Science, Technology and Research Scholars
STARS

Annual STARS II Research Symposium

Yale

Rosenfeld Hall
109 Grove St., Room 101
April 23 & 26, 2018

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STARS Program Academic Director, Kenneth Nelson
Graduate Coordinator, Robert W. Fernandez

Molecular Line Observations of First Hydrostatic Core Candidates CB 17 and L1451mm

Stephanie Spear[1], Maria Jose Maureira[2], and Hector Arce[2]

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The First Hydrostatic Core (FHSC) is a theoretical stage between the prestellar and protostellar phases of star formation. Several candidate FHSC have been identified observationally but none have been definitively proven to be true first cores. This is due to a combination of factors such as the short duration of the FHSC phase and low-resolution images, which make it difficult to identify the particular molecular lines associated with the FHSC spectral energy distribution. The observational detection and confirmation of the FHSC is of prime importance for understanding of the evolution of dense cores and star formation. Radio observations of the FHSC candidates can be used to probe the physical properties (temperature, density and kinematics) and chemistry of the surrounding gas. Multi-line observations of four FHSC candidates located in or near the Perseus star-forming region were conducted using the Very Large Array (VLA). I will present preliminary maps of ammonia (1,1) for two of these objects: CB 17 and L1451mm. These maps show the distribution and kinematics of the dense gas surrounding the core. In the future, these results will be discussed in the context of the evolutionary state of these sources.

Hydrophobic CuO Nanosheets Functionalized with Organic Adsorbates

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Cupric oxide (CuO) nanosheets were functionalized with organic adsorbates in hopes to gain insight into the surface chemistry of the nanosheets. A new class of hydrophobic CuO nanosheets is introduced by functionalization of the cupric oxide surface with p-xylene, toluene, hexane, methylcyclohexane, and chlorobenzene. The resulting functionalized nanosheets exhibit contact angles of 146°, 134°, 30°, 29°, and 27°, respectively, due to significant changes in surface composition induced by functionalization, as revealed by XPS and ATR-FTIR spectroscopies and computational modeling. Aromatic adsorbates bind to the surface as alkoxides after being reduced on the surface of the nanosheets and are stable up to 250–350 °C as shown by FTIR spectroscopy.

Exploring Post-Transcriptional Regulation of Human *ccr5*

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C-C Chemokine Receptor 5 (CCR5) serves as the co-receptor for R5-tropic human immunodeficiency virus type 1 (HIV-1) entry into CD4+ T cells and macrophages. The importance of CCR5 in HIV-1 infection is highlighted by Timothy Ray Brown, a patient cured of his HIV-1 after receiving a stem cell transplant deficient in *ccr5*. Because *ccr5* has an uncharacteristically long 3' UTR (~2.2 kb), we questioned whether the gene is regulated at the post-transcriptional level. We developed a primary CD4+ T cell-based assay based on firefly luciferase (FFLUC) reporter to quantify gene activity. In this assay, fragments of the wild type *ccr5* 3' UTR are ligated downstream of FFLUC in CMV-driven pGL-3-basic Luciferase vector. After plasmid transient nucleofection followed by cell lysis, FFLUC activity was quantified by bioluminescence assay and normalized to co-transfected eGFP plasmid, quantified by flow cytometry. We found the strongest inhibitory DNA sequences to be localized at the 5' end of the 2.2 kb 3' UTR and weaker inhibitory DNA at the 3' end, indicating a potential regulatory region within the 3' UTR. In summary, our work serves as a platform for further characterization of *ccr5* post-transcriptional regulation, perhaps by either microRNAs or RNA-binding proteins.

Testing the Calcium Sensing Ability of Parathyroid Hormone 1 Receptor Mutants to Identify Calcium Binding Sites

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Parathyroid hormone 1 receptor (PTH1R) is a family B G-protein coupled receptor (GPCR) involved in maintaining normal Ca^{2+} homeostasis, which is important for bone development and maintenance. PTH1R is activated by parathyroid hormone (PTH), which is a treatment for osteoporosis. Examining the molecular details of PTH1R signaling can improve PTH treatment. Previous studies show that the binding of a truncated parathyroid hormone, PTH(1-34) to purified PTH1R is dependent on high concentrations of calcium, suggesting that calcium binding enhances the affinity of PTH(1-34) for PTH1R. This paper examines three PTH1R mutants, D251A, E252A, and E258A, for ligand binding affinity and activation potency to test if these residues are involved in calcium binding. Single point mutations were created to neutralize the negative charge. Ligand binding affinity and activation potency of each mutant receptor was measured using fluorescent anisotropy and cell-based cyclic AMP assays, respectively. Initial results from ligand binding assays suggest that E258 is involved in calcium binding, while E251 and E252 are not. In comparison to wild-type PTH1R, activation assays indicate that higher concentrations of PTH are needed to activate the mutant receptors. These results imply that residues not involved in calcium binding affect ligand binding through another pathway. Further work will need to be done to discover if additional residues of PTH1R contribute to the observed Ca^{2+} dependent binding. Elucidating this molecular mechanism will provide a better understanding of PTH1R signaling in the bone remodeling process, and more efficacious drugs for osteoporosis.

Differentiating the Effect of Pericytes from Fibroblasts on Microvasculature in Microfluidic Devices

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Vessel biology is essential for understanding the pathomechanisms of vascular diseases and the reconstitution of vessels in tissue engineering. One vessel cell type, pericytes, has largely eluded scientific study and its effects on vessel formation are not well documented. The emergence of the field of microfluidics has allowed for precise control and manipulation of fluids at a microscopic scale, creating an apt method to study vessels and the cells that constitute them. We have incorporated human endothelial colony forming cells, human lung fibroblasts, and human placental pericytes into microfluidic devices to create an *in vitro* model system for microvasculature. The effects of pericytes on vessel formation were determined by noting changes in vessel morphology in this model and compared to the effects of fibroblasts on vessel formation. We have observed that co-cultures of fibroblasts help maintain the microvessels, while co-cultures of pericytes alone cannot. The presence of pericytes in co-cultures of endothelial cells and fibroblasts lead to thinner vessels. Pericytes are also more associated with microvessels than fibroblasts. Microvasculature networks within microfluidic devices are a novel *in vitro* approach to studying basic vasculature biology and microvascular diseases, on that more closely replicates *in vivo* conditions.

Determining the Role of Protein Kinase-2 Signaling in Mediating Amyloid-Beta Oligomer Toxicity

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Alzheimer's Disease (AD) is a chronic, degenerative disease that impacts cognitive functions (such as memory), eventually leading to death. AD is primarily a result of plaques formed from amyloid-beta and tangles from tau. Despite efforts to find therapeutics for AD that target amyloid-beta and tau, a disease modifying therapy does not exist. PrPc as a receptor for amyloid beta oligomers (ABo) and protein kinase-2 (Pyk2) is a protein implicated in the amyloid-beta–mGluR5 pathway, in which Pyk2 is a signaling molecule. The Pyk2 gene (PTK2B) has been determined as a locus for AD. Understanding how Pyk2 signaling mediates ABosupports understanding of AD. Human embryonic kidney (HEK) cells provide a well-characterized cellular system that allows for study of fundamental molecular interactions. HEK cells were transfected with different amounts of recombinant Pyk2 using lipofectamine; however, results were negative when confirming transfection for all cell populations when western blots were used to quantify Pyk2 contents for harvested cells. Troubleshooting for an effective genetic modification method is important to establish in HEK cells to eventually use on neurons, cells that are more relevant to AD, as HEK cells are often used as a first step in accessing intramolecular modifications. APP/PSI are mice that have greater expression of amyloid-beta precursor and are used as AD models in mice. The mice brains were harvested and molecular stress markers along with Pyk2 activity were measured using a western blots. The data show an increase in JNK and eEF2 phosphorylation in APP/PS1 hippocampus and cortex but a non-significant, trending rescue in Pyk2. The larger implication of the experiments lies in filling in the picture of AD molecular characteristics because Pyk2 studies could improve the development of AD therapeutics to help AD patients. Pyk2 might be successful target for therapeutic intervention.

The Role of FANCJ in Homologous Recombination DNA Repair in the Context of Breast and Ovarian Cancer and Fanconi Anemia

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FANCJ is a DNA helicase involved in breast and ovarian cancer, where it directly interacts with the BRCT repeats in the BRCA1 protein. Mutations in the helicase domain of FANCJ interfere with double-stranded break (DSB) repair that is dependent on BRCA1 binding function. FANCJ also interacts with other proteins involved in DNA repair and damage tolerance, which implicates a potential role of FANCJ in influencing DNA synthesis by the DNA polymerase δ complex (Polδ). Here, the role of FANCJ in Polδ-mediated DNA synthesis and repair was investigated within the context of hereditary breast and ovarian cancer, and also of Fanconi anemia, a progressive bone marrow disease that is related to FANCJ function. Currently, molecular cloning of Polδ and two subunit variants, Polδ* (lacking the POLD3 subunit) and POLD3 alone, was achieved. Protein purification of these subunits is ongoing, and these proteins will be subjected to protein co-immunoprecipitation with homologous recombination (HR) proteins, including FANCJ, to identify binding partners that may be important for repair DNA synthesis. In addition, DNA extension activities of Polδ and Polδ* will be examined to determine the effect of POLD3 on repair DNA synthesis. These experiments seek to elucidate the molecular mechanism of FANCJ and POLD3 in DNA synthesis when repairing DNA damage and preventing mutagenesis.

An Analysis of the June 2017 CUORE Calibration Data

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It is currently unknown whether or not the neutrino is a MAJORANA or Dirac particle, that is, whether or not the neutrino is its own antiparticle. Observing neutrinoless double-beta decay, a process only possible if neutrinos are MAJORANA particles, can answer this question. If observed, this process would indicate that Lepton number is not conserved. CUORE's (Cryogenic Underground Observatory for Rare Events) is a bolometer based detector with $Te0_2$ crystal bolometers that is used to search for neutrinoless double-beta decay in ^{130}Te. A key indication for the performance of the detector is an energy resolution of 5 keV at 2615 keV. To ensure that this detector will reach this 5 keV energy resolution, the detector must be calibrated with gamma sources. To calibrate the detector, twelve strings carrying the calibration source ^{232}Th were cooled from 300K to 10mK and installed within and around the bolometer towers. Six strings are distributed around the outside of the towers, and six strings are among the towers. This organization of strings was chosen because the gamma ray radiation from the source strings cannot penetrate more than one or two crystals at low energy. Starting in June of 2017, the collaboration began collecting calibration data and the collaboration expects this data to be similar to simulated calibration data.

Patterns of Arousal-Associated Activity in Cortical Interneurons

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This project evaluates which populations of neurons in the mouse neocortex are activated by behavioral arousal, as induced by brief foot shock. We focus on determining how different classes of GABAergic interneurons are activated by arousal, enabling us to gain insight into mechanisms by which the neocortex is modulated by behavioral context. To achieve this goal, we utilized the iDISCO tissue clearing protocol to visualize the modulation of c-Fos and Calretinin protein in the neocortex. This method provides information about modulation in the neocortex in a more enhanced form than traditional immunohistochemical staining methods. Preliminary results confirm that both c-Fos and calretinin were successfully stained in cleared brains. Further tests will need to be conducted to maximize the results of the Calretinin stain. Following this confirmation of the iDISCO tissue clearing and staining method, experiments can investigate the nature of modulation of cortical interneurons by focusing on the modulation of c-Fos and Calretinin. This study lays the groundwork for further analysis of the spatial distribution and molecular identity of neurons activated by arousal.

Predicting Antipsychotic Treatment Response in Patients with Schizophrenia

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Schizophrenia is a mental illness that affects approximately 2 – 6 million people in the U.S. Although several types of antipsychotic medications treat the symptoms of schizophrenia, patients still experience a rate of 71.7% treatment failure — defined as psychiatric re-hospitalization, discontinuation or switch to a different medication, or death. One possible solution to high treatment failure rates may be machine learning, which has been used in other fields of medicine to make more effective, personalized treatment decisions. The goal of this project is to use machine learning models to predict whether a patient will respond to a specific medication. For our analysis, we used data from five multi-site randomized controlled trials of antipsychotic efficacy (total N = 1511). We include 109 predictor variables collected at baseline in all five trials, including demographics, psychological/behavioral scales (AIMS, BARS, CGI, PANSS, and SARS), vital signs, complete blood count, blood chemistry, and urinalysis. Using elastic net regularization, we predicted 4-week treatment outcomes using the subjects' Positive and Negative Syndrome Scale (PANSS) scores, which were converted into a binary outcome of remission vs. non-remission according to Remission in Schizophrenia Working Group (RSWG) guidelines. Our elastic-net regression model achieved a balanced accuracy of 54.8% (CI: 52.2% – 57.3%). Possible methods of improving predictive balanced accuracy include using a different machine learning algorithm and collecting data on social/environmental factors related to recovery, such as social connection and employment status.

Identifying Novel Protein Partners for OLE RNAs

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Noncoding RNAs (ncRNAs) play important roles in prokaryotic and eukaryotic cellular processes ranging from catalysis and gene regulation. One such large bacterial ncRNA of unknown function, termed OLE (ornate, large, extremophilic), has been found in exclusively in extremophilic, anaerobic bacterial species. Nearly 800 unique OLE RNAs have been identified that are ~600 nucleotides long on average. These RNAs possess no sequence homology to other large noncoding bacterial RNAs, implying that they represent a novel bacterial RNA class. OLE RNAs are upregulated almost 5-fold under short chain alcohol stress, and localize to the cell membrane as part of a ribonucleoprotein (RNP) complex with two proteins of unknown function, OapA and OapB. A mutation of the OapA protein (PM1) has a more deleterious phenotype than that of the *oapA* gene knockout when grown under stress conditions (6% ethanol and at 20˚C), implying that this mutation interferes with additional unknown proteins, possibly involved in the RNP complex. Therefore, an RNA pulldown method, Capture Hybridization Analysis of RNA Targets (CHART), was conducted to determine the identity of additional proteins in the RNP complex to determine the biochemical pathway in which OLE RNAs play a role. After modifying the CHART protocol to improve yield, proteins were successfully isolated on an SDS-PAGE gel. This result implies that the CHART pulldown can be successful in bacterial cells and lends further evidence to the existence of additional proteins in the OLE RNP. The proteins isolated from gel fragments have been submitted to the Keck Proteomics Center at Yale for protein sequence identification by mass spectrometry.

Experiments Probing the Viability of Isoquinuclidines as Potent and Selective Ligands for G–Protein Coupled Receptors

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G–protein coupled receptors (GPCRs) are the largest family of eukaryotic cell surface receptors, making them the most targeted protein class by therapeutic drugs. The emergence of the crystal structures of almost 40 GPCRs has enabled a computational docking–based virtual screening approach for providing novel synthetic targets as potential ligands. This work focuses on the synthesis of densely functionalized isoquinuclidines, via a Rh(I) C–H activation/electrocyclization cascade followed by a Diels–Alder reaction of the resulting dihydropyridine (DHP) and subsequent functionalization of the compound. The ease of synthesis, coupled with the rigid nature of the bridged, bicyclic system makes the scaffold an extremely attractive substrate for the high–throughput docking screen. Although the scope of the Rh(I) C–H activation cascade is quite broad, it suffers from the limited compatibility of basic heterocycles as N-substituents. Therefore, an alternative approach was attempted involving cleavage of the easily accessible *N*-benzyl derivative. Unfortunately, cleavage was unsuccessful, presumably due to steric hindrance of an adjacent methyl group. This hypothesis is currently being examined. Simultaneously, direct functionalization of the nitrogen substituent of the imine prior to synthesis of the DHP using basic pyridines as *N*–substituents is effective, preventing the need for benzyl cleavage and subsequent reductive amination to functionalize the nitrogen in some instances. These investigations provide a two–pronged approach in accessing a large scope of highly functionalized isoquinuclidines as attractive pharmacological leads towards novel and unique therapies and treatments.

Precision Medicine in Endometriosis: Quantifying Progesterone Receptor Status to Predict Progestin Response

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Endometriosis is a gynecological disease impacting 10% of women of reproductive age. It is characterized by the growth of endometrial-like tissue outside of the uterus. The symptoms include chronic pelvic pain and infertility, with treatment options including hormonal therapy and surgical excision. The first-line hormonal treatment is a progestin-based therapy, which acts to suppress the growth of endometriotic lesions. However, not all women respond to this therapy. Thus, a more personalized and non-invasive approach to treating endometriosis is needed. We hypothesize that progesterone receptor status can predict response to progestin-based therapy. Paraffin-embedded endometriotic lesions were collected from women undergoing surgery for endometriosis at Yale-New Haven Hospital. Immunohistochemistry was performed to quantify the presence of progesterone receptor. The primary antibody used was a rabbit polyclonal IgG for detection of the progesterone receptor (PR). The antibody concentration was optimized in normal Day 15 endometrium, which was used as a positive and negative control. The receptor status was quantified using the Histo-score (H-score), which takes into account the percentage of the sample stained at a given intensity, ranging from no staining (0), minimal (1), moderate (2), and intense (3). Two blinded reviewers independently scored the histology specimens. Response to hormonal therapy was defined as not requiring a change from progestin to non-progestin based therapy, or not requiring surgery while on progestin-based therapy. Using this definition and a Receiver Operator Characteristics (ROC) curve, PR status was divided into three groups: low (H-Score: 0-5), medium (H-score: 6-85), and high (H-score: >85). For a cohort of 52 patients with one sample from each, the number of patients in each PR status group were: low (n=18), medium (n=28), high (n=6). All patients with high PR scores responded to progestin-based therapy; most patients in the medium group did not respond to progestin-based therapy (18% response rate); nearly all patients in the low PR group did not respond to progestin based therapy (11% response rate). These results show that progesterone receptor status can be used to predict response to progestin-based therapy, which will help in tailoring hormonal therapy to each individual patient.

BCL-2 Ovarian Killer Promotes Erythropoiesis in a Mouse Model of Myelodysplastic Syndrome

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BCL-2 Ovarian Killer (BOK) is a pro-apoptotic member of the BCL-2 family of proteins that induce apoptosis in response to Endoplasmic Reticulum (ER) stress. Although ER stress activates the unfolded protein response (UPR), in BOK-disrupted cells, downstream effector signaling, including ATF4 and CHOP, is defective. A functional role for BOK as a tumor suppressor is suggested by its genetic location in one of the twenty most frequent, focally deleted chromosomal regions across all human cancers. To evaluate the consequences of BOK loss in the pathogenesis of myelodysplasia (MDS) and Acute Myeloid Leukemia (AML), we used the Nup98-HoxD13 (NHD13) transgenic mouse model of MDS/AML. AML developed in 36.7% of NHD13 mice lacking BOK between the age of 8 and 13 months with a similar overall survival to the NHD13 mice. However, the loss of BOK exacerbated anemia in NHD13 mice, which raised a potential connection between BOK and the regulation of erythropoiesis in cells experiencing stress from the NHD13 translocation. NHD13 mice deficient for BOK exhibited significantly lower hemoglobin (Hgb), lower mean cell hemoglobin concentration (MCHC), and higher mean cell volume (MCV) than NHD13 mice, whereas other lineages were unaffected. Mouse colony forming unit assays revealed that there is a decreased amount of erythroid progenitor stem cells (BFU-E) in the bone marrow of NHD13- transgenic/BOK-deficient mice, which hinted at a diminished ability to produce RBCs in the absence of BOK. Isolation of various stages of erythroid progenitors in the bone marrow revealed that both NHD13 and NHD13-transgenic/BOK-deficient mice have an increase in proerythroblasts. Preliminary RT-QPCR analysis shows decreased expression of UPR components and erythropoietin target genes in the RBC progenitors of both BOK-deficient and NHD13-transgenic/BOK-deficient mice. In addition to its pro-apoptotic function, these results suggest that BOK may regulate erythropoiesis when RBC progenitors experience ER stress.

Analysis of RNA Structural Elements in β-globin mRNA Stabilization

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The element for nuclear expression (ENE) is a *cis*-acting RNA structural element that stabilizes RNAs via formation of a triple helix structure with the poly-A tail. Various forms of ENEs have been identified and their stabilizing effects were studied using an intronless β-globin reporter gene. However, the role that the distance between ENEs and the poly-A tail has on mRNA stabilization has not been investigated. It is not known whether reduced ENEs containing only the U-rich loop predicted to form the triple helix with the poly-A tail are sufficient to confer mRNA stabilization. We aim to investigate the effects of ENE location and ENE structure on mRNA stabilization. The Rhesus Rhadinovirus (RRV) canonical ENE or a putative Anopheles C virus (ACV) reduced ENE was inserted into the 3' UTR of β-globin mRNA at various distances relative to the poly-A tail and tested for its stabilization in human tissue culture cells. Increased accumulation of reporter transcripts was observed for RRV ENE whereas no significant increase was seen for ACV ENE. Our results demonstrate that insertion of RRV ENE closer to the poly-A tail enhances mRNA stabilization and that the structure of the ENE also has significant influence on mRNA accumulation.

Verification of Mitochondrial Genome in Galapagos Tortoises using Sanger Sequencing

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The Galapagos archipelago is home to a variety of unique species and taxa, including the Galapagos giant tortoises. These tortoises are of ecological and historical importance, since they were a source of inspiration for Darwin's theory of evolution. The phylogenetic tree of Galapagos tortoises still contains areas of contention, which was exemplified by the discrepancies between two sets of whole mitochondrial genome sequencing data generated by two different methods. Previous Galapagos tortoise mitochondrial DNA (mtDNA) shotgun-Sanger sequences, which were completed at Joint Genome Institute in California, were compared to a *de novo* assembled genome from one species Galapagos giant tortoises, *Chelonoidis ehpyppium*, endemic from the island of Pinzon. Several discrepancies, in the form of insertions or deletions (indels), were detected between the two set of whole mtDNA genome sequences when sequences alignments were compared. The indels ranged from 1 bp to over 25 bp in length and were located in different regions of the genome. To evaluate which sequences were the correct ones, we designed PCR primers spanning the putative indels and PCR and Sanger sequenced the obtained PCR fragments. We did this for 9 specimens which represent one each of the extant Galapagos giant tortoises and for a total of 11 PCR fragments along the mtDNA molecule. The PCR fragments produced the same DNA sequence as the *de novo* assembly produced by the Illumina method, suggesting that the 2003 Sanger sequences were incorrect. We will use this information to correct the whole genome data produced by the shot-gun approach and analyzed the mtDNA DNA sequence of all Galapagos giant tortoises to produce a mtDNA phylogeny to compare with the nuclear one base on SNP data. Give the different inheritance and recombination patterns of the mtDNa and nuclear genomes as well as difference in substitution rate, the comparisons of the phylogenies produce using two genome types will provide insights in the evolutionary processes that have shape the patterns of genetic diversity in this iconic species group.

Structural Characterization of the E3 Ubiquitin Ligase Doa10 by Cryo-electron Microscopy

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The ubiquitin-proteasome system (UPS) is a cellular machinery that can selectively target misfolded or damaged proteins for degradation. In this system, proteins are degraded by the proteasome after undergoing ubiquitylation, the covalent attachment of ubiquitin to a protein. Endoplasmic reticulum-associated degradation (ERAD), a branch of the UPS, is the process by which proteins are ubiquitylated at the ER and subsequently degraded by the proteasome. The ERAD machinery is highly conserved and enables the turnover of short-lived regulatory proteins and damaged proteins. Defects in ERAD have been linked to numerous diseases, such as diabetes and Parkinson's disease. A greater understanding of the molecular mechanisms governing ERAD is crucial for developing strategies to maintain protein homeostasis in these diseases. We aim to uncover the molecular mechanisms regulating Doa10, a conserved transmembrane E3 ubiquitin (Ub) ligase that senses and ubiquitylates ERAD substrates, which are then degraded. We will focus on determining the cryo-EM structure of the human Doa10 ortholog TEB4 and *Kluyveromyces lactis* Doa10, a naturally split version of Doa10 encoded as two fragments that assemble to form a functional E3 Ub ligase. The TEB4 and *K. lactis* Doa10 constructs have been cloned successfully, and expression of the TEB4 construct was verified in *Saccharomyces cerevisiae*. Preliminary data also suggests the *K. lactis* Doa10 constructs are expressed properly. Determining the structure of TEB4 or *K. lactis* Doa10 will provide insight into how ERAD substrates are recognized and modified and removed from the membrane. Understanding these basic ERAD mechanisms will be useful for developing therapeutics to treat the many diseases affected by ERAD deficiency.

Creation and Characterization of PEG Hydrogels to Observe Neural Stem Cell Chemotaxis

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The subventricular zone (SVZ) is the largest area of the adult brain where neurogenesis, the creation of new neurons, occurs. From the SVZ, activated neural stem cells (NSCs) travel through the rostral migratory stream (RMS) where they differentiate to respond to brain injury. Interactions between NSCs and microvascular endothelial cells (EC), suggest that vascular cells play a role in NSC maintenance. However, there is a lack of evidence regarding the specific roles that ECs play in NSC migration. Therefore, we have developed a porous poly-ethylene glycol (PEG) hydrogel system to investigate vascular cues directing NSC migration. PEG, a biologically inert material, can be rendered adhesive via conjugation of the cell-adhesive proteins found in the brain, including fibronectin (Fn). The leaching of zinc oxide crystals creates a network of tortuous pores throughout hydrogel. Preliminary studies demonstrate that NSCs cluster and migrate throughout the modified hydrogel towards an EC monolayer. NSC clustering and directional migration through the hydrogel are not seen in the absence of ECs, suggesting that soluble factors produced by ECs regulate NSC neurosphere formation and chemotaxis. Future studies will incorporate oxygen-glucose deprivation to understand how vascular cells probe NSC migration during brain injury *in vitro*, in particular post ischemic stroke.

Expression of the Diphtheria Toxin Fragment A to Ablate Postnatal Microglia

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Recent work from several labs has shown that microglia play a critical role in mediating synaptic pruning. However, additional studies are needed to clarify the role that microglia play in other developmental processes such as myelination, astrocyte maturation and neurogenesis, and how these developmental processes program complex behaviors later in life. To answer these questions, we have set out to create and validate a tool for specific, efficient and non-toxic elimination of hippocampal microglia in the postnatal brain of mice using cre-mediated expression of the diphtheria toxin fragment A (DTA). Upon confirming the successful ablation of microglia, we will move onto our second aim: determining the effects of this elimination on behavior. For both aims, we will use a cohort of mice exposed to Brief Daily Separation (BDS). BDS mimics several aspects of Early Life Stress (ELS) in children including increased anxiety and abnormal hippocampal-dependent learning. Exposure to BDS increases the number of microglia in the developing hippocampus raising the question of whether blocking this process can reverse the behavioral abnormalities seen in BDS mice.

The Role of miR-223 in Zebrafish Hematopoietic Stem Cell Differentiation

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Many disorders of the blood, such as leukemia, are difficult to treat and pose a major problem for modern medicine. While hematopoietic stem cells (HSPC) hold the potential to treat these disorders, no method for mass-producing HSPCs is currently available as the regulators of HSPC differentiation have not been completely identified. Recently, microRNA (miR)-223 was identified as an important inhibitor of the HSPC transdifferentiation from hemogenic endothelial cells (ECs) in the zebrafish model organism, a process termed the endothelial-to-hematopoietic transition (EHT). Notably, many putative miR-223 target genes are N-glycogenes, which suggests that regulation of levels of N-glycosylation, a process that has previously been associated with stem cell differentiation states, may be essential for HSPC differentiation from dorsal aorta. In order test the hypothesis that modulation of N-glycosylation in zebrafish results in increased HSPC differentiation, the effects of 3 glycosylation inhibiting drugs, each affecting different steps of the glycosylation pathway, were tested in zebrafish embryos. The number of HSPCs was marked by Tg(Gata2b;UAS-lifeactGFP; Kdrl:mCherry) embryos, as gata2b is an early marker of blood cells and kdrl is marker of the vasculature of the fish. Nascent HSPC counts using fluorescent microscopy show that embryos exposed to drug treatment show similar levels of HSPC formation compared to controls. As a result, we conclude that miR-223 inhibition of N-glycosylation HSPC differentiation observed in zebrafish does not take place during this early step during EHT regulated by gata2b. The next steps will be to determine whether the effects of miR-223 on HSPC proliferation from the hemogenic epithelium are due to effects on N-glycosylation at later points. Ultimately, this work aims to shed light on the previously unidentified molecular and cellular regulators of HSPC differentiation, opening the path for the development of potential applications in regenerative medicine.

Identifying the Binding Region of anti-MuSK Monoclonal Antibodies in Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune disease in which autoantibodies target specific protein on the muscle endplates and disrupt neuromuscular signaling. In a subtype of the disease, specific autoantibodies target muscle specific tyrosine kinase (MuSK), which is a membrane receptor that plays an essential role at the neuromuscular junction. The O'Connor lab isolated short-lived antibody producing cells called plasmablasts that produce anti-MuSK autoantibodies. These anti-MuSK antibodies show evidence of affinity maturation, which is a process that introduces mutations in the heavy and light chain variable regions that improve an antibody's affinity for a particular antigen. However, the effects of these mutations on the autoantibody binding pattern have not been investigated. Our project's aim is to determine whether MuSK is the driving antigen of these mutations. We hypothesized that reverting the mutated sequences in the heavy and light chain variable regions will affect the binding activity of the autoantibodies to MuSK by lowering their affinity to the epitope on the receptor. If reverting these autoantibodies to germline does not affect binding, it might suggest that MuSK binding existed in naïve B-cell antibodies and were not eliminated by proper immune tolerance mechanisms. Conversely, a decrease in binding would indicate that somatic hypermutation stimulated by MuSK antigen is necessary for shaping and increasing the antibody's binding affinity against MuSK. The experiment consisted of reverting the light chain sequence back to the germline sequence via site-directed mutagenesis, expressing the mutated/reverted antibodies in human embryonic kidney (HEK) 293 cells, and testing their activity in a MuSK binding assay. Preliminary results indicate that the light chain of IgG1 MuSK did not affect binding activity, which suggests that components of the heavy chain are likely involved in the binding to the receptor. Reversion of heavy chain regions CDR2 and CDR3 had no significant impact on the binding. Determining the autoantibody binding regions will provide insight on the development of anti-MuSK autoantibodies and work towards predicting a molecular mechanism of their pathogenicity.

Examining the Role of Exosomes on Neuronal Morphology in the Context of TSC Disease

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that causes cortical malformations. Phenotypes of TSC patients include seizures, autism spectrum disorders, developmental delay, and epilepsy. TSC is caused by inactivating mutations in the *tsc1* or *tsc2* genes, which subsequently cause the hyperactivity of the mechanistic target of rapamycin (mTOR), a major converging point in cell signaling. TSC is characterized by cortical tubers, which are composed of a mosaic of wildtype and mutant cells. While tubers can be removed in TSC patients, it is possible that neighboring wildtype cells are affected by surrounding mutant neurons. Whether and how mutant neurons impact the properties of wild type neighboring neurons is unknown. As such, exosomes, or vesicles that transfer molecules for cell-cell communication, are a possible signaling modality contributing to these alterations. We will examine whether exosomes from mutant neurons affect neuronal morphology of surrounding cells. Defects on neuronal properties will be investigated following the addition of exosomes from *tsc* mutant cells to wildtype neurons. This project aims to investigate whether exosomes from mutant *tsc* neurons alter the morphogenesis and synaptic integration of neighboring wildtype neurons. By understanding the effects of exosomes secreted by mutant cells on surrounding wildtype neurons, the mechanism behind TSC malformations and disease could be better understood, allowing for more targeted therapeutic intervention.

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