Online Proceedings

Poster Session 1

Commons East, Easel 74

11:00 AM to 1:00 PM

Analyzing Star Formation Histories to Find High Mass X-ray Binary Candidates

Jacob Alexander Gross, Senior, Astronomy, Physics: Comprehensive Physics
Mary Gates Scholar, UW Honors Program
Mentor: Benjamin Williams, Astronomy

High Mass X-ray Binaries (HMXBs) are some of the most physically extreme sources in the universe. They comprise of a compact object such as a black hole or a neutron star, and a high mass companion star. The compact object accretes matter from the companion star and forms an accretion disk of material that has temperatures in excess of ten million degrees Kelvin. This accretion disk then radiates out in the X-ray energy band and allows us to ascertain physical properties for the source. However, the very nature of an HMXB constrains the age of the source to a very high degree–this is because the source needs to be old enough for the compact object to form yet it needs to be young enough for the high mass companion star to still have fusion fuel in its core. In this project, we have created star formation histories at the locations of unknown X-ray emitting objects using optical observations from *Hubble Space Telescope*. These star formation histories determine if there are stars that have an age consistent with HMXBs present. The age of these unknown sources may be associated with these stars, which means they could be HMXBs. We then looked for star formation histories that have constrained ages consistent with an HMXB to try and give more evidence for an unknown object being an HMXB.

Poster Session 1

MGH 241, Easel 161

11:00 AM to 1:00 PM

Characterization of New Probes Designed to Reveal the Neural Circuitry Responsible for Stress-Induced Behaviors

Sanne Marie Casello, Sophomore, Pre-Sciences
Mentor: Charles Chavkin, Pharmacology
Mentor: Benjamin Land, Pharmacology
Mentor: Allisa Song, Pharmacology

Chronic stress induces the release of hormones and neuropeptides including dynorphin, which activates Kappa Opioid Receptors (KOR) to encode the anxiogenic components of the stress response. Previous studies have identified the specific dynorphin-KOR neurochemical signaling responses in the regions of the brain known for regulation of reward systems, and thus may be potential therapeutic targets for stress-related conditions such as depression, anxiety, and substance use disorders. Formally, cells containing dynorphin-KOR have been difficult to study due to technical limitations of the available reagents. However, the generation of new tools including mice expressing promotor-driven cre-recombinase (e.g. KOR-Cre and prodynorphin-Cre) have allowed cell-specific gene expression using Cre-dependent viral expression of fluorophores combined with in-situ staining. In this study, the reliability of a phospho-KOR antibody was tested. The specificity of the immunostaining was verified by administering a KOR agonist, U50,488, in wild-type animals or transgenic animals lacking the G protein-coupled receptor kinase 3 (GRK3), which phosphorylates the KOR. Additionally, wild-type mice with a pre-treatment of norBNI, a KOR antagonist, followed by U50 were also tested. Immunohistochemistry of animal brain tissue showed that the norBNI pre-treated, wild-type animals and GRK3 knock-out animals had lower KORp-IR fluorescence compared to their wild-type counterparts treated with U50,488, verifying that the KORp antibody was reliable. After verification, this KORp antibody was utilized to characterize the dynorphin-KOR circuitry in the mouse brain. To do so, an excitatory opsin was injected into the DR of pDyn-Cre mice with an optic fiber implant. The DR region was then optically stimulated with blue light and dynorphin-releasing projections into the ventral tegmental area were verified by measuring KORp fluorescence. This experiment details one specific dynorphin-KOR pathway in the mouse brain that was not previously studied, and future experiments will utilize this approach to map the extent of dynorphin/KOR localization within the reward circuitry.

SESSION 1E

FROM VIRAL PATHOGENESIS TO GENETIC DISEASES TO BUILDING A BETTER KIDNEY

Session Moderator: Michael Lagunoff, Microbiology
MGH 231
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Using CRISPR-Cas9 Gene Editing to Discover Which Transporters Are Responsible for Glucose Absorption in Kidney Organoids

James David Whiteley, Junior, Biology (Molecular, Cellular & Developmental)
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Ramila Gulieva, Nephrology

Kidneys are responsible for regulating blood glucose levels in the body through the reabsorption of sugars in the proximal tubule of the nephron. Issues with glucose absorption can lead to diabetic nephropathies where too much glucose is excreted. To study kidney glucose absorption, the lab made kidney organoids, which are miniature stem cell-derived in vitro organs that mimic the structure and function of kidneys. We used induced pluripotent stem cells (iPSCs), a type of stem cell that can be generated directly from one's own adult stem cells. In a previous experiment, the lab found glucose absorption was present through the Sodium Glucose Co-Transporter 2 (SGLT2). The lab wanted to see if this was the only channel responsible for glucose absorption. Using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, we knocked out SGLT1 and SGLT2 separately and also knocked out Glucose Transporters (GLUT1 and GLUT2) separately in the WTC11 kidney stem cell line. We selected a guide RNA corresponding with each glucose transporter in the cell membrane of kidney organoids. Then RNA-guided Cas9 created the mutation by breaking the sequence for each glucose transporter in a specific site of the genome. We verified our mutations were done correctly by sending our results to a DNA sequencing company. We expect to find mutant lines will have less glucose absorption than our control and hope to learn which transporters are more active. We are further using this method to create double and quadruple knockout lines with multiple glucose transporters knocked out at once. We hope to learn which transporters are most responsible for glucose absorption, and in the future, can assess when certain transporters are used over others, which will open the doors for disease models for diabetes not only in kidneys but also in other organs.

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12:30 PM to 2:15 PM
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Building a Better Kidney Organoid from Human Pluripotent Stem Cells

Laura Victoria Islas, Junior, Biochemistry
Mary Gates Scholar
Mentor: Benjamin Freedman, Medicine/Nephrology

Treatment options for kidney disease are limited to dialysis and transplantation, which are of limited efficacy and availability. Human pluripotent stem cells (hPSCs) can be used to generate immunocompatible kidney organoids, which contain the major proximal structures of the nephron, including podocytes, proximal tubules, distal tubules, and endothelial cells. They can be made on-demand to study kidney disease and regeneration. Unfortunately, these organoids lack a collecting duct system, a crucial component of the kidney. To address this need, we have developed a protocol to differentiate hPSCs into ureteric bud (UB) cells, which are the precursors of collecting duct cells. First, we identified markers for the collecting duct lineage in developing kidneys. Immunofluorescence analysis of human kidney tissue revealed co-expression of cytokeratin 8 and *Dolichos biflorus* agglutinin (DBA) in the collecting ducts. These markers were not expressed in the proximal tubules. Next, we investigated the ability of hPSCs to express these markers. Undifferentiated hPSCs were treated with a concentration gradient of small molecules known to induce kidney lineage differentiation, including CHIR99021 and glial cell line-derived neurotropic factor (GDNF); their effects were jointly and individually examined. Immunofluorescence was used to characterize the resulting cells, which were initially stained with DBA and *Lotus tetragonolobus* lectin (LTL), a proximal tubule marker. We found that CHIR99021 alone was sufficient to produce DBA positive and LTL negative cells *in vitro*. Notably, the DBA levels in these cells were lower than observed in kidney tissue, indicating that the structures we obtained were not yet fully mature. These studies provide the first evidence that collecting duct cells can be generated from hPSCs. Optimization of this differentiation protocol will yield kidney organoid structures that include the collecting duct system, which will make them ideal for kidney disease modeling and regenerative medicine approaches to reduce the need for kidney transplants.

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Session Moderator: Michael Lagunoff, Microbiology
MGH 231
12:30 PM to 2:15 PM
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Using Induced Pluripotent Stem Cells to Change Urine into Kidney Tissue
Kezia Caroline Philip, Senior, Bioengineering
Mary Gates Scholar
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Nelly Cruz, Medicine
Mentor: Ramila Gulieva, Nephrology

Approximately 14% of the United States population is currently living with chronic kidney disease (CKD), demonstrating the importance of developing effective treatments. Presently, the mechanisms and driving forces of the many diseases that comprise the spectrum of CKD are not well understood. Current methods for acquiring kidney cells for research purposes require an invasive biopsy taken directly from the kidney. We theorized that patient kidney cells can be acquired from a significantly less invasive urine sample, be expanded, and subsequently differentiated into new kidney tissues. We developed a method for accomplishing this, which enabled the collection of cells from the urine samples of over 40 different patients located across the globe with various subtypes of CKD. Reverse transcription polymerase chain reactions (RT-PCRs) probing for the expression of XIST, a RNA gene exclusively expressed in females, were conducted on urinary cells from patients who have had kidney transplants with donors of the opposite gender. Using this assay, we show that these urinary cells originated in the donor kidney. By introducing a set of transcription factors expressed in embryonic stem cells, urinary cells were further reprogrammed to induced pluripotent stem cells (iPSCs), an undifferentiated, stem cell-like state. Subsequently, the iPSCs were differentiated into patient-specific kidney organoids, marking the first time that new kidney-like structures have been generated from a urine sample. Hollow tubules, which better mimic the architecture of the kidney, were created by incorporating these urinary cells or iPSC-derived kidney organoid cells into three-dimensional microfluidic devices wherein normal cell viability and morphology was maintained up to twenty days. Immunohistological stainings indicate similar protein expression between the cell types, which will be further investigated in the upcoming months. These studies improve our ability to regenerate kidney tissue and disease processes from patients' own bodies, starting with a simple urine sample.

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FROM VIRAL PATHOGENESIS TO GENETIC DISEASES TO BUILDING A BETTER KIDNEY

Session Moderator: Michael Lagunoff, Microbiology
MGH 231
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Generation of Human Pluripotent Stem Cells without Cilia Establishes a Novel Model of Polycystic Kidney Disease
Christine Vu Tran, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Nelly Cruz, Medicine

Primary cilia are antenna-like structures on the surface of diverse cell types. Cilia are important for signal transduction and processes such as cell division and development, but their precise role in the cell remains poorly understood. Defects in cilia result in a wide spectrum of genetic disorders called ciliopathies, which commonly involve polycystic kidney disease (PKD), the formation of fluid-filled sacs in the kidney which eventually result in kidney failure. To understand the role of cilia in these diseases, we generated human pluripotent stem cells (hPSCs) with defects in cilia formation for the first time. We used the CRISPR-Cas9 genome editing system to introduce mutations in two different genes that are required for cilia formation, kinesin family member 3A (KIF3A) and kinesin family member 3B (KIF3B). We isolated four independent cells lines with indel mutations in exon 3 of the KIF3A gene and two in exon 2 of KIF3B. The introduced mutations led to frameshifts and protein truncations. Immunoblot analysis confirmed that no full-length protein was produced in these mutant cells. The cells were stained with acetylated alpha-tubulin, a marker of cilia. No cilia were detected in the mutant hPSCs, while an average of 48% cilia were detected in controls of identical genetic background. Surprisingly, the absence of cilia did not alter hPSC pluripotency, self-renewal, amniotic cavity formation, or growth. Further, we differentiated these cell lines into kidney organoids. The cilia deficient kidney organoids formed cysts reminiscent of PKD, while the isogenic controls did not. In conclusion, we have generated human cells and organoids lacking an entire organelle, the primary cilium, and used these to conclusively link cilia to PKD. These cilia deficient hPSC lines can be used to study cilia function, to model various ciliopathies, and to screen for

drugs that can ameliorate the disease phenotypes associated with them.

POSTER SESSION 2

Commons East, Easel 49

1:00 PM to 2:30 PM

Investigating the Cyst-Inducing/-Inhibiting Effects of Drugs on Kidney Organoids Differentiated from PKD Mutant Human Pluripotent Stem Cells

Kosuke Winston, Senior, Bioengineering
Mary Gates Scholar
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Nelly Cruz, Medicine

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder in which fluid-filled cysts form in the kidneys and other organs. Mutations in the PKD1 and PKD2 genes, encoding the polycystin-1 (PC1) and polycystin-2 (PC2) proteins respectively, result in ADPKD. The underlying mechanisms that connect these mutations to ADPKD pathogenesis are poorly understood. Therefore, there is a great need to understand the mechanism of this disease and find an effective treatment. Our laboratory has developed an ADPKD cellular model by differentiating human pluripotent stem cells (hPSC) with mutations in PKD1 or PKD2 into kidney organoids. We are using this model to screen drugs that potentially induce or inhibit cyst formation in a dish. The cyclic AMP signaling pathway is hypothesized to contribute to cystogenesis in PKD. To test this, PKD mutant hPSC lines and isogenic controls were differentiated into kidney organoids and treated with forskolin, a strong cAMP agonist. Images of live organoids in adherent cultures were acquired in intervals and quantified for cyst formation using the cell counter feature of ImageJ. Organoids were subsequently fixed, stained, and imaged with fluorescent microscopy. Forskolin, induced a rapid and dose-dependent swelling in kidney organoids derived from both PKD hPSCs and isogenic controls. After removal of the drug, the tubules returned to its original form. These studies show that chemical modifiers of cystogenesis can be successfully tested using this system. Surprisingly, cyclic AMP does not appear to have effects that are specific to PKD. We are currently testing other drugs and culture conditions for cyst-inducing or –inhibiting effects. Interestingly, PKD kidney organoids form cysts in suspension cultures at significant higher rates than adherent cultures, revealing a role for the microenvironment in cystogenesis. These findings help us gain insight into the disease pathophysiology, which will ultimately guide us to discoveries of better treatments for PKD.

POSTER SESSION 2

Balcony, Easel 88

1:00 PM to 2:30 PM

Using Collateral Sensitivity to Combat Antibiotic Resistance in *Escherichia coli*

Reilly Virginia (Reilly) Falter, Junior, Comparative Religion
Mary Gates Scholar
Mentor: Olivia Kosterlitz, Department of Biology
Mentor: Benjamin Kerr, Biology

Antibiotic resistance is an enormous public health problem and causes death for 23,000 Americans each year due to ineffective treatments. Many strategies to target resistance have emerged, the majority of which target resistance machinery directly. In *Escherichia coli*, the TEM-1 gene encodes for the β-lactamase enzyme which can degrade penicillins, a subclass of β-lactam antibiotics. Alleles of this gene can either improve or weaken the ability of β-lactamase to degrade other subclasses of β-lactam drugs. One strategy that can potentially slow the evolution of bacteria and select against antibiotic resistance is exploiting collateral sensitivity – mutations that confer resistance to one drug and confer sensitivity to another. Utilizing a technology called Deep Mutational Scanning, we are screening every amino acid variant of the TEM-1 gene, and examining growth patterns in twelve different β-lactam antibiotics. This will allow us to quantitatively measure the effect of every single protein variant on the fitness of the organism in hopes of identifying patterns of collateral sensitivity. This is done by first optimizing growth parameters and next sequencing the DNA of each amino acid variant before and after exposure to drug. The large data set produced allows us to uncover common evolutionary trends. This foundational knowledge will potentially allow us to predict drug pairings that exploit collateral sensitivity, leading to more successful treatments.

SESSION 2F

PLANT FORM AND FUNCTION: FROM MOLECULES TO FOSSILS

Session Moderator: Caroline Stromberg, Biology

MGH 242

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Evolution of Flower Symmetry Genes in Genus *Rhododendron*

Ryan William Koning, Senior, Biology (General)
Mentor: Benjamin Hall, Biology
Mentor: Elizabeth Ramage, Biology

Flowering plants exhibit striking diversity in their floral symmetry due to independent genetic changes throughout their evolutionary history. Diversity is also observed within the flowers of genus *Rhododendron*, which displays a variety of radially and bilaterally symmetric flowers across its approximately 1000 species. Floral symmetry is governed by an interaction between one TCP transcription factor, CYCLOIDEA (*CYC*), and two MYB-class genes, RADIALIS (*RAD*) and DIVARICATA (*DIV*). This interaction initiates when *CYC* is expressed in the floral meristem, leading to the activation of *RAD* in the dorsal domain. *RAD* then antagonizes *DIV* activity in the dorsal regions, leading to radial symmetry. In other flowering plants, duplications in *CYC* have been associated with changes in floral symmetry, but the role of *RAD* and *DIV* remain unstudied. A number of transitions to and reversals from radial symmetry have occurred throughout *Rhododendron* from a bilaterally symmetric ancestor. Focusing on *RAD* and *DIV*, the goal of this study is to better understand the evolutionary history of these floral symmetry genes in rhododendrons, and their correlation to floral symmetry changes. I sampled eight different species throughout *Rhododendron* in addition to various outgroups and reconstructed phylogenies of these genes. I obtained sequences from genomic and transcriptomic databases, and used these sequences to design primers for amplifying and sequencing these genes from laboratory samples from wild and cultivated specimens. I found two distinct copies resulting from one duplication, in both *RAD* and *DIV*. In future studies, we will expand our sampling of species to investigate the phylogenetic placement of the origin of these duplications in tandem with changes in flower symmetry, to determine whether duplications in *RAD* or *DIV* are associated with symmetry changes as has been shown for *CYC*.

POSTER SESSION 3

Balcony, Easel 90

2:30 PM to 4:00 PM

Designing a Web Application for the Presentation of Muscle Synergies for Clinicians

Claire Lindsey Mitchell, Senior, Bioengineering
Mentor: Katherine Steele, Mechanical Engineering
Mentor: Benjamin Shuman, Mechanical Engineering

Patients with cerebral palsy tend to have altered muscle activations during walking compared their unimpaired peers. Quantifying these changes is important for clinicians to be able to proscribe treatment protocols. Every brain injury with cerebral palsy is unique, requiring patient specific measures of motor control. Muscle synergies are used to quantify a patient's motor control by using the electrical activity of individual muscles during walking. The resulting electrical activity of the muscles, electromyography, are processed using matrix factorization algorithms. These algorithms identify weighted groupings of muscles which are commonly activated during activities such as walking. However, across clinical institutions differences in data gathering protocols and resources, as well as muscle selection can make the interpretation of synergies challenging. Moreover, calculation of synergies requires programing and data processing expertise not uniformly available to clinicians. The aim of this research was to design a web application that could intuitively represent synergy results for clinicians and patients based upon the data they collect at their institution. This web application uses processing and factorization scripts in Python and integrates the results into JavaScript and HTML for an accessible and flexible display. The application allows the user to select the muscles they want to include in the analysis, as well as how many synergies they want calculated. Each synergy solution is displayed by presenting the weighting of every muscle in each synergy. Additionally, the application shows how accurately the synergies reconstruct the electromyography signals. Generated results from the application can be exported as a report for the patient and clinician to refer to. Because of the differences in data gathering practices across institutions, this research also looked to identify if there are specific ways to quantify how individual muscles impact synergy results so that synergy results can be more translatable across institutions.

POSTER SESSION 3

MGH 241, Easel 162

2:30 PM to 4:00 PM

The Effects of Pond Leveler Devices on Salmon Migration through Restored Riverine Beaver Ponds Complexes

Helena Marie (Lena) Wilson, Senior, Environmental Science & Resource Management (Landscape Ecology & Conservation)
Bridger Machus, Senior, Environmental Science & Resource Management (Landscape Ecology & Conservation)
Mentor: Joshua Lawler, School of Environmental and Forest Sciences
Mentor: Benjamin Dittbrenner

The North American beaver (*Castor canadensis*) is a keystone species and ecosystem engineer, capable of modifying the landscape and creating diverse habitats for other species. Through dam-building, beavers impound streamflow and create ponds and wetland complexes behind dams. The presence of beavers has been shown to increase biodiversity, reduce downstream flooding, and improve water quality. While beavers alter landscapes greatly, often in ways beneficial to native habitats and species, their dam-building can also create conflict with human land-use and infrastructure. One way to reduce human-beaver conflict is to adopt non-lethal approaches such as the use of pond leveler devices. Pond level-

ers, consisting of a pipe through the beaver dam with a cage around the inlet, control the water level of the pond by establishing a desired level while allowing beavers to persist, thereby reducing flooding. Little observational evidence exists on whether pond leveling devices create fish barriers or otherwise impede fish passage for migrating salmon. The objective of this research was to evaluate whether migrating salmon can pass through or around beaver dams fitted with standard pond leveling devices, under high flow conditions. We tested this by observational analysis collected from five pond levelers on Big Spring Creek, King County, Washington during the 2017 autumn salmon migration. Target migrating species include Chinook (*Oncorhynchus tshawtscha*) and Coho salmon (*Oncorhynchus kisutch*). During prolonged precipitation events it was determined that stream stage overtopped dams, providing unhindered passage in our study areas. Additionally, during the 2017 migration period, overdam stream velocity and through-dam velocity (i.e. within the pipe) were passable by salmonids during 100% of the flows we observed.