides sensitive endpoints for toxicity tests. Killifish embryos from a Bar Harbor, ME, population were exposed to 7.5 to 75 ug As/mL. The time to hatching, length at hatch, and growth rate were determined. Individual embryos had heart rates recorded at 8 dpf (days post fertilization), and were examined for heart, growth, and developmental defects at 10 dpf. Once hatched larval killifish were recorded for ability to catch prey. Results show that arsenic exposure at high concentrations impairs overall embryo development, and causes severe heart deformities. Arsenic exposed embryos hatched later than controls, and were slower to locate and consume prey compared to controls. These results document As effects on development and confirm the usefulness of early killifish developmental endpoints to explore toxic mechanisms.
The Role of the BMP Signaling Family in *Drosophila melanogaster* in the Induction of Allodynia

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According to the NIH, more than 100 million people suffer from chronic pain in the United States, yet the mechanism of this pain sensitivity lacks understanding. The ultimate goal of this research is to develop better drugs that can block the activity of candidate molecules, thus blocking the sensitized pain state.

This research utilized a candidate gene approach to identify novel components required for modulation of the pain sensitization pathway in *Drosophila melanogaster*. Components of The Bone Morphogenetic Protein (BMP) signaling pathway were investigated by using an RNAi knock-down approach. The larval epidermis was damaged with UV light, and then touched with a normally non-noxious thermal stimulus. The response latencies of the mutants and the controls were recorded, thereby testing for allodynia, or a response to a normally non-noxious stimulus. The results found that the knockdown of BMP ligands significantly decreased the formation of sensitization compared to the controls which indicates that BMPs are necessary in the formation of allodynia.
Genetic and environmental interactions play integral roles in the innate immune response to *Pseudomonas aeruginosa* infection in zebrafish

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The innate immune system is the first responder to pathogens. This can be compromised by genetic or environmental events, such as mutations in the *cystic fibrosis conductance transmembrane regulator* (*CFTR*) gene, which causes Cystic Fibrosis (CF), or exposure to arsenic. Similarly, zebrafish embryos with dysfunctional *cftr* expression have impaired innate immune responses. The leading cause of death in CF patients is infection by *Pseudomonas aeruginosa*. The goal of our experiment was to determine the effect of environmental and genetic factors on genes involved with the innate immune system. We hypothesized that innate immune response genes in *P. aeruginosa* infected zebrafish would be down regulated upon arsenic exposure and *cftr* knockdown. High-throughput RNA sequencing data show the expression of several genes that were affected significantly by these immune compromising conditions. One of these genes was the innate immune gene *tlr5b*. 
Multiplexed SERS imaging in biological systems using biocompatible Raman active nanostars

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The objective of this study is to design a nanoparticle labeling system suited for enhanced Raman micro-spectroscopic imaging to directly probe the concentration, chemical dynamics, and spatial distribution of individual Raman active nanoprobes at nanometer length scales in biological systems. Surface-enhanced Raman spectroscopy (SERS), using specifically engineered spherical metallic nanoprobes for chemical sensing, has received considerable attention recently. It is considered a powerful alternative to fluorescence labeling, offering several advantages over traditional methods, including increased photostability, narrower emission peaks, allowing for simultaneous observation of a larger number of “channels”, and ease of bioconjugation. Unfortunately, most existing nanoparticle geometries require mean diameters on the order of 50-80 nm to provide single particle signals comparable to those observed via competitive fluorescence methods. We propose to investigate a new nanoparticle geometry based on a star-shaped architecture which shows further enhancement over spherical particles. We further propose to create 10 spectrally distinct Raman active nanostars, overcoming the channel limitations encountered using standard fluorescence labeling methods. We will also develop the enhanced software required to obtain and analyze the Raman signal data by improving the computational efficiency of our current scanning software while adding spectral multiplexing algorithms.
The role of CGRP in LP-BM5 infection of astrocytes and microglia

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CGRP (calcitonin gene related protein) is a neuropeptide mainly expressed by primary sensory neurons. LP-BM5 infection induces profound immunodeficiency in B6 mice and has been used as a murine model of HIV infection. Previously, we showed that LP-BM5 induced increased CGRP expression in the spinal cords of infected mice. Further in vitro study indicated a microglia dependent, CGRP-induced reduction of LP-BM5 viral loads in primary mixed glial cells. To further delineate CGRP’s effects on glial cells upon LP-BM5 infection, we examined CGRP’s effects on in individual types of glial cells (astrocyte and microglia) upon LP-BM5 infection using specific murine cell lines. We found that LP-BM5 was capable of infecting both microglia and astrocytes alone. CGRP’s antiviral effect was observed in astrocyte-only cultures, but not in microglia-only cultures. LP-BM5 infection did not change significantly affect the viability of either astrocytes or microglia and CGRP antiviral effects in astrocytes did not seem to be due to decreased astrocyte viability. We are currently testing whether CGRP’s antiviral effects in astrocytes can be further enhanced when astrocytes and microglial cells are cultured together. The involvement of specific signaling pathways will be investigated in the future.
Bacteriophages (phages) play important roles in bacterial evolution and virulence. Temperate phages insert their linear genomes into the host cell cytoplasm where it circularizes. The two genomes now in the cell then integrate using phage and bacterial genomic sites, attP and attB respectively. The mycobacteriophage ChipMunk shows an altered phenotype of circularizing but not integrating into the host genome. We believe this is because of a mutation in the integrase gene. The results of several experiments suggested there might be errors in the sequence. It also suggested that at least some of the DNA from the phage particles was circular. Raw sequence data was reassembled and confirmed that the sequence was correct. It also confirmed that there was circular DNA obtained from the phage particles. The development of a program to analyze the sequence structure led to adaptation of the program to rapidly search for potential promoter sequences.
Mapping the genome of *C. elegans* to find where *dpy-5* is located


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At a developmental genetics short course hosted by Mount Desert Island Biological Laboratory three experiments were completed. Mapping of the *C. elegans* genome was conducted to see where the gene *dpy-5* was located. The gene *dpy-5* was found on chromosome one. Another experiment conducted was crossbreeding wild-type males and hermaphrodites, group A and B, which carried either rol-6 dominant gene or rol-6 recessive gene to establish which gene was dominant. The results determined that rol-6 group A was the dominant gene. The last experiment conducted was the use of RNAi for suppression of GFP and Unc-33, a gene that allows normal mobility. Gene expression was suppressed with the Unc-33 showing decreased mobility and fluorescence.
Lowering eukaryotic initiation factor 4G improves proteostasis and helps explain the associated longevity phenotype in Caenorhabditis elegans

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Regulation of protein synthesis is known to impact aging and the ability to respond to environmental changes in model organisms. Reducing expression of the eukaryotic initiation factor 4G (eIF4G), a crucial mediator of protein synthesis, elicits an increase in lifespan accompanied with enhancement of factors that promote cellular homeostasis. Some of these factors are known to be essential for lifespan extension. We hypothesized that reducing the expression of eIF4G increases lifespan by maintaining proteostasis. Expression of eIF4G was reduced in C. elegans and two proteostasis pathways were tested: 1) heat shock response under heat stress and 2) unfolded protein response (UPR) induced by tunicamycin stress. While lowering eIF4G improved survival under each condition, we found that the UPR was resolved more rapidly under tunicamycin stress than in controls and that factors important for increased longevity were also important for survival under UPR stress conditions. These results suggest that the longevity benefits of eIF4G may be due to enhanced protein homeostasis.
Mycobacteriophage (phage) are viruses that infect the bacterial genus *Mycobacterium*. They are classified into lettered clusters and numbered subclusters based on genome sequence similarity. A temperate phage integrates its genome into the host genome after infection, forming a lysogenic bacterial cell, or lysogen. Ollie, a lysogenic phage, was isolated from a soil sample taken from the University of Maine, Orono. Ollie DNA was sequenced and determined to belong to cluster-A3, but the full annotation of the genome is incomplete. The aim of my project is to use bioinformatics tools to identify this integrase cassette region in Ollie. I will then closely examine its isolation host, *M. smegmatis*, to find genomic similarities with other bacteria of the genus *Mycobacterium*. Ultimately, this project will broaden my understanding of Ollie’s host range and how it interacts with these alternative hosts.
Impact of *Lyst* and *Dock7* mutations on retinal pigment epithelial cells

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The retinal pigment epithelium (RPE), a monolayer of highly polarized pigmented cells located between the retina and the choroidal vasculature, serves as a blood–retinal barrier and is necessary for the maintenance of the outer retina. RPE dysfunction and cell death drives the pathogenesis of multiple retinal diseases. Our research revealed that murine *Lyst* and *Dock7* double mutations lead to retinal pigment epithelial atrophy (RPEA) characterized by RPE hypopigmentation and photoreceptor degeneration. To further characterize the interaction of these genes and their contribution to RPEA, the retinal structure, RPE cell and melanosome morphology of mice bearing *Lyst*, *Dock7* or both mutations were compared with wild-type (WT) controls. In RPE flat mounts and transmission electron microscopy (TEM) analysis, *Lyst* mutants showed enlarged melanosomes that were unevenly distributed within the RPE cells compared with WT and *Dock7* mutants. However, only mice bearing both *Lyst* and *Dock7* mutations exhibited RPE lesions and photoreceptor degeneration as assessed by optical coherence tomography (OCT) and histology. Likewise, the onset of RPE cell morphological alteration was more pronounced in mice with *Lyst* and *Dock7* double mutations than *Lyst* alone, and *Dock7* mutants did not differ from WT in RPE morphology. These results suggest that the *Dock7* mutation has little effect on the RPE on its own, however, it is able to significantly enhance the pathological consequences of the *Lyst* mutation.
Cystic Fibrosis (CF) results from mutations in the *cystic fibrosis transmembrane conductance regulator* (*cftr*) gene. Previous research in the Kim laboratory demonstrated that *cftr*-morphant zebrafish have altered innate immunity, evidenced by reduced respiratory burst and increased bacterial burden (Phennicie et al 2010). The role of *cftr* in the host innate immune response to viral infection remains largely unknown. Influenza A virus (IAV) infections are responsible for over 200,000 hospitalizations and 36,000 deaths yearly. We propose to characterize the role of *cftr* in altering the neutrophil response to an IAV infection, which has not yet been completed. We use three models for *cftr* disruption in the zebrafish, a *cftr* mutant, morpholino-mediated *cftr* knockdown, and a chemical *cftr* inhibitor. We aim to determine the role of *cftr* in the neutrophil behavior, neutrophil response to systemic IAV infections, and neutrophil migration to localized infections in the swimbladder. These aims may uncover novel mechanisms by which CF patients have altered immunity to viral infections.
Suppression of germline programs in the soma of Caenorhabditis elegans

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Cancer cells acquire a number of germline-specific traits, including cellular immortality and the ability to self-renew. A subset of genes exclusively found in the germ cells of the testis and/or ovary, which are collectively referred to as cancer-germline (CG) genes, are overexpressed in 70% of melanomas, 46% of breast tumors, and are frequently found in bladder, lung, and hepatocellular cancers.¹² A number of CG proteins are components of germ granules, which are found in the germ-cell cytoplasm from worms to humans. We have previously shown that C. elegans germ granules, called P granules, function to maintain germline pluripotency.³ In the soma, components of the synMuv B chromatin remodeling complex, which include homologs of Retinoblastoma (RB) and Malignant Brain Tumor (MBT), actively repress P-granule expression.⁴⁵ In an EMS mutagenesis to identify mutations with abnormal P granules, we isolated eight mutants with somatic P-granule expression. While five of these alleles exhibited a synthetic multivulva phenotype characteristic of synMuvB genes, three mutant alleles (sam4, sam12, and sam13) did not. We used Hawaiian Variant Mapping to clone and map these three alleles and report on their role in repressing germline identity in the soma.

The long-range goal of our research is to dissect the molecular regulation of vertebrate limb regeneration, and apply this information toward designing therapies that restore regenerative responses in humans who have lost limbs to trauma or disease. Previous studies of vertebrates that can regenerate appendages have shown that multiple genetic programs are modulated through regulatory factors. MicroRNAs (miRNAs) are short highly conserved non-coding genes that suppress expression of target genes and thereby control multiple genetic programs. Given their important regulatory roles and evolutionary conservation, we hypothesize that miRNAs define a conserved genetic regulatory circuit important for appendage regeneration. We compared miRNA expression in zebrafish caudal fins, bichir pectoral fins and axolotl forelimbs and found five up-regulated and five down-regulated common miRNAs. MiR-21 was most highly up-regulated and candidate target genes were predicted that were commonly down regulated. Candidates include the tumor suppressor gene, pdcd4. Knowledge of how miRNAs govern regeneration significantly advances our knowledge of this complex process.
Environmentally relevant transplacental arsenic exposure effects on mouse (Mus musculus) hepatic protein expression

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Inorganic arsenic has been linked to cancers, diabetes, cardiovascular disease, and other metabolic diseases, but the molecular mechanisms of toxicity are poorly understood. This project explores the toxic effects of arsenic using a mouse (Mus musculus) model organism. The Van Beneden lab has shown that mice respond to low-dose, transplacental arsenic exposure in a dose-, sex- and generation-dependent manner. This study addresses a potential mechanism of toxicity by determining relative expression levels of pAKT/AKT, a serine/threonine kinase that is activated via phosphorylation. Previous studies have shown arsenic exposure is linked to altered expression of gene products associated with cell cycle regulation and glucose metabolism. AKT is known to be involved in these conserved pathways, making it the subject of cancer and diabetes research. Initial data show a trend of reduced AKT activity at 50ppb transplacental arsenic exposure, but no sex-dependent response or statistically significant effects of the treatment levels. Analysis of pAKT/AKT expression provides insight to the molecular pathways involved in arsenic toxicology when partnered with existing literature and results of the ongoing study in the Van Beneden laboratory.
Photoreceptors are indispensable for normal vision. NR2E3, an orphan nuclear receptor, is critical for proper differentiation and homeostasis of photoreceptors. Mutations of NR2E3 in humans are associated with enhanced S-cone syndrome and retinitis pigmentosa, characterizing by the overproliferation of S-cone cells, a specific type of photoreceptor. Likewise, a recessive murine mutation, Nr2e3\textsuperscript{rd7} results in excessive S-cone proliferation, which ultimately results in photoreceptor degeneration. The mouse bearing the Nr2e3\textsuperscript{rd7} mutation has been crossed to different genetic backgrounds, in an attempt to search for the contributors to the regulation of photoreceptor growth and development. It has been previously reported that there are modifiers that are capable of rescuing photoreceptors from aberrant development that results from the Nr2e3\textsuperscript{rd7} mutation. Identifying and characterizing novel modifiers in mice will not only further our exploration on the transcriptional regulatory network that governs neuroretina homeostasis, but may also facilitate therapeutic interventions for photoreceptor retinopathies.

Through a sensitized chemical mutagenesis screen by ENU, we have successfully established at least seven lines of mouse in which Nr2e3-associated phenotypes are remarkably suppressed (e.g. fewer observable white spots compared to Nr2e3\textsuperscript{rd7} mice by fundus examination). Initially, next-generation sequencing has been performed on modified mouse lines and QTL analyses carried out to discover the modifier genes. We have identified several potential modifier genes, of which Frmd4b is currently under further verification by genome-wide scan and fine mapping analyses. Additionally, we are mechanistically investigating its role in determining the cell fate of photoreceptors, as well as its protective effects in retina. We hope that our study will deepen our knowledge about the development and homeostasis of photoreceptor cells, and provide potential strategies for therapeutic interventions.
Mycobacteriophage (phage) are a diverse group of viruses that infect bacteria of the genus *Mycobacterium*. Mycobacteriophage are classified into 22 clusters based on sequence similarity. Using bioinformatics tools, Misomonster, a A3 cluster phage, was annotated and analysed. Misomonster has 10 repeat sequences with a 14 base pair core repeat sequence found in genomes of A cluster phage and in *M. smegmatis*. Repeat sequences are hypothesized to play regulatory roles in cluster K phage. The 21–24 base pair conserved repeats of Misomonster are located immediately adjacent to the start of the protein coding region. Each repeat includes the ribosomal binding site, the sequence to which the ribosome binds and initiates translation of the mRNA. Six of the repeats are intergenic and located in gaps of 23–53 base pairs gaps. The other 4 repeats are in a large 1100 base pair sequence with no predicted genes. We are currently looking for the presence of unannotated genes in this genome region.
Clarifying the protein interactions of lycopene elongase and bacterioopsin in *Halobacterium salinarum*

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Integral membrane proteins are known to play vital roles in cellular functions, yet further clarification of these protein interactions is needed. Two such proteins in the halophilic microbe *Halobacterium salinarum* are lycopene elongase (Lye) and bacterioopsin (BO). While previous research demonstrated that BO inhibits Lye activity, this study is testing if the inhibition is due to direct protein interaction.

If Lye and BO interact, they should be in close proximity of each other in the membrane, so that the addition of formaldehyde will cross-link them. In order to test this possible cross-linking event through immunoblotting, a new strain of *H. salinarum* with a modified BO gene that encodes a histidine tag on the C terminus and an epitope tag containing Lye gene is being constructed.

Phylogenetics was also used to investigate a possible co-evolution of the two proteins. The analysis of phylogenetic trees based on protein sequences has shown good indication of co-evolution, supporting the existence of BO-Lye direct interactions.
Computational identification and biological validation of FOXJ1 regulatory regions in *Strongylocentrotus purpuratus*

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In this work, we used computational methods to identify candidate regulatory regions near the FOXJ1 ciliogenesis gene, and then investigated the biological activity of these candidates in the sea urchin *Strongylocentrotus purpuratus*. We found that 5/9 computationally derived candidate regulatory regions were biologically active at the times measured.

The study of ciliogenesis in *S. purpuratus* may lead to advances in treatment for cilia-related diseases in humans. The FOXJ1 gene, a member of the forkhead family of transcription factors, functions as a master regulator of ciliary genes. Our goal is to computationally identify candidate CRMs for the FOXJ1 gene and investigate the expression of these elements at 14, 16, and 18 hours post-fertilization, the period when cilia first form. We used computational tools, such as GAMI (Genetic Algorithms for Motif Inference), to identify candidate CRMs *in silico*. We then investigated the top 12 candidate CRMs *in vivo* using a DNA-tag reporter system. Of the 12 candidate CRMs, 9/12 were successfully amplified and ligated to reporter constructs. The resulting constructs were then microinjected into *S. purpuratus* egg cytoplasm, and the expression of these constructs was later measured via qRT-PCR. Of the 9 candidate CRMs accessed, 5 were found to be biologically active in all three time points measured.
Circadian rhythms maintain an organism’s daily sleep-wake cycle by conserved regulatory pathways, inducing profound effects in metabolic activity. *Drosophila* circadian period is maintained in a 24-hour cycle with peaks of activity at dawn and dusk. Ethanol exposure causes disruptions in a variety of physiological processes including circadian rhythms. We hypothesized that defects in circadian rhythm might lead to altered behavioral responses to ethanol and to disruptions in ethanol metabolism. To investigate this hypothesis, we used *Drosophila* strains bearing mutations that resulted in circadian periods which are longer (*perL*) or shorter (*perS*) compared to wild type rhythms. We analyzed ethanol metabolism by measuring alcohol dehydrogenase (Adh) activity, an enzyme that converts alcohol to aldehyde, and characterized behavioral responses to ethanol exposure by measuring sedation time, recovery from sedation, and tolerance after repeated exposure. This study will contribute to the understanding of *Drosophila* ethanol exposure on circadian rhythm modulations, which may facilitate the explanation of ethanol intoxication consequences on human circadian changes.
The Role of Serine Protease Inhibitor Kunitz Type 1a (spint1a) in Innate Immune Response to Influenza Virus Infection in Zebrafish

Influenza is a widespread, devastating disease that causes over 100,000 hospitalizations each year in the United States alone. Influenza begins primarily as a respiratory infection of the lung epithelial cells but in immunosuppressed individuals it can progress into a systemic infection, causing multi-organ failure and even death. In order to better understand the mechanisms of disease progression it is critical to study this process in vivo. The zebrafish (Danio rerio) is regarded as an excellent model organism for several human diseases because of the ease with which it can be genetically manipulated, the potential for development of transgenes, and its sole reliance on the innate immune system during the first 4-6 weeks of development (Kanther et al., 2010).

The Kim laboratory has recently developed a zebrafish model for human influenza infection, allowing direct examination of gene interactions and regulation of the host immune response to viral infection (Gabor et al., 2014). Serine peptidase inhibitor Kunitz type 1a (spint1a, also known as hail) is a gene that has previously been implicated in neutrophil chemotaxis (Carney et al., 2007). The role of spint1a in the host innate immune response to influenza infection is largely unexplored. Preliminary analysis demonstrates upregulation of spint1a upon influenza infection. By employing the zebrafish as a model organism, further characterization of spint1a in response to influenza infection would be possible. These findings further our understanding of host immunity and viral pathogenesis and will potentially assist in the development of effective antiviral therapies.
Molecular determinants of JC polyomavirus attachment and entry
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JC polyomavirus (JCPyV) is a ubiquitous human pathogen and the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). Viral infection is initiated upon attachment to cellular receptors and entry into host cells; thus, these interactions regulate viral infection and govern critical outcomes in viral disease. Utilizing a glycan array screen, we determined that the viral attachment receptor is α2,6-linked lactoseries tetrasaccharide c (LSTc). Structure-function analysis revealed that specific contacts between the major viral capsid protein VP1 and the α2,6-linked sialic acid of LSTc are required for infection. Further analysis demonstrated that while JCPyV can bind to multiple glycan receptors, JCPyV has the highest affinity for LSTc and retains specificity for this glycan. After binding to LSTc, JCPyV utilizes the 5-hydroxytryptamine (5-HT₂) receptors to mediate internalization. These findings highlight that the specificity and affinity of virus-carbohydrate interactions together with the function of viral entry receptors plays an important regulatory role in viral tropism and pathogenesis.
Insulin-like Growth Factor Binding Protein 4 (IGFBP4) is expressed in high levels by human adipose tissue and is the most abundant IGF binding protein produced by osteoblasts. In vitro, IGFBP4 is known to inhibit IGF-I action on pre-osteoblasts. Previous reports showed that locally injected IGFBP4 diminishes IGF-I effects on bone while systemic injection induces bone formation in mice; however, it is unclear whether IGFBP4 enhances or inhibits IGF-I signaling in vivo. The purpose of this study was to determine how IGFBP4 mediates adipose differentiation and if this impacts skeletal acquisition. Thus, we investigated the skeletal and adipose phenotypes of IGFBP4 null (IGFBP4\(^{-/-}\)) mice at 8 and 16 wks of age using DXA, MicroCT, gene expression and protein analysis. Whole body DXA revealed a significant decrease in fat proportion and lean mass in 8- and 16-wk-old BP4\(^{-/-}\) males and females (p<0.05). IGFBP4\(^{-/-}\) females had a reduction (p<0.04) of areal and femoral bone mineral density (BMD) and content (BMC) while males appeared unaffected by the loss of IGFBP4. At 16 weeks, IGFBP4\(^{-/-}\) mice showed reductions in femur length, inguinal (iWAT), gonadal white adipose tissues, and interscapular brown fat. Femurs of IGFBP4\(^{-/-}\) females had reduced BV/TV (p=0.06), trabecular and cortical thickness (p=0.05). However, males had higher connectivity density (p=0.02) and trabecular number (p=0.005) with decreased trabecular spacing (p=0.02). Serum IGF-I and IGFBP6 were both increased in IGFBP4\(^{-/-}\) females (p=0.03). However, in males, serum IGF-I was decreased (p=0.02) and IGFBP6 was unchanged. Gene expression analysis showed that Sost expression was decreased in femurs of IGFBP4\(^{-/-}\) males but not females. Ppar\(\gamma\) expression was reduced in iWAT of both genders. 8-week-old mice were challenged with a high fat diet (HFD, 60%kcal) or a control diet (10%kcal). After 6 weeks of HFD, wild type males gained more weight than IGFBP4\(^{-/-}\) males (13.7g vs. 6.3, p=0.004). Surprisingly, IGFBP4\(^{-/-}\) and wild type females appeared to gain a similar amount of weight (5.1g). To measure adipogenesis, outer ear mesenchymal stem cells (eMSCs) were isolated from 3-wk-old female mice and differentiated into adipocytes. IGFBP4\(^{-/-}\) eMSC showed a significant decrease in adipogenesis compared to cells isolated from wild type mice (p<0.001). In summary, our data suggest that BP4 is an important modulator of bone and fat development, but there is clear gender and tissue specificity. Indeed, males appeared to be protected against the reduction in bone parameters and the HFD-induced weight gain observed in females. Reduction of Sost expression in males but not in females may contribute to this gender-specific phenotype. Also, impaired adipocyte differentiation may partially explain the generalized reduction of adipose tissue. Taken together, our results support the hypothesis that BP4 likely mediates mesenchymal cell progression through several mechanisms, which may be linked to IGF-I and tissue specificity.
Smad4 is Required for Normal Kidney Development in Mice

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Receptor-regulated Smad proteins (R-Smads) are phosphorylated by transmembrane serine-threonine receptor kinases in response to TGF-β superfamily signaling. Activated R-Smad heteromeric complexes (Smad2/3 and Smad1/5/8) are translocated to the nucleus by the common mediator Smad (co-Smad) Smad4, where they regulate transcription of genes involved in cell growth, differentiation, and development. Nuclear translocation of R-Smads by Smad4 is believed to be non-redundant, and Smad4 global knockout mice are embryonic lethal. Foxd1 (forkhead transcription factor D1) is expressed in the cortical interstitium during mouse kidney development. Lineage tracing has shown that Foxd1-positive cells give rise to renal pericytes, including peritubular pericytes, perivascular pericytes, and mesangial cells in the glomerulus. The Foxd1\textsuperscript{+/Cre}; Smad4 mouse model allows for conditional deletion of Smad4 and, presumably, R-Smad transcriptional regulation in Foxd1-expressing pericyte precursors. Immunohistochemical and immunofluorescent staining shows that Foxd1\textsuperscript{+/Cre}; Smad4 mice exhibit a distinct kidney phenotype at postnatal day zero. Mutant mice display disorganized expression of smooth muscle actin and an increase in stromal cells and platelet-derived growth factor-β in the inner and outer medullary interstitium compared to wild type. Preliminary experiments also indicate defects in collecting duct arrangement and glomeruli differentiation. These results suggest that Smad4 plays an important role in kidney patterning and development.
Opioid abuse accelerates the onset of human immunodeficiency virus (HIV)-associated cognitive deficits and increases central nervous system (CNS) viral load in HIV-1 patients. Opiates such as morphine mediate this effect by attenuating the inflammatory response of glia, which initiate and maintain innate immune defense in the CNS. Using the LP-BM5 murine AIDS (MAIDS) model, we are investigating the effect of chronic morphine on the glial immune response in the context of an active viral infection. Key regions of interest include the hippocampus and striatum — regions which are highly susceptible to HIV infection in humans — and the frontal lobe, which is less susceptible to HIV infection.

Our model has shown that morphine significantly increases BM5 viral load in the hippocampus in the absence of significant changes in blood-brain barrier permeability. We also observed a significant decrease in the proinflammatory chemokine CCL5 in the frontal lobe, suggesting morphine may exert an immunosuppressive effect. In the hippocampus, we have shown that morphine exposure prevents an increase in the expression of type 1 interferons.

The type 1 interferon response activates somatic cells’ innate antiviral capabilities and amplifies the response of innate immune cells, such as glia. By inhibiting this response, morphine may inhibit cells’ ability to ward off viral infection and suppress replication. In our ongoing work, we are supplementing morphine-treated animals with an intranasal dose of interferon-beta to boost the type 1 interferon response and prevent the morphine-induced increase in hippocampal viral load. In addition, we are studying the influence of these local changes on the pro- vs. anti-inflammatory balance in glia, which may further impair the CNS immune response. With this work, we hope to provide fresh insight and reveal new avenues for treatment in morphine-potentiated HIV memory disorders.
We know that the oceans are packed with marine viruses and they have an important role in the rise and fall of plankton populations but current mathematical models do not accurately account for virus-host interactions when predicting plankton blooms. Therefore I am using model optimization and comparison techniques to evaluate current models and reassess how the virus-host system should be described. Preliminary analysis of in situ blooms has revealed previously unreported trends occurring over the course of an *E. huxleyi* bloom. Inclusion of virus-host interactions in models describing the calcifying coccolithophore *E. huxleyi* may give insight into global carbon cycling, as large-scale blooms are known to draw down significant amounts of atmospheric carbon.
Influenza A virus (IAV), a public health threat, can cause up to 500,000 deaths worldwide annually. In addition, IAV can undergo antigenic shift, a mechanism of genetic mutation that allows the virus to evade the host immune response. As a result, pandemic influenza could arise and have a detrimental impact on the world population and global economy. Thus, IAV has remained the subject of extensive interdisciplinary research. However, the role of microRNAs (miRNAs), a rather recently characterized class of cellular regulators, during IAV infection has remained largely unknown. Previous studies have established that many miRNAs are differentially expressed upon IAV infection in mice (Li et al.), highlighting their possible importance during viral infection. Others have demonstrated that miRNAs are utilized by both the host immune system as well as the invading pathogen (Li et al.). The zebrafish Danio rerio has emerged as a mainstream model for the study of genetics, development, cancer, and infection and immunity. Our laboratory has recently established a zebrafish model for human influenza, recapitulating mammalian infection (Gabor et al.). I hypothesize that IAV may be using host miRNAs to manipulate its cellular networks, allowing immune evasion, and subsequent viral propagation. Using the zebrafish model, I hope to characterize an immune function for miR-223, a candidate miRNA, in the context of IAV infection and host immunity. While additional studies are required, understanding the mechanisms underlying miRNAs regulation during IAV infection could lead to the development of targeted antiviral therapeutics.

Source:


The goal of the present study was to characterize the behavioral and neural effects of a biallelic deletion within the Disc1 (disrupted in schizophrenia 1) gene in female rats as a function of chronic stress exposure or control conditions. Mutations in the Disc1 gene are associated with increased incidence of psychiatric illness, particularly schizophrenia but also depression and bipolar disorder. A common feature of these disorders is the contributing factor of stress. Thus, this study tested the hypothesis that exposure to chronic unpredictable stress would significantly worsen the effects of the gene deletion. To do this, DISC1 knockout and wildtype rats were exposed to either 3 weeks of chronic unpredictable stress or maintained under normal colony conditions. At the end of the stress period, all rats underwent a battery of tests to evaluate emotionality and cognition. Rats were then sacrificed and brains retained for analyses of neural and genomic markers. The preliminary behavioral findings offer some, but incomplete, support for the hypothesis; the neural assays are underway.
R-spondins, are a family of four cysteine-rich secreted proteins known to play a critical role in development. In fact, R-spondin-2 has been shown to be a positive regulator of skeletal myogenesis via a canonical Wnt signaling dependent manner in C2C12 cells as well as primary satellite cells. However, intriguingly, Tg(MCK-Rspo2) mice, a transgenic mouse line overexpressing R-spondin-2 in postnatal skeletal muscle displayed impaired muscle growth during young adult age instead of expected muscle hypertrophy. Thus, we sought to determine a novel role of R-spondin-2 in muscle hypertrophy. The administration of R-spondin-2 recombinant protein to myofibers derived from C2C12 myoblast cells reduced AKT and S6 kinase phosphorylation, two components of the IGF-1/AKT pathway known to catalyze skeletal muscle growth by regulation of protein synthesis, in a time-dependent manner. In addition, Wnt3a, a known canonical Wnt pathway activator, recombinant proteins, similarly decreased AKT and S6 kinase phosphorylation in cultured myofibers. Furthermore, the reduction in AKT and S6 kinase phosphorylation was synergized with dual administration of R-spondin-2 and Wnt3a and ameliorated when cultures were additionally treated with IGF-1 recombinant proteins. Taken together, our results suggest that, in possible cooperation with Wnt signaling, R-spondin-2 catalyzes muscle atrophy by inhibiting the IGF-1/AKT pathway and thus, skeletal muscle growth.
Identification and Expression of GnRH2 and GnRH3 in the Black Sea Bass (*Centropristis striata*), a Hermaphroditic Teleost

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We cloned two cDNAs for two gonadotropin-releasing hormones, GnRH2 (chicken GnRH-II) and GnRH3 (salmon GnRH), respectively from the black sea bass (*Centropristis striata*). Black sea bass are protogynous hermaphroditic teleosts that change from females to males between 2 and 5 years of age. Similar to other GnRH precursors, the precursors of black sea bass GnRH2 and -3 consisted of a signal peptide, decapeptide, a downstream processing site and a GnRH-associated peptide. Our analyses failed to identify GnRH1. GnRH3 precursor transcript was more widely distributed in a variety of tissues compared to GnRH2. Further examination of GnRH expression and gonadal histology was done in black sea bass from three different size groups: small (11.4 to 44.1 g); medium (179.4 to 352.2 g) and large (393.8 to 607.3 g). Interestingly, GnRH3 expression occurred only in the pituitaries of males in the small and medium groups compared to expression of GnRH2. Future functional studies of the sea bass GnRHs will be valuable in elucidating the potential underlying neuroendocrine mechanisms of black sea bass reproduction, and may ultimately contribute to management advances in this commercially-important fish. This work was supported by NSF-0849569 to SAS. Partial funding was provided by the New Hampshire Agricultural Experiment Station as well as a University of New Hampshire Undergraduate Research Award (URA).
Genetic diversity and the 2013 decline of eelgrass (*Zostera marina*) beds of the Frenchman Bay, ME, coastlines

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Abstract

Eelgrass beds serve a vital marine role invaluable to commercial supplies of seafood in Maine by forming estuaries for young fish and shellfish. Since satellite imaging done in 1996, the coverage of eelgrass in the Frenchman Bay, ME, has shown reduction from 3,000 to 200 acres. In the summer of 2013, major restoration areas, established by ecologists to counter this loss, disappeared. The invasive species of European green crabs, which voraciously target young shellfish and clip through eelgrass, may be contributing to loss. Genetic diversity of eelgrass may explain differences in survival between populations, imparting more resistance to physical disturbance, comparable to destruction caused by crabs. The genetic diversity of six populations across Mount Desert Island was quantified using microsatellite size analysis and correlated with loss susceptibility. The genotype profiles of individuals from each population were determined by size analysis of six microsatellite loci previously described by Reusch *et al.* and F-statistics population genetics were performed. The quantified genetic diversity of the six populations was further correlated with previous research, including abundance and susceptibility to decline, biomass and tensile strength of eelgrass shoots, and prevalence of green crabs in the area.
**BMP7-TAK1-JNK-JUN signaling pathway governs the proliferation of nephron progenitor cells**

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Balancing self-renewal and differentiation of nephron progenitor cells is essential for formation of a full complement of nephrons during kidney development. The cellular fate of the nephron progenitors is controlled by multiple signaling cascades and complex molecular interactions with the ureteric bud as well as other progenitor cell types in the niche. We have previously shown that BMP7 promotes proliferation of the nephron progenitor cells through the MAPK pathway and differentiation of the progenitors via the SMAD transcription factors. However, the cellular and molecular mechanisms for these distinct effects of BMP7 on the progenitor cell fate are yet to be elucidated.

In this study, we show that BMP7 activates the TAK1-JNK-JUN signaling cascade in the highest order (CITED1+) nephron progenitor cells *in vitro*. We also demonstrate that *in vivo*, BMP7 and TAK1 genetically interact and function in the same pathway to govern the self-renewal of the nephron progenitor cells. We found that conditional deletion of *Tak1* in the nephron progenitor population results in strikingly smaller but morphologically normal kidneys as a result of coordinated reduction in proliferation in the progenitors and the collecting duct. More importantly, we show that TAK1 is required for renewal of the CITED1+ nephron progenitor cells throughout nephrogenesis. Further, we identified c-JUN and c-MYC (Cell cycle regulatory genes) as key targets downstream of TAK1-JNK signaling that may potentially be involved in regulation of progenitor proliferation. *In vivo* and *in vitro* experiments are underway to determine if c-JUN is required for self-renewal of nephron progenitors. Taken together, our findings suggest that BMP7-TAK1-JNK-JUN signaling pathway regulates the proliferation of highest order nephron progenitor cells during kidney development.
This study evaluates Maine Prescription Monitoring Program prescribing trends for opioids, sedatives, and stimulants, 2010-2014. Approximately 2.5 million controlled-substance prescriptions were dispensed each year. Of these, 50% were opioids, 36% were sedatives, and 15% were stimulants. Opioid prescribing was highest in 2010 with a decline from 2010-2013 and an increase in 2014. Of the three most commonly prescribed opioids, hydrocodone and oxycodone consistently declined, while buprenorphine prescriptions and recipients of these prescriptions increased. Sedative prescriptions peaked in 2010, followed by a steady decline through 2014. Stimulants were the fastest growing category with a 24% increase in annual prescriptions from 2010 to 2014. All age groups experienced an increase in the number of stimulant prescriptions, with the highest rates of increase (40%) among adults 30 years or older. Benzodiazepine prescribing rates were highest for adults 60 years or older with the highest rates among women. For this age group, 20% received one or more benzodiazepine prescriptions in 2014.
Modeling Behavior of *Hemigrapsus sanguineus* in the Delaware Bay

Kiera O’Donnell¹, Charles Tilburg²

In this study, we follow previous studies of simulating the Delaware Bay using a coastal modeling program. This program models the physical properties of Delaware Bay, including those important to transporting larvae. However in order to more closely study the transport of *Hemigrapsus sanguineus*, we create a biological model in the computer program Matlab that simulates their growth and vertical movement. Therefore, we use these two models to study the effects that discharge, winds and the biological model have on simulated *Hemigrapsus sanguineus*. These modeled trajectories eventually allow us to predict where larvae will settle and move throughout the Delaware Bay.

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**ABSTRACT:**

SEF (similar expression to fgf) is a transmembrane protein which functions as an inhibitor of the fibroblast growth factor signaling pathway. In the context of cancer, recent reports have shown that downregulation of SEF is associated with highly invasive and metastatic human carcinomas, thereby outlining a role for Sef as a tumor suppressor gene. Ongoing studies in our lab show that hSEF regulates epithelial mesenchymal transition (EMT) in breast cancer cell lines. Since the expression of β-catenin is associated with EMT, we postulated that SEF regulates EMT in breast cancer by interacting with β-catenin. Preliminary results from co-immunoprecipitation experiments showed that SEF associated with β-catenin and overexpression of SEF downregulated the endogenous activation of β-catenin in mammary epithelial cells. Ongoing experiments are aimed at determining if SEF regulates β-catenin signaling by blocking the transport of active β-catenin into the nucleus to function as a tumor suppressor gene in the context of breast cancer.
Effects of Waste Water Treatment Plant Effluent on Stress Tolerance in the European Green Crab, *Carcinus maenas*

Gwendolyn Pelletier, Claire Whalen, Chris Goodchild, Markus Frederich

Wastewater treatment plant effluent (WTPE) is commonly discharged into marine environments. We investigated the effect of WTPE on the stress tolerance of the green crab *Carcinus maenas*. Crabs were placed in 5 and 20% of WTPE and incubated for 4 days and then underwent an anoxia challenge for 1 hour. 20% WTPE-exposed crabs showed decreased treadmill running endurance (145±76 and 316±179 sec, respectively), righting response (8.9±8.4 and 3.5±5.7 sec), and oxygen consumption (0.067±0.012 and 0.064±0.009 µmol/g/min). GST activity, as an indicator for oxidative stress, was increased in WTPE exposed crabs (0.9±0.46, 3.3±0.52 and 2.25±1.84 units/mg protein for control, 5% and 20%, respectively). Gene expression assessed by qPCR revealed only a 1.5 fold increase in AMPK, as an indicator of cellular energy stress, and a 1.3 fold increase in HSP70, as a general stress marker, for the 20% WTPE treated animals. Overall, there was very little change in the selected stress parameters after exposure to WTPE, paired with anoxia. This is different than observed in a parallel study using the blue mussel, *Mytilus edulis*, which showed diminished performance at the whole animal and cellular level after exposure to WTPE. Our data indicate that WTPE does not diminish the performance of the green crab, but that of other species. Therefore, WTPE might aid in the success of the invasive green crab in dominating new environments. Further studies will continue to explore this.
Notch signaling in vascular remodeling following injury

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Notch signaling is a highly conserved signaling pathway that plays an important role in vascular development and disease. Mammals have five Notch ligands and four Notch receptors, and there are significant gaps in the understanding of downstream effects resulting from receptor-specific Notch activation. Our laboratory has previously demonstrated a receptor-specific function for Notch2 in promoting vascular smooth muscle cell quiescence (Boucher et al., \textit{Circ Res.}, 113:975-985, 2013). We predicted that one \textit{in vivo} role of Notch2 during remodeling would be to limit hyperproliferation in response to injury. Surprisingly, we found that conditional deletion of Notch2 in vascular smooth muscle cells resulted in decreased neointimal lesion formation following injury. We are analyzing this finding further by evaluation of changes in proliferation markers, smooth muscle cell markers, other Notch receptors, miR-143/145 levels and matrix remodeling. Understanding the role of Notch2 in vascular remodeling will lead to more effective and targeted treatments for vascular obstructive pathologies, which are a major cause of morbidity and mortality in the United States and abroad.
Planar polycyclic hydrocarbons can interfere with genetic fidelity by causing DNA adducts. Two gene families that encode transcription factors, that of the Aryl hydrocarbon receptor (Ahr), and the Nuclear factor erythroid 2-related factor (nrf), regulate genes involved respectively in phase I and II of chemical metabolism. There is evidence for crosstalk between Ahr and Nrf, demonstrated by the reduction of nrf expression upon Ahr knockout in developing zebrafish. This project aims to explore the cis-regulatory mechanism between the two transcription factors using Chromatin Immunoprecipitation (ChIP) in zebrafish embryos. ChIP will determine if there is an interaction between the Ahr-1b protein and the regulatory region of nrf genes.
Knockout of the bap gene increases bacteriorhodopsin production in Halobacterium salinarum
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Bacteriorhodopsin (BR) is an integral protein complex in the halophilic microbe Halobacterium salinarum, which provides the cell with energy by acting as a light-driven proton pump. BR is composed of the retinal cofactor and the bacterioopsin (BO) protein, encoded by the bop gene. In the H. salinarum genome, downstream of bop, there is a gene that encodes the bacteriorhodopsin-associated protein (bap), whose function is unknown. Due to its proximity to bop in H. salinarum and homologous genes in other organisms, it is believed that it affects the production of BR.

To study the potential effect of bap on BR biosynthesis, a bap knockout strain of H. salinarum was generated, and its BR production was quantified through absorption spectroscopy. Preliminary results show an increase of 51% in the BR levels for the Δbap strain compared to the wild-type strain, indicating that the presence of bap inhibits BR biosynthesis. Further studies will be conducted to determine the mechanism of this inhibitory effect.
Abstract

Diquat and tert-Butyl hydroperoxide (tBOOH) are common toxins that can induce oxidative stress leading to free radical formation. Free radicals can denature proteins, lipids, and nucleic acids. The appropriate concentration of diquat and tBOOH to induce oxidative stress remains untested in the zebrafish (Danio rerio) model organism throughout development. To test induction of oxidative stress, the common oxidative stress regulating genes: nqo1, gsr, gclc, txnrd1 and hmox1 were analyzed against a range of diquat and tBOOH concentrations at 2, 48, and 96hpf. Concentration curves were conducted utilizing the most affected gene and time point.
MicroRNAs (miRNAs) are ~22 nucleotide non-coding RNAs that function as posttranscriptional regulators by modulating mRNA translation and stability. The human genome encodes ~1900 miRNA genes with each miRNA having up to 200 predicted mRNA targets impacting a variety of biological pathways that are important in development and oncogenesis. In the canonical miRNA biogenesis pathway, the RNase III enzyme DICER cleaves the ~70nt pre-miRNA “hairpin”, to produce a duplex dsRNA that is loaded into the RNAi effector mechanism, the RNA induced silencing complex (RISC). DICER binds two double-stranded RNA binding protein (dsRBP) co-factors, TARBP2 and PRKRA, which modulate pre-miRNA “dicing” kinetics, cleavage site selection and loading of the miRNA duplex into the RISC. Disruptions in the pre- to mature miRNA processing step can impact mammalian development and cancer. Dicer1 mutant mice show an early embryonic lethal phenotype and haplo-insufficient Dicer1 cells promote tumorigenesis. Furthermore, recent studies with the small molecule enoxacin reveal a cancer cell-specific growth inhibition response mediated by the DICER co-factor TARBP2. We have tested the in vivo function of the DICER co-factors in the context of mammalian development as well as the target specificity of enoxacin using mouse cell lines genetically ablated for the DICER co-factors. To determine if in vitro-derived miRNA biogenesis models are accurate in vivo, we created single and double mutant Tarbp2 or Prkra mice to determine unique and redundant functions in development. The discordance between the phenotypes of single and double mutant Tarbp2 and Prkra mice compared with Dicer mutant mice suggest that these cofactors are not functionally redundant and that TARBP2 and PRKRA may be important for processing specific classes of miRNA’s at certain stages or have a novel function in post-transcriptional gene regulation apart from miRNA biogenesis. Finally we present a strategy using immortalized fibroblasts derived from Tarbp2 and Prkra embryos to test if the two structurally related proteins can equally mediate enoxacin’s effect on miRNA biogenesis and post-transcriptional gene regulation.
Mechanisms underlying stretch feedback in the heart of American lobster, *Homarus americanus*
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Central pattern generators are neuronal networks that endogenously produce rhythmic motor outputs. The crustacean heart is neurogenic and is controlled by a simple pattern generator, the cardiac ganglion (CG). The CG of the American lobster consists of nine neurons that are both electrically and chemically coupled. Four interneurons serve as pacemakers, providing excitatory input that initiates activity in the five motor neurons. Previous studies have suggested that stretch feedback relays information to the CG about the degree of filling in the heart through mechanosensitive dendrites of the CG neurons. To determine the cellular mechanisms underlying stretch feedback in the lobster heart, we recorded intracellularly from motor neurons while stretching the myocardium connected to the pacemaker cells. Stretch elicited a decrease in the period and duration of driver potentials in the motor neurons. The extent of these changes was a function of the baseline driver potential duration, suggesting that the stretch response is state-dependent.
Rapamycin with metformin treatment ameliorates comorbidities in a type 2 diabetic mouse model, NONcNZO10/LtJ

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Rapamycin delays glucose clearance in B6 mice implying that it would have deleterious effects in those susceptible to type 2 diabetes (T2D). We treated a mouse model of T2D, NONcNZO10/LtJ, with rapamycin, metformin, or both. Although rapamycin exacerbated hyperglycemia, it inhibited weight gain, prevented hyperinsulinemia and hepatic lipidosis, and reduced inflammation and developing nephropathy. Metformin-treatment alone did little to alleviate most diabetic phenotypes, but did reduce hyperinsulinemia. Combination treatment showed phenotypes similar to rapamycin treatment but also reduced rapamycin-driven hyperglycemia. Rapamycin-mediated gene expression changes in liver and fat were consistent with improved health and metabolism. Rapamycin inhibited insulin production, but combination treatment improved this phenotype. Insulin tolerance tests showed that NONcNZO10/LtJ mice are insulin insensitive, however rapamycin increased insulin sensitivity and, in combination with metformin, normalized insulin sensitivity. These results suggest that rapamycin can have multiple beneficial effects even in the context of type 2 diabetes and that combination with metformin can promote insulin sensitivity and ameliorate rapamycin-associated hyperglycemia.
Assessing the Link between Antibiotic-Resistance Genes in Health Care Facilities and Environmental Reservoirs
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Environmental soil microbes make up a reservoir of antibiotic resistant genes. Research shows these genes from bacteria in soils are capable of dispersing to human pathogenic bacteria. We will determine if there is a linkage of B-lactam resistance between pathogens collected from Aroostook area healthcare facilities, and soils collected adjacent to these facilities. The genes will be sequenced and compared to allow for quantification of gene dispersal between the medical centers and their local environments. Methodology is being developed starting with isolation and screening for antibiotic resistance in soil bacteria from Aroostook county farms. Pathogenic bacteria have been obtained from Cary Medical Center and DNA extraction, along with PCR amplification of B-lactamase genes is ongoing. Upon spring we will sample soils and pathogenic bacteria from the health care facilities and conduct experiments according to the methodology. The information obtained can assist in development of protocols to lessen gene dispersal, and therefore slow the spread of antibiotic resistance to human pathogens.
Environmental Studies and Biology classes have begun a restoration project of the Alder Stream on the University of Maine at Presque Isle campus to improve the habitat for trout. Genetics students collected samples to obtain pre-restoration baseline data on invertebrate abundance. Samples were identified first by dichotomous key identification and DNA barcoding was then tested as an alternate identification strategy using the COI gene. Some samples showed DNA matches at the correct taxonomic level such as isopods and oligochaetes, but some showed incorrect matches or unsuccessful DNA amplification. All of this information can be used for comparison in later studies as restoration of Alder Stream continues.
Post-transcriptional processing is increased upon dietary restriction in C. elegans: a possible role for nonsense mediated decay and alternative splicing in lifespan extension

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Dietary restriction (DR) extends lifespan in a myriad of organisms ranging from single celled yeast to complex mammals. By combining analysis of total and polysome-bound mRNA from dietary restricted C. elegans with RNAseq, we differentiated changes in gene expression due to mRNA transcription from those arising due to altered translation. Our results indicate a fundamental shift in post-transcriptional regulation under DR. Among the genes down-regulated post-transcriptionally under DR were components of the ribosome. Several of the ribosomal components, including rpl-12, display significantly more intron retention under DR, which has been previously shown to target them to degradation via nonsense mediated decay after a pioneering round of translation. We hypothesized that the accumulation of unproductive spliced ribosomal components could be due to a decrease in NMD and/or an increase in alternative splicing under DR. Using a reporter construct containing a PTC, we determined that NMD is moderately inhibited due to DR and in silico analysis has implicated the splicing factor rsp-3 in intron retention under DR.
**Congenital Muscular Dystrophy with Megaconial Myopathy**  
*(MDCMC)*

Congenital muscular dystrophies include over 30 types of muscle disorders, all having an onset at or just after birth. Most CMDs are autosomal recessive disorders with rapidly progressing symptoms that have clinical manifestations resulting in muscle weakness and delayed/arrested motor abilities. Many of these disorders include progressive respiratory involvement, cardiac illness and delayed speech development. In spite of an increase in the number of patients detected with CMD, no definite cure or therapy exists. We plan to concentrate on Congenital Muscular Dystrophy with Megaconial Myopathy (MDCMC), a type of CMD in which the patients manifest enlarged mitochondria and progressive muscle degeneration as a result of mutations in the choline kinase beta *(CHKB)* gene, which leads to defective CHKB enzyme production. In this project, we will make use of a spontaneous null mutation and transgenic mouse models in order to determine the time point at which megamitochondria emerge and the rate of progression of MDCMC disease symptoms. We will also aim to determine if the mitochondrial structural abnormalities are a direct cause of the MDCMC phenotype or if they arise as a secondary symptom of Chkb deficiency by establishing crosses with known gene mutations affecting mitochondrial fusion. Time point analysis will partly help fill the knowledge gap in disease biology and will also help in formulating better intervention strategies. Overall, through this project, we hope to formulate a potential mechanism for the rescue of disease phenotypes in our preclinical model of MDCMC.

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Mycobacteriophage (phage), are viruses that infect bacteria. All bacteria are infected by phage, and each bacterial species has unique phage that infect them, making phage prime candidates for studying viral diversity and evolution. Some phage integrate into the host genome upon infection (prophage). During excision of phage from host genome, there is potential for regions of phage DNA to be left behind. The purpose of this research is to identify vestigial phage genes within the genome of bacterial host *Mycobacterium smegmatis*. DNA sequences from certain genes in phage Ollie aligned with the host genome. Three regions within *M. smegmatis* JS623 contained genes related to Ollie. In addition there is evidence for two cryptic prophage in *M. smegmatis* MC2 155. Experiments are underway to determine if any vestigial phage genes are expressed. Computational analysis of additional mycobacterial hosts will extend the study of the coevolution of phage and their hosts.
Mycobacteriophage Ollie is a lysogenic virus that infects *Mycobacteria*. Lysogenic phage genomes integrate into their host’s genome and under stressed conditions can shift to lytic growth, while lytic phage only use host enzymes to produce replicates of itself. A putative repressor, gp77, was identified that regulates between lytic and lysogenic growth. Structural analysis suggests the Ollie repressor is inactivated differently than well-studied CI repressor of bacteriophage Lambda. This is supported by the observation that the drug mitomycin C fails to induce Ollie lytic growth. To verify if gp77 has repressor function, the gp77 sequence will be cloned and expressed in *Mycobacterium smegmatis*. If gp77 is a repressor, mycobacterial cells expressing gp77 will be immune to infection by Ollie.
Ollie, a mycobacteriophage, is a virus that infects mycobacterial hosts including the non-pathogenic *Mycobacterium smegmatis* and fish pathogen *M. chelonae*. Ollie can integrate its DNA into the host’s genome (lysogeny). Lysogenic phage can impact the virulence of the host by providing virulence factors; genes that increase the host’s fitness. A putative lipase gene was found in the Ollie genome. Lipases hydrolyze fat for energy, that could increase host virulence. If the lipase increases host fitness, it would need to be expressed during lysogeny. A potential promoter (transcriptional start site) has been identified upstream using a promoter search program. RNA has been isolated from *M.smegmatis* and Ollie lysogens. Primers were designed that will amplify the gene sequence. If the lipase gene is expressed during lysogeny, a PCR product will be amplified from cDNA that was made from bacterial RNA.
Peripheral neuropathy impacts an estimated 1 in 15 adults in the United States, and can be the result of an inherited mutation, exposure to neurotoxic agents, or another primary disease state. All forms cause a specific and progressive degeneration of peripheral axons. Both the wide array of causes and the technical challenge of studying this unique cell compartment contribute to the lack of a cure. Among the many types of peripheral neuropathies, Charcot-Marie-Tooth (CMT) is the most common inherited form. We are specifically investigating CMT type 2D, which is caused by dominant mutations in glycyl tRNA synthetase (GARS). The goal of this project is to determine how mutant GARS causes neuropathy. We are testing the hypothesis that the mutant protein contains an inherent toxicity that results in impaired axonal translation. While local translation in adult axons is not yet established, we have preliminary data showing that ribosomes are available in the axon and are associated with potentially dozens of transcripts. Our lab is now performing an in vivo analysis of translation in motor axons of two mouse models of CMT type 2D using three techniques: (1) non-canonical amino acid tagging, (2) ribo tagging, and (3) thiouracil tagging. Past technology limited this study to whole tissues, while our current approach allows both cell type-specificity and temporal control. Linking defects in axonal translation to a clinically significant neurodegenerative disease would be a major step forward in axon biology, and could provide a common mechanism for several types of peripheral neuropathies.
Arterial stiffness is a mechanism relating lipoprotein levels to executive function and working memory

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Arterial stiffening in the brain is a significant form of vascular brain injury. Pulse wave velocity (PWV; m/s) measures the speed of the forward pressure wave generated by heart systole and is an indirect measure of arterial stiffness. HDL cholesterol is associated with lower PWV and triglycerides to higher PWV. HDL has been related to better cognitive performance and higher triglycerides to lower levels of cognitive performance, specifically Executive Functioning (EF) and Working Memory (WM). In this study we ask the question as to whether arterial stiffness, as measured by PWV, is a mediator of relations between these cholesterols and cognition, i.e. cholesterol→PWV→cognition. To test the mediation hypothesis we analyzed cholesterol, PWV, and cognitive outcome data for 694 adults (mean age=64.8, SD=12.8) from the Maine-Syracuse Longitudinal Study (MSLS). As predicted, PWV was a significant positive mediator of the relationship (p<.05) between HDL and WM (b=.002) and EF (b=.002) and a negative mediator of the relationship (p<.05) between TRIG and WM (b=-.001) and EF (b=-.001). Arterial stiffness may be a mechanism through which lipids can affect cognitive function, though additional mechanisms are likely.
Functional characterization of non-canonical translational GTPases during ribosome stalling

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Ribosome recycling is the terminal step of the cyclic process of mRNA translation and is mediated by two directly interacting eukaryotic release factors, eRF1 and eRF3. In addition to ribosome recycling at the canonical stop codon after effective elongation and termination, it also occurs when ribosomes aberrantly stall during translation elongation due to the absence of stop codons, damaged or structured mRNAs, mRNA cleavage, polypeptides that stably interact with the exit tunnel of the ribosome, limited availability of charged transferRNAs, or nutritional stress. The mRNA surveillance pathways ensure recovery of stalled ribosomes for further rounds of translation to limit their energetically costly replacement.

Little is known about the components and the functional significance of ribosome recycling pathways in higher eukaryotes. However, Pelo, Hbs1l, GTPBP1 and GTPBP2, which share structural similarity with canonical eukaryotic release factors, have been postulated to act as mRNA surveillance modulators in mammals. Indeed, we recently demonstrated that GTPBP2/Pelo recycles stalled ribosomes in the mouse brain. In spite of their structural homology and cellular co-expression, our studies demonstrate that loss of Hbs1l or GTPBP2 result in different phenotypes, suggesting that these proteins may have distinct functions. We propose to utilize loss-of-function alleles of these different murine translational GTPases to determine their involvement in mRNA surveillance pathways and their potential targets during ribosome stalling.
Fibroblast Growth Factor Receptors, FGFR’s, are proteins located on the surface of animal cells. Orthologs of such receptors have been found throughout the Animal Kingdom from worms to humans. Growth factors stimulate these FGFR’s to induce localized physiological responses. Growth factor responses can include cell proliferation, differentiation and cell death. Using five cell line models, three mammalian (human, mouse, dog), an amphibian (frog), a teleost (zebrafish) as well as tissue from a cartilaginous fish (dogfish shark) we isolated FGFR 1, 2, and 3 specific mRNA to study the evolution of the tyrosine kinase domain of expressed FGFR’s. We used reverse transcriptase PCR to make cDNA and prepared the cDNA for sequencing. Analysis of the sequences reveals that the number of FGFR paralogs increased from one to four probably through gene or genome duplications early in vertebrate evolution. Phylogenetic trees of the tyrosine kinase domain reveal orthologous evolution of the ancestral genes over the course of vertebrate history.
Role of Tbx1 in Late Stage Stria Vascularis Development

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Early studies have shown that Tbx1 play important roles for inner ear development during embryonic stage. In this study, we will apply a hypomorphic Tbx1\(^{wdm1}\) mutation to investigate a previously unknown role of TBX1 in regulating late-stage inner ear development. We found that Tbx1\(^{wdm1}\) mice are deaf due to abnormal stria vascularis development. We hypothesize that TBX1 plays an important role during late stage inner ear development by regulating the maturation of non-sensory epithelial cells in the stria vascularis, and the Tbx1\(^{wdm1}\) missense mutation prevents this late stage function. We found Tbx1 mRNA and protein expression in stria vascularis. Immunostaining and in-situ hybridization with markers specific for stria vascularis cell layers indicate absence of marginal cells. We will apply yeast two hybrid assay to identify proteins interact with TBX1 during this stage. This study will lead to discovery of novel roles of TBX1 during late stage stria vascularis development and identify other proteins that are involved in this process.
Regulation of Energy Balance by the Bone Morphogenetic Proteins

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Energy balance is maintained by regulating both energy intake and energy expenditure, through complex processes involving coordination by the central nervous system as well as communication between the brain and peripheral organs and tissues. Recently, we have demonstrated that the bone morphogenetic proteins (BMPs) are capable of beneficially regulating both arms of energy balance, thereby mitigating both obesity and diabetes. Specifically, we showed that BMP7 decreases appetite via a central mTOR-p70S6K pathway and acts through the BMPR1A receptor in anorectic POMC neurons of the hypothalamus. Furthermore, we demonstrated that activation of central BMPR1A increases sympathetic nerve activation of brown adipose tissue, thereby increasing thermogenesis and energy expenditure, without affecting blood pressure or heart rate. Finally, we have shown that BMP signaling is important for the development and activation of brown adipocytes in a cell autonomous manner. Taken together, our data provide evidence for a novel signaling pathway that could be exploited to treat metabolic diseases such as obesity and diabetes.
Antimicrobial agent triclosan is a mitochondrial uncoupler in rat and human mast cells

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Triclosan (TCS) is an antimicrobial that is used widely in hospitals, consumer goods, and personal care products, at concentrations \textasciitilde10 mM. TCS is readily absorbed into human skin. Mast cells, ubiquitous in the body, are key players both in physiological processes and in disease, including eczema, infectious disease, carcinogenesis, and autism. We previously showed that non-cytotoxic, \textmu M levels of TCS inhibit degranulation, the release of histamine and other mediators, from both rat (RBL-2H3) and human (HMC-1.2) mast cells. Here, we show that TCS disrupts ATP production in RBL-2H3 cells cultured in glucose-free, galactose-containing media, with an EC\textsubscript{50} of 7.5-9.6 \textmu M (95\% CI), without causing cytotoxicity. The same experiments were performed with human HMC-1.2 cells, yielding similar results: disrupted ATP production with an EC\textsubscript{50} of 4.2-13.7 \textmu M (95\% CI) and no cytotoxicity. TCS also increased oxygen consumption rate in RBL-2H3 cells at 10 \textmu M. As expected, ATP depletion was not observed in experiments utilizing glucose, wherein cells produce sufficient ATP via glycolysis. Known mitochondrial uncouplers (e.g., CCCP) previously were found to inhibit mast cell function, and we have confirmed those findings. The reduction in ATP with no change in plasma membrane integrity and the increase in oxygen consumption indicates that TCS is a mitochondrial toxin. Using these same glucose-free conditions, 15 \textmu M TCS dampens RBL-2H3 cell degranulation by 40\%. TCS-methyl, which has no ionizable proton but, instead, a methyl group in its place, affects neither degranulation nor ATP production. Thus, triclosan’s effects on mast cell function are due, at least in part, to its ionizable proton. Also, 5 \textmu M TCS inhibits thapsigargin-stimulated degranulation of RBL-2H3 cells: further evidence that TCS disrupts mast cell signaling and Ca\textsuperscript{2+} influx. As a proton ionophore, TCS likely affects numerous cell processes which depend on electrochemical gradients, in diverse cell types.
Identification and characterization of mycobacteriophage Ukulele integration site attP
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Mycobacteriophage (phage) are a diverse group of viruses that infect bacteria in the \textit{Mycobacterium} genus. Two phage life styles are lytic and lysogenic. Temperate phage follow the lysogenic lifecycle and integrate their genome into that of the host at the phage integration site \textit{attP}, forming a lysogen. \textit{attP} has been identified and characterized in some phage but has not yet been identified in Cluster E phage. Ukulele is a temperate cluster E phage isolated at the University of Maine in 2011. Using bioinformatic analysis, I have identified a region in the Ukulele genome that potentially contains \textit{attP}. This region is similar to previously identified \textit{attP} sites including those in the well-studied lambda and L5 phages. This region is located in an intergenic space upstream of the Ukulele integrase gene. To determine if this region contains \textit{attP}, I will clone the sequence into a plasmid and test for integration of the plasmid in \textit{Mycobacterium smegmatis} that expresses the Ukulele integrase.
Native to Europe and Asia, *Arabidopsis thaliana* has been introduced to North America. Northern Maine appears to be just beyond the northeastern edge of its range and climate change could potentially accelerate its expansion. To investigate the genetic basis of adaptation to local climates, a series of common garden experiments was run in Presque Isle, Maine with a set of recombinant inbred lines and ecotypes of *A. thaliana* from Europe and northern Asia, some from the same latitude and some from a similar climate, to compare flowering time responses and seed production. The summer of 2013 identified the vernalization gene *FRIGIDA* and heavy rains as playing important roles in the timing of flowering and survival. On the other hand, the summer of 2014 saw extended dry periods which encouraged predation by flea beetles. Fall-germinated seedling saw vigorous rosette growth, however, with plants alive going into winter. Ecotypes from the same latitude showed differential response than ecotypes from more northern climates.
Sulforaphane Enhances the Anti-Cancer Activity of Paclitaxel against Triple Negative Breast Cancer by Killing Cancer Stem Cells

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Triple negative breast cancer (TNBC) is a breast cancer subtype with high morbidity and mortality rates. Patients after traditional chemotherapeutic treatments are often present with residual disease resulting in relapse. The purpose of the project was to examine the combination of anti-cancer stem cell (CSC) compound, sulforaphane (SFN), with chemotherapeutic drug, paclitaxel (PAC), to increase the anti-cancer efficacy and prevent relapse of TNBC. MTS assay showed that IC50s of the SFN and PAC in TNBC cell lines ranged from 5-10μM, and 1-10nM, respectively. The combination killed more tumor cells compared with using PAC alone. The Aldeflour assay showed that PAC increased the aldehyde dehydrogenase–positive (ALDH+) cell population, and the addition of SFN decreased ALDH+ cells. The mammosphere assay showed that the combination reduced the number and size of mammospheres formed, suggesting diminishment of the self-renewal capacity. These results support the combination enhances the efficacy against TNBC. The underlying molecular mechanisms will be investigated to elucidate the signaling pathways involved.
Maine Biological and Biomedical Sciences Symposium
April 24-25, 2015

MDI Biological Laboratory
Salisbury Cove, ME 04672

Abstracts of Presentations

Abstracts are in alphabetical order, you may open the bookmarks menu in the left navigation column to easily skip to the abstract of interest.
Drosophila model of Frontotemporal Dementia: A view from fly eyes

Frontotemporal dementia (FTD) is associated with a progressive decline in the cognitive functions due to age-dependent loss of neurons in frontal and temporal lobes of the brain. The development of early therapeutic interventions has been hampered by the lack of genetic markers. One genetic marker associated with a hereditary form of FTD is a mutation in CHMP2B - a protein involved in the endosomal-lysosomal pathway that delivers cell surface proteins for recycling and degradation. However, it remains unclear how these mutant proteins lead to neurodegeneration. My lab utilizes fruit fly Drosophila, a well-established model system for studying human neurodegenerative diseases to understand such problems. We use the genetic tools to ectopically express human mutant CHMP2B in Drosophila and analyze the effects at molecular, cellular, and behavioral level. This presentation will provide an update on our efforts to characterize the effects of expression of FTD-associated human mutant protein in Drosophila.

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Identification of novel genes regulating germ granule function in *C. elegans*.

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Germ-granule components in *C. elegans*, are associated with RNA metabolism, and function to maintain the totipotent and immortal properties of the germ cells. The Argonaute protein CSR-1 is a core component of germ-granules and acts to suppress sperm-gene expression in the hermaphrodite germline. To identify additional CSR-1 pathway components we performed an EMS mutagenesis in a PGL-1::GFP strain and analyzed 4000 haploid genomes for large/missshaped germ-granules. From this screen we isolated ten alleles that fall into six complementation groups. We have shown that multiple endogenous germ-granule components are enlarged and overexpressed in all our mutants. Using Hawaiian Variant Mapping combined with CloudMap we found that two alleles, *sam15* and *sam18*, contain mutations in the PAZ domain of *csr-1* itself, validating our approach. We focus on a novel gene mutated in *sam3* and *sam6* that alters germ-granule appearance and expression, but is not required for fertility. Our results contribute to further understanding of epigenetic regulation and germ-granule function in germ cells.
Characterization of lysogeny regulation in the Cluster E mycobacteriophage Ukulele

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Mycobacteriophage (phage) are viruses that infect species of the genus *Mycobacterium*. The temperate Cluster E phage Ukulele was isolated at the University of Maine in 2011. Temperate phage follow the lysogenic lifecycle. After the phage genome is injected into the host cell it integrates into the host genome, forming a lysogen. Though Ukulele is temperate, its genome does not obviously encode repressor or excise genes. The repressor protein is required to maintain the lysogenic state. Under certain conditions, the excise promotes excision of the phage genome and the phage enters the replicative lytic cycle. I identified putative Ukulele excise (gp52) and repressor (gp88) genes. Ukulele gp52 also has a similar predicted fold as a cro-like protein found in several other phage. I deleted gp52 and gp88 independently using the Bacteriophage Recombineering of Electroporated DNA system. Gp88Δ mutants are not viable and therefore I am using complementation to isolate them and to determine whether over or under expression of gp88 has an effect on plaque morphology. Gp52Δ mutants have been detected in plaques containing a mix of wild type and recombinant phage. Gp52Δ mutants must be isolated before performing additional experiments to determine whether gp52 may or may not encode the excise or a cro-like protein.
Characterization of the murine lung transcriptome during pre- and postnatal development in three strains of laboratory mice

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Lung development requires the precise spatiotemporal coordination of a suite of genomic programs to direct the formation of a highly branched airway that maximizes gas-exchange surface area. To capture changes in gene expression correlated with lung development, and compare strain-dependent differences in expression between common strains of laboratory mice, we performed genome wide transcriptional profiling of A/J, C57BL/6J, and C3H/HeJ mice at 26 timepoints ranging between embryonic day 9.5 to postnatal day 56. Using principal components analysis (PCA) we identified 10 factors (principal components) that define the majority of sample variation within the dataset. Mining the genes contributing to these components yielded patterns of gene expression in common across developmental time. Ontological term enrichment analysis using the Visual Annotation Display (VLAD) hosted by the Mouse Genome Informatics (MGI) database was used to identify processes and phenotypes enriched in each principal component. Genes associated with lung development (GO:0030324) included Fgf1, Pdgfa, and Gli1/2/3, as well as a host of genes previously unassociated with lung development.
**GenomePatternScan: Computational identification of genome-wide candidate binding sites for FOXD1**

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We have developed GenomePatternScan (GPS), a computational tool that identifies the locations of a DNA pattern, such as a transcription factor binding site (TFBS), throughout a genome or other long genetic sequence. In this work, we use GPS to identify putative genes regulated by FOXD1, an important transcription factor in kidney development.

The program searches a genome for a TFBS represented using the standard A, C, G, T alphabet or the extended IUPAC notation. Output includes the genetic context of each candidate hit as well as a link into the UCSC Genome Browser to simplify further investigation of the genomic context. Cross-referencing is also provided, e.g., to look for hits in the same gene across different species and the ability to input a listing of genes of interest, such as the results from microarray experiments.

Using GPS, we identified 512 candidate locations of the FOXD1 binding site in the noncoding regions for the same genes in human, rat, and mouse. We further reduced this listing by cross-referencing with literature searches and are confirming the resulting short list of genes at the bench using qRT-PCR and chromatin immunoprecipitation (ChIP).
Despite the importance of insect pollinators, honey bees are being threatened by commercial agricultural practices, such as pesticide treatment and migratory bee keeping. Traditional pharmacological tests, such as LD$_{50}$ which measures death rates, or measures of learning, such as the classic olfactory classical conditioning paradigm (PER), may not be sensitive enough to identify subtle changes in bees as they become vulnerable. The Dobrin lab is working to develop analytical methods for use in a research laboratory to measure subtle changes in the health of honey bees as well as a field test for commercial bee keepers to easily identify weak colonies while an intervention might still be beneficial.
BMP signaling is required for allodynia in *Drosophila*

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In the United States alone over 100 million people suffer from chronic pain and unfortunately, even still, there is a lack in scientific understanding for the mechanisms of abnormal pain sensitivity. The present study utilized a candidate gene approach to identify novel components required for modulation of the tissue damage induced pain sensitization pathway in *Drosophila melanogaster*. We have shown that nociceptor specific RNAi silencing of several members of the Bone Morphogenetic Protein (BMP) signaling pathway significantly attenuated sensitization. Furthermore, overexpression of BMP 2/4 in nociceptor neurons was sufficient to induce sensitization. We then showed that the effects of BMP signaling were largely specific to the sensitization pathway and not to normal nociception or dendritic morphology. Thus, we have shown that the BMP family has a crucial and novel role in sensitization. Because the BMP family is so strongly conserved between vertebrates and invertebrates we are confident that the genes we discover will represent potential therapeutic targets applicable to humans.
Bacteriophages (phages) play important roles in bacterial evolution and virulence. Temperate phages insert their linear genomes into the host cell cytoplasm where it circularizes. The two genomes now in the cell then integrate using phage and bacterial genomic sites, attP and attB respectively. The mycobacteriophage ChipMunk shows an altered phenotype of circularizing but not integrating into the host genome. We believe this is because of a mutation in the integrase gene. The results of several experiments suggested there might be errors in the sequence. It also suggested that at least some of the DNA from the phage particles was circular. Raw sequence data was reassembled and confirmed that the sequence was correct. It also confirmed that there was circular DNA obtained from the phage particles. The development of a program to analyze the sequence structure led to adaptation of the program to rapidly search for potential promoter sequences.
Lowering eukaryotic initiation factor 4G improves proteostasis and helps explain the associated longevity phenotype in *Caenorhabditis elegans*

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Regulation of protein synthesis is known to impact aging and the ability to respond to environmental changes in model organisms. Reducing expression of the eukaryotic initiation factor 4G (eIF4G), a crucial mediator of protein synthesis, elicits an increase in lifespan accompanied with enhancement of factors that promote cellular homeostasis. Some of these factors are known to be essential for lifespan extension. We hypothesized that reducing the expression of eIF4G increases lifespan by maintaining proteostasis. Expression of eIF4G was reduced in *C. elegans* and two proteostasis pathways were tested: 1) heat shock response under heat stress and 2) unfolded protein response (UPR) induced by tunicamycin stress. While lowering eIF4G improved survival under each condition, we found that the UPR was resolved more rapidly under tunicamycin stress than in controls and that factors important for increased longevity were also important for survival under UPR stress conditions. These results suggest that the longevity benefits of eIF4G may be due to enhanced protein homeostasis.
Computational identification and biological validation of FOXJ1 regulatory regions in Strongylocentrotus purpuratus

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In this work, we used computational methods to identify candidate regulatory regions near the FOXJ1 ciliogenesis gene, and then investigated the biological activity of these candidates in the sea urchin Strongylocentrotus purpuratus. We found that 5/9 computationally derived candidate regulatory regions were biologically active at the times measured.

The study of ciliogenesis in S. purpuratus may lead to advances in treatment for cilia-related diseases in humans. The FOXJ1 gene, a member of the forkhead family of transcription factors, functions as a master regulator of ciliary genes. Our goal is to computationally identify candidate CRMs for the FOXJ1 gene and investigate the expression of these elements at 14, 16, and 18 hours post-fertilization, the period when cilia first form. We used computational tools, such as GAMI (Genetic Algorithms for Motif Inference), to identify candidate CRMs in silico. We then investigated the top 12 candidate CRMs in vivo using a DNA-tag reporter system. Of the 12 candidate CRMs, 9/12 were successfully amplified and ligated to reporter constructs. The resulting constructs were then microinjected into S. purpuratus egg cytoplasm, and the expression of these constructs was later measured via qRT-PCR. Of the 9 candidate CRMs accessed, 5 were found to be biologically active in all three time points measured.
JC polyomavirus (JCPyV) is a ubiquitous human pathogen and the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). Viral infection is initiated upon attachment to cellular receptors and entry into host cells; thus, these interactions regulate viral infection and govern critical outcomes in viral disease. Utilizing a glycan array screen, we determined that the viral attachment receptor is α2,6-linked lactoseries tetrasaccharide c (LSTc). Structure-function analysis revealed that specific contacts between the major viral capsid protein VP1 and the α2,6-linked sialic acid of LSTc are required for infection. Further analysis demonstrated that while JCPyV can bind to multiple glycan receptors, JCPyV has the highest affinity for LSTc and retains specificity for this glycan. After binding to LSTc, JCPyV utilizes the 5-hydroxytryptamine (5-HT2) receptors to mediate internalization. These findings highlight that the specificity and affinity of virus-carbohydrate interactions together with the function of viral entry receptors plays an important regulatory role in viral tropism and pathogenesis.
We know that the oceans are packed with marine viruses and they have an important role in the rise and fall of plankton populations but current mathematical models do not accurately account for virus-host interactions when predicting plankton blooms. Therefore I am using model optimization and comparison techniques to evaluate current models and reassess how the virus-host system should be described. Preliminary analysis of in situ blooms has revealed previously unreported trends occurring over the course of an *E. huxleyi* bloom. Inclusion of virus-host interactions in models describing the calcifying coccolithophore *E. huxleyi* may give insight into global carbon cycling, as large-scale blooms are known to draw down significant amounts of atmospheric carbon.
Investigation of Disc1 gene status and stress exposure in female rats on behavior and hippocampal plasticity

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The goal of the present study was to characterize the behavioral and neural effects of a biallelic deletion within the Disc1 (disrupted in schizophrenia 1) gene in female rats as a function of chronic stress exposure or control conditions. Mutations in the Disc1 gene are associated with increased incidence of psychiatric illness, particularly schizophrenia but also depression and bipolar disorder. A common feature of these disorders is the contributing factor of stress. Thus, this study tested the hypothesis that exposure to chronic unpredictable stress would significantly worsen the effects of the gene deletion. To do this, DISC1 knockout and wildtype rats were exposed to either 3 weeks of chronic unpredictable stress or maintained under normal colony conditions. At the end of the stress period, all rats underwent a battery of tests to evaluate emotionality and cognition. Rats were then sacrificed and brains retained for analyses of neural and genomic markers. The preliminary behavioral findings offer some, but incomplete, support for the hypothesis; the neural assays are underway.
Genetic diversity and the 2013 decline of eelgrass (*Zostera marina*) beds of the Frenchman Bay, ME, coastlines

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Abstract  
Eelgrass beds serve a vital marine role invaluable to commercial supplies of seafood in Maine by forming estuaries for young fish and shellfish. Since satellite imaging done in 1996, the coverage of eelgrass in the Frenchman Bay, ME, has shown reduction from 3,000 to 200 acres. In the summer of 2013, major restoration areas, established by ecologists to counter this loss, disappeared. The invasive species of European green crabs, which voraciously target young shellfish and clip through eelgrass, may be contributing to loss. Genetic diversity of eelgrass may explain differences in survival between populations, imparting more resistance to physical disturbance, comparable to destruction caused by crabs. The genetic diversity of six populations across Mount Desert Island was quantified using microsatellite size analysis and correlated with loss susceptibility. The genotype profiles of individuals from each population were determined by size analysis of six microsatellite loci previously described by Reusch *et al.* and F-statistics population genetics were performed. The quantified genetic diversity of the six populations was further correlated with previous research, including abundance and susceptibility to decline, biomass and tensile strength of eelgrass shoots, and prevalence of green crabs in the area.
BMP7-TAK1-JNK-JUN signaling pathway governs the proliferation of nephron progenitor cells

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Balancing self-renewal and differentiation of nephron progenitor cells is essential for formation of a full complement of nephrons during kidney development. The cellular fate of the nephron progenitors is controlled by multiple signaling cascades and complex molecular interactions with the ureteric bud as well as other progenitor cell types in the niche. We have previously shown that BMP7 promotes proliferation of the nephron progenitor cells through the MAPK pathway and differentiation of the progenitors via the SMAD transcription factors. However, the cellular and molecular mechanisms for these distinct effects of BMP7 on the progenitor cell fate are yet to be elucidated.

In this study, we show that BMP7 activates the TAK1-JNK-JUN signaling cascade in the highest order (CITED1+) nephron progenitor cells \textit{in vitro}. We also demonstrate that \textit{in vivo}, BMP7 and TAK1 genetically interact and function in the same pathway to govern the self-renewal of the nephron progenitor cells. We found that conditional deletion of \textit{Tak1} in the nephron progenitor population results in strikingly smaller but morphologically normal kidneys as a result of coordinated reduction in proliferation in the progenitors and the collecting duct. More importantly, we show that TAK1 is required for renewal of the CITED1+ nephron progenitor cells throughout nephrogenesis. Further, we identified c-JUN and c-MYC (Cell cycle regulatory genes) as key targets downstream of TAK1- JNK signaling that may potentially be involved in regulation of progenitor proliferation. \textit{In vivo} and \textit{in vitro} experiments are underway to determine if c-JUN is required for self-renewal of nephron progenitors. Taken together, our findings suggest that BMP7-TAK1-JNK-JUN signaling pathway regulates the proliferation of highest order nephron progenitor cells during kidney development.
Analysis of the Maine Prescription Monitoring Program, 2010-2014

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This study evaluates Maine Prescription Monitoring Program prescribing trends for opioids, sedatives, and stimulants, 2010-2014. Approximately 2.5 million controlled-substance prescriptions were dispensed each year. Of these, 50\% were opioids, 36\% were sedatives, and 15\% were stimulants. Opioid prescribing was highest in 2010 with a decline from 2010-2013 and an increase in 2014. Of the three most commonly prescribed opioids, hydrocodone and oxycodone consistently declined, while buprenorphine prescriptions and recipients of these prescriptions increased. Sedative prescriptions peaked in 2010, followed by a steady decline through 2014. Stimulants were the fastest growing category with a 24\% increase in annual prescriptions from 2010 to 2014. All age groups experienced an increase in the number of stimulant prescriptions, with the highest rates of increase (40\%) among adults 30 years or older. Benzodiazepine prescribing rates were highest for adults 60 years or older with the highest rates among women. For this age group, 20\% received one or more benzodiazepine prescriptions in 2014.
Planar polycyclic hydrocarbons can interfere with genetic fidelity by causing DNA adducts. Two gene families that encode transcription factors, that of the Aryl hydrocarbon receptor (Ahr), and the Nuclear factor erythroid 2-related factor (nrf), regulate genes involved respectively in phase I and II of chemical metabolism. There is evidence for crosstalk between Ahr and Nrf, demonstrated by the reduction of nrf expression upon Ahr knockout in developing zebrafish. This project aims to explore the cis-regulatory mechanism between the two transcription factors using Chromatin Immunoprecipitation (ChIP) in zebrafish embryos. ChIP will determine if there is an interaction between the Ahr-1b protein and the regulatory region of nrf genes.
Rapamycin with metformin treatment ameliorates comorbidities in a type 2 diabetic mouse model, NONcNZO10/LtJ

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Rapamycin delays glucose clearance in B6 mice implying that it would have deleterious effects in those susceptible to type 2 diabetes (T2D). We treated a mouse model of T2D, NONcNZO10/LtJ, with rapamycin, metformin, or both. Although rapamycin exacerbated hyperglycemia, it inhibited weight gain, prevented hyperinsulinemia and hepatic lipidosis, and reduced inflammation and developing nephropathy. Metformin-treatment alone did little to alleviate most diabetic phenotypes, but did reduce hyperinsulinemia. Combination treatment showed phenotypes similar to rapamycin treatment but also reduced rapamycin-driven hyperglycemia. Rapamycin-mediated gene expression changes in liver and fat were consistent with improved health and metabolism. Rapamycin inhibited insulin production, but combination treatment improved this phenotype. Insulin tolerance tests showed that NONcNZO10/LtJ mice are insulin insensitive, however rapamycin increased insulin sensitivity and, in combination with metformin, normalized insulin sensitivity. These results suggest that rapamycin can have multiple beneficial effects even in the context of type 2 diabetes and that combination with metformin can promote insulin sensitivity and ameliorate rapamycin-associated hyperglycemia.
Post-transcriptional processing is increased upon dietary restriction in C. elegans: a possible role for nonsense mediated decay and alternative splicing in lifespan extension

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Dietary restriction (DR) extends lifespan in a myriad of organisms ranging from single celled yeast to complex mammals. By combining analysis of total and polysome-bound mRNA from dietary restricted C. elegans with RNAseq, we differentiated changes in gene expression due to mRNA transcription from those arising due to altered translation. Our results indicate a fundamental shift in post-transcriptional regulation under DR. Among the genes down-regulated post-transcriptionally under DR were components of the ribosome. Several of the ribosomal components, including rpl-12, display significantly more intron retention under DR, which has been previously shown to target them to degradation via nonsense mediated decay after a pioneering round of translation. We hypothesized that the accumulation of unproductive spliced ribosomal components could be due to a decrease in NMD and/or an increase in alternative splicing under DR. Using a reporter construct containing a PTC, we determined that NMD is moderately inhibited due to DR and in silico analysis has implicated the splicing factor rsp-3 in intron retention under DR.
Regulation of Energy Balance by the Bone Morphogenetic Proteins

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Energy balance is maintained by regulating both energy intake and energy expenditure, through complex processes involving coordination by the central nervous system as well as communication between the brain and peripheral organs and tissues. Recently, we have demonstrated that the bone morphogenetic proteins (BMPs) are capable of beneficially regulating both arms of energy balance, thereby mitigating both obesity and diabetes. Specifically, we showed that BMP7 decreases appetite via a central mTOR-p70S6K pathway and acts through the BMPR1A receptor in anorectic POMC neurons of the hypothalamus. Furthermore, we demonstrated that activation of central BMPR1A increases sympathetic nerve activation of brown adipose tissue, thereby increasing thermogenesis and energy expenditure, without affecting blood pressure or heart rate. Finally, we have shown that BMP signaling is important for the development and activation of brown adipocytes in a cell autonomous manner. Taken together, our data provide evidence for a novel signaling pathway that could be exploited to treat metabolic diseases such as obesity and diabetes.
Poster Sessions

Poster Session A

Megan Beauchemin, Ashley Smith and Viravuth P. Yin
MicroRNA-101 controls cardiomyocyte proliferation and scar tissue resolution during heart regeneration

Campbell, AC, Updike, DL
CSR-1 and P granules suppress sperm-specific transcription in the Caenorhabditis elegans germline

Curtis CD, Foster A, Goodney C, Sewell E, Soohey S
Genetic screening of CYP2C19 can determine drug efficacy

Demers, C, Soundararajan, P, Cox, G, Smith, R, Collins, S
Microengineering the Neural Tube

Duncan Bailey and Jane Disney
Anecdata: A Platform for Crowdsourced Public Health Data and Health Research Studies

Marissa Giroux, Diane Nacci, Rebecca Van Beneden, Bryan Clark, Ashley Bertrand, Denise Champlin
The Effects of Arsenic on the Development and Behavior of Fundulus heteroclitus

Taylor Follansbee, Kayla Gjelsvik, Michael Galko, Geoffrey Ganter
The Role of the BMP Signaling Family in Drosophila melanogaster in the Induction of Allodynia

Genetic and environmental interactions play integral roles in the innate immune response to Pseudomonas aeruginosa infection in zebrafish

Eliza Grlickova-Duzevik, James Vaughn, Ling Cao
The role of CGRP in LP-BM5 infection of astrocytes and microglia
Jurczyszak DR, Bowman AR and Kim CH
Characterizing a Role for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene in Altering the Innate Immune Response to Influenza A Virus Infections

Ashley Kelly, Michael Senter-Zapata, Dustin Updike
Suppression of germline programs in the soma of Caenorhabditis elegans

King, BL, Carlisle HR and Yin, VP
MicroRNA Control of Appendage Regeneration

Knowlton, JK and Van Beneden, R
Environmentally relevant transplacental arsenic exposure effects on mouse (Mus musculus) hepatic protein expression

Kong, Y, Charette, J, Naggert, JK, Zhao, LH and Nishina, PM
Identification of Modifier Genes of Retinal Dysplasia in Nr2e3rd7/rd7 Mice

Adam A. Lavertu, Ron F. Peck
Clarifying the protein interactions of lycopene elongase and bacterioopsin in Halobacterium salinarum

Jennifer Liao, Tariq Ahmad
The Effects of Drosophila period Mutations on Ethanol Metabolism

Jacob R. Longfellow
The Role of Serine Protease Inhibitor Kunitz Type 1 a (spint1a) in Innate Immune Response to Influenza Virus Infection in Zebrafish

David Maridas, Victoria DeMambro, Phuong Le, Casey Doucette, Subburaman Mohan, Clifford Rosen
Gender-Specific Effects of Insulin-like Growth Factor Binding Protein 4 in Body Composition and Skeletal Maturation

McCarthy, SS, Oxburgh, L
Smad4 is Required for Normal Kidney Development in Mice

Cambell Miller
The Role of miR-223 During Influenza A Virus Infection

Kathryn H. Morelli, Xianghua Han, & Jeong Yoon
The Novel Role of R-spondin 2 in Myofibril Atrophy

Morin, SJ, Decatur, WA, Breton, TS, Marquis, TJ, Hayes, MK, Berlinsky, DL, and Sower, SA
Identification and Expression of GnRH2 and GnRH3 in the Black Sea Bass
(Centropristis striata), a Hermaphroditic Teleost
Kiera O'Donnell, Charles Tilburg
Modeling Behavior of Hemigrapsus sanguineus in the Delaware Bay

Shivangi Pande, Qing He, Lindsey Gower and Robert E. Friesel
SEF regulates Epithelial-Mesenchymal transition in breast cancer by modulation of β-catenin signaling

Gwendolyn Pelletier, Claire Whalen, Chris Goodchild, Markus Frederich
Effects of Waste Water Treatment Plant Effluent on Stress Tolerance in the European Green Crab, Carcinus maenas

Alex M. Plesa, Ron F. Peck
Knockout of the bap gene increases bacteriorhodopsin production in Halobacterium salinarum

Nicholas W. Pray
Diquat and tert-Butyl hydroperoxide induction of oxidative stress within early embryonic development of zebrafish (Danio rerio)

Erica Sewell, Sally Dixon, Keith Hutchison
Exploring the Coevolution of Mycobacteriophage and their Hosts

Kevin J. Sullivan, Merrill F. Elias, Michael A. Robbins
Arterial stiffness is a mechanism relating lipoprotein levels to executive function and working memory

Markus Terrey, and Susan L. Ackerman
Functional characterization of non-canonical translational GTPases during ribosome stalling
Poster Sessions

Poster session B

Sarah, Alamer and Robert, Gundersen
The regulatory role of the phosphorylated G protein: Implication of lipid microdomains

Lucy D. Algeo, Alexis R. Bowman, Conner R. Lajoie, Kathryn A. Liberman, Spencer E. Traxler
Downregulation of the cystic fibrosis transmembrane conductance regulator negatively impacts the innate immune response to Pseudomonas aeruginosa infection in zebrafish

Browning, CL, Xie, H, Jasin, M, Prakash, R, Kelly, DF and Wise Sr, JP
The role of RAD51 in chemical carcinogenesis: prolonged exposure to particulate chromate inhibits filament formation and induces cytoplasmic accumulation

Carter, EV, Belanger, JJ, Pasquarella, ME, Archambault, LS, Henry CA
The Effects of NAD+ on a Secondary Dystroglycanopathy in Zebrafish

Grant, J., Allgeyer, E., Browne, M., Pongan, A., Mason, M.
Multiplexed SERS imaging in biological systems using biocompatible Raman active nanostars

Mapping the genome of C.Elegans to find where dpy-5 is located

Emily Illingworth, Keith W. Hutchison, Sally D. Molloy
Towards understanding the host range of Ollie, an A3 mycobacteriophage

Ji, X, Chang, B, Krebs, MP, Bernard FitzMaurice, Naggert, JK, and Nishina, PM
Impact of Lyst and Dock7 mutations on retinal pigment epithelial cells

Conner Lajoie
Towards understanding the possible regulatory effects of repeat sequences in the mycobacteriophage Misomonster
Virginia D. McLane, Ling Cao, and Colin L. Willis
The Role of Type 1 Interferons in Morphine-Potentiated LP-BM5 Murine AIDS

Sarah Peterson, Jacqueline Turner, Anne Harrington, Josh Boucher, Lucy Liaw
Notch signaling in vascular remodeling following injury

Sri Ramulu N. Pullagura, Nichelle Gray, Lindsey C. Krening, Bill Buaas and
Robert E. Braun
Functional Analysis of Dicer Cofactors in Mammalian Development and Cancer

Qu, X; Dickinson, ES; Harmon, K; Johnson, AS; Ellers, O; Dickinson, PS
Mechanisms underlying stretch feedback in the heart of American lobster, Homarus americanus

Riitano, A., Feinstein, L.
Assessing the Link between Antibiotic-Resistance Genes in Health Care Facilities and
Environmental Reservoirs

Ireland III, E., Jensen, I., Sewell, J., Yankowsky, N., Johnston, J., Putnam, D., Roe, J.L.
Pre-Restoration Barcoding of Invertebrates in Alder Stream

Ambreen Sayed
Congenital Muscular Dystrophy with Megaconial Myopathy (MDCMC)

Robert Soohey, Keith W. Hutchison, and Sally D. Malloy
Understanding distinct Mycobacteriophage mechanisms for regulating the lytic growth
cycle
Stephen Soohey, Keith W. Hutchison, Sally D. Molloy
Examining Phage Contributions to Bacterial Virulence

Spaulding, Emily and Burgess, Robert
An in vivo Analysis of Axonal Translation in two Mouse Models of Charcot-Marie-Tooth
Disease Type 2D

Theriault, Derek and Hayden, Lauren
Phylogeny of FGF Receptors

Cong Tian, Chantal M. Longo-Guess, Kenneth R Johnson
Role of Tbx1 in Late Stage Stria Vascularis Development

Weatherly, LM, Shim, J, Kennedy, RH, Hashmi, HN, McGillicuddy, SE, Gosse, JA
Antimicrobial agent triclosan is a mitochondrial uncoupler in rat and human mast cells
Emily E. Whitaker, Gwendolyn M. Beacham, Keith Hutchison, Sally Molloy
Identification and characterization of mycobacteriophage Ukulele
integration site $attP$

Yankowsky, N, Botting, C and Roe, JL
Variation in phenology responses to climate in ecotypes and recombinant inbred lines of
Arabidopsis thaliana at the edge of its range in northern Maine

Gi Lim, Yanyan Li, Tao Zhang
Sulforaphane Enhances the Anti-Cancer Activity of Paclitaxel against Triple Negative
Breast Cancer by Killing Cancer Stem Cells