Posters

A Group of Inner Kinetochore Proteins Regulate the Spindle Assembly Checkpoint

Presenter: Michael Bokros
Biomedical Sciences, Florida State University, Tallahassee, FL, 32301
The spindle assembly checkpoint (SAC) ensures faithful chromosome segregation during mitosis by preventing anaphase onset when the kinetochore (KT) is unattached to the microtubule. The mechanism for activation of the SAC has been well studied yet it remains ambiguous how the SAC is silenced after chromosome bipolar attachment. Here we identify a group of inner kinetochore proteins, named the Constitutive Centromere Associated Network (CCAN) in S. cerevisiae, which function to prevent SAC silencing in the presence of tensionless attachments. In addition to the CCAN, Ybp2 was identified as a KT protein which interacts with CCAN proteins and inhibits CCAN and outer-KT interactions. We find that ybp2? displays delayed SAC silencing and partially suppresses the SAC silencing mutant ipl1-321 indicating that Ybp2 functions to promote SAC silencing. Together our data uncovers a new group of KT proteins involved in regulating SAC silencing.

Myelin Debris Induces Bone-Marrow-Derived Macrophage Migration: Roles in Spinal Cord Injury

Presenter: Dale Bosco PhD
Biomedical Sciences, Florida State Univeristy, Tallahassee, FL, 32306
Using chimeric mouse models, we have previously demonstrated that infiltrating bone-marrow-derived macrophages (M?) migrate in high numbers towards the epicenter of spinal cord injuries (Wang et al. 2015). Consequently, we wanted to determine what factors could be attracting M? to the lesion. Since, almost all of the resident cells are killed during the injury event, we examined if myelin debris, which is produced in large quantities following an SCI, was responsible. Our preliminary data shows that myelin debris is a potent stimulator of M? migration. Since myelin debris is a relatively complex mixture of lipid, proteins, and other factors, we investigated individual components for their ability to induced migratory response. We found that myelin basic protein (MBP) was a robust stimulator of M? migration. This is in contrast to other myelin components such as myelin oligodendrocyte glycoprotein (MOG). Further investigations reveled that MBP may be inducing migration through the activity of G-protein coupled receptors and extracellular signal–regulated kinase (ERK)1/2. Consequently, our examinations have determined that MBP has a central role in myelin-debris-induced migration.

Activation of the centrosome by the Zika virus (ZIKV)

Presenter: Rebecca Buchwalter
Biomedical Sciences, FSU, Tallahassee, FL, 32306
Recent outbreaks of the Zika virus (ZIKV) in the Americas has resulted in a public health crisis due to its links to microcephaly and additional neurological defects such as Guillain–Barré syndrome. Our lab investigates the congenital forms of microcephaly, which are due to mutations in centrosome protein-encoding genes. The centrosome, an organelle in most animal cells, is the major microtubule-organizing center (MTOC). However, the functional link between centrosomes and the development of microcephaly remains unclear. We are investigating how ZIKV infection affects the centrosome's function. During infection, we observe an increase in the centrosome's MTOC activity and a close association between the centrosome and the organization of virus assembly into a pericentrosomal compartment called the 'viroplasm'. We also observe higher levels of the centrosomal protein Ninein at the centrosomes of ZIKV infected cells compared to uninfected cells. We propose that ninein is recruited to the centrosome in response to the Zika virus, and that it is required for centrosomal activation during viral infection. We further propose that ZIKV-mediated centrosome activation is required for ZIKV proliferation.

Proteomic analysis of the CD63 interaction network reveals important functions of CD63 in LMP1-dependent protein trafficking

Presenter: Mujeeb Cheerathodi Ph.D.
Authors: David Meckes Jr.
Biological Sciences, FSU COLLEGE OF MEDICINE, Tallahassee, FL, 32306
CD63 is a common exosome marker belonging to the tetraspanin family of proteins, which are important in extracellular vesicle cargo sorting and protein trafficking within the cell. Indeed, our previous work has demonstrated the importance of CD63 in exosomal targeting and subcellular localization of the Epstein-Barr virus oncoprotein LMP1, and in positively regulating small extracellular vesicle production. However, very little is known about the protein-protein interactions that could be driving these important CD63 functions. Here we sought to utilize the recently developed proximity based -BioID approach and LC-MS/MS to identify CD63 interacting proteins. Mass spectrometry analysis detected more than one thousand potential direct or indirect CD63 interacting partners. Bioinformatics analysis revealed the identified proteins are enriched in protein trafficking, vesicle transport, exosome targeting and cell signaling. Selected known and novel interaction partners were verified by immunoblot analysis. Interestingly, two proteins previously known to be regulated by LMP1, EGFR

and vimentin, were identified as CD63 interacting proteins and efficient trafficking of these proteins to extracellular vesicles required CD63. Overall, this study defines the protein interaction network of CD63 and provides new insights into the functions of CD63 in protein trafficking, vesicle biogenesis, and signal transduction in context of LMP1 expression.

Exosomes as Potential Diagnostic Marker for Neurodegenerative Pathology

Presenter: Kalonji Cole BS
Authors: James Olcese Ph.D, David Meckes Ph.D
Biomedical Sciences, 1994, Talllahassee, FL, 32312

Investigation into how exosomes released from a variety of cells affect physiology and disease progression has proliferated as a field of interest among biomedical researchers within the last decade. Here, we aim to elucidate the connection between neuro-derived exosomes and neurodegenerative pathologies. Since amyloid plaque deposits are a major diagnostic marker for Alzheimer disease, we compared AD 5X mice brains with 2X mice brains at corresponding ages from 2 months to 10 months using immunohistochemistry (IHC) to determine at what age each model would have significant plaque deposition. The goal here was to demonstrate which mice model would be best to use for exosome collection. The 5X mice model had a significantly faster rate of AD pathology than the 2X. Since exosomes can reflect the disease state of cells, we developed a protocol to harvest exosomes from whole brain tissue of wildtype and 5X AD mice. We used common exosome identification markers via western blots to confirm that our collection was of exosomes and not similarly sized cellular vesicles. Our future goal is to compare protein and RNA profiles of exosomes from both 5X and WT mice brains using mass spectroscopy and to identify distinguishable biological molecules of healthy and diseased brains. Successful identification of neuro-derived exosomes opens the potential to identify and isolate exosomes in different fluids of the body, such as blood, urine and CSF.

Phosphorylation of the Transcription Factor Yin Yang 1 by c-Abl Tyrosine Kinase

Presenter: Susan Daraiseh
Biomedical Sciences, FSU, Tallahassee, FL, 32306

Tyrosine phosphorylation controls multiple aspects of cell and organism growth, differentiation and function by modulating cellular signaling pathways, and if deregulated can result in various types of cancer and disease. Yin Yang 1 (YY1) is a multifunctional zinc finger transcription factor that can activate or suppress gene expression depending on both the promoter and co-factors it recruits. In addition to the transcriptional functions of YY1, it has been found to regulate a broad spectrum of biological processes such as development, apoptosis, DNA repair, autophagy, oncogenesis and X-chromosome inactivation. Several of the functions carried out by YY1 have been found to be regulated by post translation modifications. Using biochemical and cellular studies we report that YY1 is phosphorylated by c-Abl kinase. We show that this phosphorylation can be inhibited by Imatinib a Bcr-Abl tyrosine kinase inhibitor, used as a medication to treat chronic myeloid leukemia (CML). Additionally, we have demonstrated that this phosphorylation targets tyrosine residue 254 in the spacer region of YY1. We have preliminary data that suggests this phosphorylation regulates YY1's transcriptional activity. Currently, we are using CRISPR/Cas9 genome editing and mass spectrometry, with non-phosphorylatable (Tyr254Phe) and phosphomimetic (Tyr254Glu) to further understand how the signaling pathway of c-Abl kinase regulates the function of YY1. In conclusion, we demonstrate the novel role of YY1 in the c-Abl proto-oncogene signaling pathway..

A Transcriptomic Analysis of the Female Mouse Brain: Effect of the Estrous Cycle in 4 Brain Regions

Presenter: Lisa DiCarlo
Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, 32306

For many years biomedical and, in particular, neuroscience research, has often focused on male subjects. Female subjects have frequently been excluded due to the perceived complications of the hormonal changes across the estrous cycle and the potential need to include appropriate control groups. To examine changes in gene expression in the female brain, we utilized transcriptomic analysis of the hypothalamus, hippocampus, neocortex, and cerebellum of C57BL/6J (B6) mice using 12 animals, 3 from each of the 4 stages of the estrous cycle. At a false discovery rate (FDR) less than 0.05, we found that there are ~10,000 differentially expressed genes (DEGs) between each of the six possible pairs of brain region comparisons, which is ~50% of the total number of genes detected. Within each of the four brain regions, between 0.5% and 1% of genes are differentially expressed as a result of the estrous cycle, and only 3 genes are differentially expressed in all 4 brain regions. These results demonstrate that despite large differences in gene expression between the four brain regions >99% of the transcriptome is unchanged across the 4 stages of the estrous cycle. We expect that our results will be a useful guide for researchers in the field of neuroscience as females are incorporated in future experiments as well as shedding light on the interactions of hormones and gene expression in different brain regions.

Estrous cycle surpasses sex differences' regulation of the medial prefrontal cortex transcriptome in rats and reveals an important underlying role of early growth response 1 (Egr1).

Presenter: Florian Duclot PhD
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
Males and females differ in cognitive functions and emotional processing, which in part have been associated with baseline sex differences in gene expression in the medial prefrontal cortex. Nevertheless, a growing body of evidence suggests that sex differences in medial prefrontal cortex (mPFC)-dependent cognitive functions are attenuated by hormonal fluctuations within the menstrual cycle. Despite known genomic effects of ovarian hormones, the interaction of the estrous cycle with sex differences in gene expression in the mPFC remains unclear and warrants further investigations. We undertake a large-scale characterization of sex differences and their interaction with the estrous cycle in the adult mPFC transcriptome and report that females with high and low ovarian hormone levels exhibit a partly opposed sexually biased transcriptome. The extent of regulation within females vastly exceeds sex differences, and supports a multi-level reorganization of synaptic function across the estrous cycle. Genome-wide analysis of the transcription factor early growth response 1 (Egr1) binding highlights its role in controlling the synapse-related genes varying within females. In addition to illustrating the importance of accounting for the estrous cycle in females, our data set the ground for a better understanding of the female specificities in cognition and emotional processing.

Physiological consequences of prenatal exposure to valproic acid in prairie voles

Presenter: Lindsay Elvir BS
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304
Previous studies have shown that rats and mice prenatally treated with sodium valproate (valproic acid, VPA) exhibit deficits in social behaviors that resemble some aspects of autism spectrum disorders. Although significant discoveries on the embryopathology of VPA have been proposed, not one study has assessed its effects on social bonding, a complex behavior not exhibited by rats and mice. In this study, we aimed at validating the socially monogamous prairie vole (Microtus ochrogaster) model for the study of the effects of prenatal VPA exposure. Male control and VPA-prenatally exposed subjects were assessed on a battery of behavioral tests to evaluate the VPA-induced social deficits and anxiety-like behavior. VPA-pretreated voles engaged in fewer affiliative behaviors, displayed reduced social interaction with novel conspecifics, and showed enhanced anxiety-like behavior, compared to control animals. While spine density in the medial prefrontal cortex (mPFC) was not affected, spine morphology was significantly altered by VPA treatment. Currently we are examining mRNA expression of genes, in the mPFC, that modulate social bonding in prairie voles, such as the oxytocin and vasopressin receptors, as well as genes largely implicated in neurodevelopmental disorders and involved in synaptic formation and signaling, such as Shank3, Nlgn1, and MeCP2.

TGFB-dependent ciliogenesis

Presenter: John Gonzalez
Biomedical Sciences, FSU, Tallahassee, FL, 32304
We study the role of TGF-beta signaling during the process of ciliogenesis in frog embryos, for the purpose of advancing knowledge in cilia biology to find more effective methods of treating ciliopathies in humans.

Thin Filament-Mediated Modulation of Mouse Cardiac Cross-Bridge Kinetics by Ca2+-Sensitizing Mutation cTnC-A8V or Bepridil

Presenter: David Gonzalez-Martinez BS
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304
Genetic and functional data with the Ala8Val missense mutation in cardiac troponin C (cTnC-A8V) suggest its pathogenicity for development of hypertrophic and restrictive cardiomyopathy (HCM/RCM) in humans and mice. This study aims to further understand the molecular mechanisms that underlie the development of HCM/RCM caused by the cTnC-A8V mutation by measuring the kinetics of tension redevelopment (kTR) and sinusoidal stiffness (SS) at two sarcomere lengths (SL), e.g., 1.9μm or 2.1μm. SL dependent changes in mechanical properties were similar in WT and cTnC-A8V preparations: increased pCa50, with increased stiffness and decreased kTR at any given activation level. Regardless of SL, cTnC-A8V preparations exhibited an increased pCa50, kTR and SS at submaximum Ca2+ levels relative to WT. Maximum SS increased 2x in WT preparations stretched from SL 1.9μm to 2.1μm, while for the cTnC-A8V preparations the SL-dependent increase in maximum SS was blunted. To determine if the difference in cross-bridge kinetics at SL 2.1μm between cTnC-A8V and WT were due to increased Ca2+-sensitivity of cTnC, WT preparations were treated with Bepridil, a Ca2+-sensitizer. As seen with cTnC-A8V, sensitizing the thin filament with Bepridil increased pCa50 and kTR, and decreased maximum SS. These data suggest an important role of the thin filament controlling not only the number of cross-bridges during Ca2+-activation, but may also influence cross-bridge kinetics.

Abnormal Cardiac Cross-Bridge Kinetics in a Troponin T Ile79Asn Transgenic Mouse Model

Presenter: David Gonzalez Martinez BS
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304
The missense mutation Ile79Asn in human cardiac troponin T (HcTnT-I79N) has been associated with phenotypic outcomes

of familial hypertrophic cardiomyopathy, arrhythmias, and sudden cardiac death. Little is known about the changes in cross-bridge kinetics, if any, that result from the mutation. This study investigates isometric force, sinusoidal stiffness and rate of tension redevelopment (kTR) of skinned papillary muscle fiber bundles at two sarcomere lengths (1.9μm and 2.1μm); isolated of non-transgenic wild-type (WT) or transgenic mice bearing the HcTnT-I79N mutation. Sinusoidal stiffness and kTR were obtained after recording steady-state isometric tension by oscillating the fiber bundle 0.2% of its relaxed length or by applying a ramped shortening followed by a quick re-stretch to original fiber bundle length, respectively. The HcTnT-I79N mutation resulted in increased Ca2+-sensitivity of isometric force although no difference in maximum force per cross-sectional area was observed. Length-dependent activation at the longer SL resulted in increased Ca2+-sensitivity of isometric force and sinusoidal stiffness, and decreased kTR for both WT and HcTnT-I79N. However, HcTnT-I79N exhibited faster kTR and greater sinusoidal stiffness at sub-maximum pCas (-log[Ca2+]) compared to WT regardless of SL. These data indicate that HcTnT-I79N mutation, and SL, markedly influence not only the dynamics of individual regulatory units, but also the kinetics of cross-bridge cycling.

Using Adeno-Associated Viruses to create mouse models of psychiatric disorders

Presenter: Kourtney Graham
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
Neurodevelopmental defects play a particularly important role in the pathophysiology of schizophrenia, a complex mental disorder affecting approximately one percent of the US population. Previously, we have reported the successful creation of a new mouse model that addresses the role of the 14-3-3 family of proteins. These transgenic mice express a 14-3-3 peptide inhibitor (YFP-difopein) that antagonizes 14-3-3 bindings to its endogenous partners and is thus considered a 14-3-3 functional knockout (FKO). We have shown that these 14-3-3 FKO mice exhibit a variety of behavioral and morphological deficits reminiscent of the core endophenotypes of established schizophrenia animal models. Recently, we created adeno-associated viruses (AAVs) expressing either the 14-3-3 peptide inhibitor or a siRNA against YFP-difopein. The 14-3-3 peptide inhibitor AAV will be used to induce the schizophrenic-like phenotypes in wildtype mice in a region-specific manner. The AAV expressing the siRNA will be used to knockdown expression of the 14-3-3 peptide inhibitor in 14-3-3 FKO mice in a region-specific manner in order to attenuate the schizophrenic-like phenotypes. Together, these new mouse models will shed some light on the role that 14-3-3 plays in the development of psychiatric disorders.

ntegrating Machine Vision Systems to Study Maize Nested Association Mapping (NAM) Collection Subjected to Abiotic Stress

Presenter: GOKHAN HACISALIHOGLU PHD
Biological Sciences, FLORIDA AGRICULTURAL AND MECHANICAL UNIVERSITY, TALLAHASSEE, FL, 32307
Abiotic stress has long being recognized as a major limiting factor to crop production in many parts of the world and it can affect early spring sowing and growth of maize seedlings. Maize (Zea mays) is one of the most important staple food crops with 354 MMT U.S. production in 87 M acres area. Maize is sensitive to abiotic stress and commonly exposed to it due to its cultivation in diverse environments. Twenty-seven genotypes of maize Nested Association Mapping (NAM) population were evaluated using standard and machine vision systems. All maize kernels were first NIRS phenotyped to measure seed density, weight, oil, protein, starch composition traits. Our results suggest that machine visions are useful indices to screen and potentially improve maize stress tolerance. The current status of this project will be presented including the further research results and analysis.

EPR Analysis of Protein-Lipid Interaction at the HIV Membrane Interface

Presenter: Zahra Hayati Ph.D. Student
Other, Florida State University, TALLAHASSEE, FL, 32306
HIV enters human T cells through the fusion of viral and host-cell membranes. This fusion process is mediated by a surface protein, gp41, and the platform provided by the cholesterol-rich viral membrane. The membrane proximal ectodomain region (MPER) of gp41 plays a critical role in this fusion process and is a major target of anti-gp41 antibodies and vaccine design. Here, EPR and NMR techniques were used to define MPER structure on the membrane, and how neutralizing anti-gp41 antibodies recognize their membrane-immersed epitopes and disrupt a hinge-related function of the MPER. The analyses of several HIV-1 clade B and clade C MPERs revealed a structurally conserved pair of helices immersed in the viral membrane separated by a flexible hinge, which include critical helix capping residues. Double alanine mutations of the capping residues result in an altered hinge structure with a deeper lipid-buried MPER middle region, as well as reduced viral fusion and infectivity. Furthermore, neutralizing anti-gp41 antibodies disrupt the MPER hinge function by perturbing MPER hinge orientation, and/or extracting part of the MPER from the membrane. In addition, MPER-membrane interaction and antibody binding are modulated by lipid composition and cholesterol content. These findings have revealed important features of gp41-antibody interaction at the viral membrane interface.

The Role of Sse1 (Hsp110) in the Clearance of Mutant Huntingtin in Yeast

Presenter: Ryan Higgins
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
Huntington's disease is a neurodegenerative disorder caused by a trinucleotide (CAG) repeat expansion in the Huntingtin gene. Since CAG codes for glutamine (Q), this repeat leads to polyQ expansion in the Huntingtin protein (Htt). PolyQ repeats greater than 40 cause protein misfolding and aggregation, resulting in Huntington's disease. The first line of defense against protein aggregation is chaperone-mediated disaggregation and subsequent degradation via the ubiquitin-proteasome system (UPS). The second line of defense is the sequestration of aggregates into cytoprotective inclusion bodies (IB) that is subsequently cleared through autophagy, but many of the components required for IB formation remain unknown. Here, we show that chaperone protein Sse1, a member of Hsp110, is required for IB formation of Huntington disease-linked polyQ protein Htt103QP. The morphology of the Htt103QP aggregates in sse1 mutant cells differs from that in WT cells, and the number of aggregates is substantially higher. Moreover, sse1 mutant cells show rapid aggregation of Htt103QP. We also found that Sse1 is required for efficient Htt103QP degradation via both proteasomal and autophagic pathways. Although the autophagy machinery appears normal in sse1 mutants, the recognition and engulfment of Htt103QP aggregates is impaired. These findings suggest that chaperone protein Sse1 (Hsp110) is essential for the efficient clearance of aggregation-prone polyQ expanded proteins

A role for the conserved sorting nexin Snx4 in the autophagic clearance of proteasomes and other multisubunit complexes

Presenter: Lauren Howell
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304
Proteasome dysfunction occurs via unknown means during the course of aging and is a major contributing factor in various proteinopathies, such as Alzheimer's and Parkinson's diseases. Restoring normal proteasome function shows great promise for treating these illnesses, but first requires an understanding of how damaged or unneeded proteasomes are typically cleared, and the consequences that ensue if they are not. Toward this goal, we performed a targeted genetic screen in Saccharomyces cerevisiae to identify novel factors essential for the autophagic clearance of the proteasome. We identified the conserved sorting nexin SNX4 to be essential for proteasome autophagy induced by both nitrogen starvation and enzymatic inhibition with the FDA-approved proteasome inhibitor bortezomib. Deletion analysis of known Snx4 binding partners suggests that Snx4 cooperates with Snx41 and the retromer complex to mediate proteasome turnover. Of particular importance, deletion of SNX4 had no effect on autophagy of small, monomeric proteins but severely compromised turnover of two unrelated multisubunit complexes, the ribosome and fatty acid synthase. These complexes share nothing in common with the proteasome, other than their size and complexity. Together, these findings highlight an important role for Snx4 and the retromer complex in autophagy of proteasomes, and unexpectedly suggest that Snx4 dependence may be characteristic of autophagy-mediated turnover of multisubunit complexes.

Epstein-Barr virus LMP1 intracellular signaling and endosomal sorting is mediated by CD63

Presenter: Stephanie Hurwitz
Authors: David Meckes Jr.
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
EBV is a gamma herpesvirus linked to malignancies such as nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin's lymphoma. EBV hijacks the exosome pathway to modulate cell-to-cell signaling by secreting viral components into host cell EVs. Trafficking of oncoprotein LMP1 into exosomes is associated with increased oncogenicity of secreted vesicles. LMP1-modified vesicles in turn enhance the growth and migration of malignant cells, likely contributing to the progression of EBV-associated cancers. Despite the significance of exosomes in cancer, little is understood about the mechanisms orchestrating LMP1 incorporation into vesicles. Here we describe a role of LMP1 in EV production that requires CD63, and provide evidence of CD63-mediated exosomal LMP1 release that is distinct from lipid raft trafficking. Knockout of CD63 severely impaired LMP1 packaging into EVs, concomitant with a disruption in the perinuclear localization of LMP1. Activation of PI3/AKT and canonical NF-?B pathways by LMP1 remained intact following CD63 knockout, while MAPK/ERK, noncanonical NF-?B, and mTOR activation were increased. These data suggest that LMP1 activation of signal transduction pathways occurs at distinct locations within the cell and is tightly linked to exosomal sorting pathways. Findings from this work will have implications in future investigations into general mechanisms of exosome biogenesis, protein trafficking, and signal transduction, especially in viral-associated tumorigenesis.

A Novel DCM-associated Mutation in The N-Helix of Cardiac Troponin C Exhibits Impaired Contractile Kinetics and Reduced Ca2+ Sensitivity In Vitro

Presenter: Jamie Johnston B.S.
Biomedical Sciences, Florida State University , Tallahassee, FL, 32306
Missense mutations in genes encoding the subunits of the cardiac troponin complex are known to result in various forms of cardiomyopathy. Recently, a clinical case of a 1-year- old female who presented in the neonatal period with severe dilated cardiomyopathy (DCM) and hypotonia has been reported. Whole exome sequencing detected a de novo heterozygous variant of uncertain clinical significance in exon 1 of the TNNC1 gene, c.12C>G (I4M). To understand the pathogenicity of this

variant, we investigated the functional effects of this mutation in vitro by measuring Ca2+-sensitivity of isometric force development and kinetics of isometric tension redevelopment (ktr). The [Ca2+] required to reach 50% maximum force (pCa50) at 21ºC for the mutant was 5.44, compared to 5.54 for WT. At 30ºC, pCa50 for TnC-I4M was 5.74, compared to 5.87 for WT, demonstrating a greater decrease in Ca2+-sensitivity near a physiological temperature. At 21ºC, TnC-I4M shows a significant decrease in ktr only at maximal Ca2+-activation compared to WT, while at 30ºC, the TnC-I4M showed a significant decrease in ktr at submaximum and maximum Ca2+ levels. Altogether, these results suggest that Ca2+-sensitivity of force development and cross-bridge kinetics are perturbed as a consequence of this mutation, and likely contribute to the pathogenesis of DCM.

Molecular Analysis of Dopa-Responsive Dystonia in Cultured Dopaminergic Neurons

Presenter: Lataisia Jones
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306

Gene expression is controlled by multiple regulatory elements including single nucleotide polymorphism (SNP)-induced modification or insertion of upstream open reading frames (uORFs). SNP-induced uORFs serve as translational repressors of the canonical ORF (cORF) and are associated with multiple diseases, making the uORFs potential targets for drug discovery. GTP Cyclohydrolase 1 (GCH1) is a rate limiting catalyst of dopamine biosynthesis. Heterozygous mutations in the GCH1 gene are associated with Dopa-Responsive Dystonia (DRD), a neurological movement disorder involving involuntary muscle contraction and painful twisting of the limbs and body. We have discovered a functional –22C>T SNP that introduces an upstream start codon (uAUG) in the 5' region of GCH1 and inhibits gene expression without altering mRNA levels. Translation of the uORF generates a novel 73 a.a. peptide. Although this peptide is targeted for rapid degradation, it leads to reduced GCH1 expression in transfected HEK293 cells. We predict that the -22C>T SNP induced uORF reduces GCH1 levels in DRD patients by impeding translational initiation at the cAUG. In cells transfected with the uORF and normal GCH1, both peptides were expressed but the 73 a.a. peptide localized in the nucleus whereas GCH1 was found in the cytoplasm. The differential subcellular localization suggests that mechanisms in addition to or independent of direct physical interaction with GCH1 may contribute to the function of the uORF peptide.

A Closer Look at the Structure and Function of the N-Helix of Troponin C: A Literature Review

Presenter: Eliani Lorenzo BA
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304

Dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are heart disease that affect 1 in 2500 and 1 in 200 persons, respectively. These maladies can be caused by mutations in sarcomeric proteins, including cardiac troponin C (cTnC), a protein responsible for triggering the muscle contractions of the heart. Several naturally occurring mutations in cTnC have been found to lead to cardiomyopathies, and three of them are found in the N-Helix of TN. Calmodulin (CaM) is a protein very similar to TnC in that they are both EF-hand proteins; and the main difference between the two is that TnC is more functionally focused, whereas CaM is more ubiquitous. Furthermore, TnC bears two striking distinctions from the latter. Foremost it contains an a-helix that encompasses amino acids 2-14; this is known as the N-helix. Secondly, it bears another notable difference, this time in the central helix where it has an additional amino acid cluster (88KGK90). Though the N-helix is not directly involved in calcium binding, it is still functionally important. Studies have suggested that the N-helix is in fact required for full TnC function due to lower calcium binding affinity to N-terminal domain; TnC mutants lacking N-helix have reduced contractile function due to lower affinity calcium binding. In this manuscript, we will review the role of the N-helix of cTnC in muscle regulation and attempt to explain how mutations in this a-helix can lead to cardiomyopathy in humans.

KSHV inhibitor of cGAS (KicGAS) encoded by KSHV ORF52 is controlled by a nuclear export signal (NES) that is significant in KicGAS inhibiting cGAS.

Presenter: Siming Ma MD
Biological Sciences, FSU, Tallahassee, FL, 32304

KSHV Inhibitor of cGAS (KicGAS) is a tegument protein encoded by Gammaherpesvirus-conserved ORF52 gene of Kaposi's Sarcoma Associated Herpes virus (KSHV). Besides its major role as negative mediator of cGAS which is a major cytosolic DNA sensor triggering type-I IFN response during viral infection, KicGAS is also reported to play roles in KSHV viral production. Co-localization of cGAS and KicGAS in cell cytoplasm suggested potential roles of KicGAS subcellular localization in KicGAS inhibiting cGAS. As KicGAS was revealed persistently cytoplasmic, a class 3 leucine-rich nuclear exporting signal (NES) was defined to contribute to KicGAS cytoplasmic localization. So far, KicGAS subcellular localization distinguished in its irresponsiveness to Leptomycine B treatment and there seems other mechanism, say KicGAS dimerization/oligomerization and cytoplasmic component association contribute to KicGAS cytoplasmic localization. Thus, KicGAS subcellular localization is under control of multiple mechanism which plays roles in KSHV viral life cycle.

Prenatal nicotine exposure and GABA neuron development

Presenter: Melissa Martin M.S.
Biomedical Sciences, Florida State University, TALLAHASSEE, FL, 32306
Smoking during pregnancy is a major public health concern because it can have detrimental effects on both the mother and the child. For example, neurobehavioral changes in children and adolescents following prenatal nicotine exposure (PNE) include increased risk for ADHD, conduct disorder, learning disabilities, anxiety, epilepsy, and depression. The GABA neurotransmitter system is known to be altered in many of these developmental disorders. During prenatal development, the majority of the GABA neurons originate in the medial ganglionic eminence of the basal forebrain and migrate tangentially to regions of the dorsal forebrain. We examined the effects of PNE on the development of the GABAergic system during the embryonic period using a GAD67-GFP mouse model. Mice were exposed to plain drinking water or water containing nicotine (100 or 200 µg/ml) beginning 3 weeks prior to conception and throughout pregnancy. We found that PNE produced a significant increase in the number of GABA neurons in the intermediate zone and cortical plate of the dorsal forebrain in 15-day old embryos in both the nicotine groups suggesting that PNE alters the GABA neuron migration in the embryonic brain. Since perturbation of developmental pathways can lead to lasting changes in the mature brain, ongoing studies are examining the effects of PNE on the number and location of GABA neurons in the mature brain as well as changes in cognitive functions such as attention and working memory.

Nicotine-induced epigenetic modifications of the paternal germline DNA and ADHD symptoms in offspring

Presenter: Deirdre McCarthy
Biomedical Sciences, FSU, Tallahassee, FL, 32306
Cigarette smoking and other forms of tobacco use remain a leading cause of disease, disability and death in the United States. Cigarette smoking during pregnancy nearly doubles the risk for ADHD in the offspring. While more men than women smoke cigarettes, little is known about the impact of father's nicotine use upon his offspring. We developed a paternal nicotine exposure mouse model in which adult male mice were exposed to nicotine (200µg/ml) in drinking water for 12 weeks. While the nicotine exposure was ongoing, mice were bred with drug naïve females. To our surprise, the offspring of the nicotine-exposed fathers displayed hyperactivity and inattention, phenotypes commonly associated with ADHD. Interestingly, the nicotine-exposed fathers did not display either of these phenotypes. The offspring also displayed region- and sex-specific alterations in dopamine receptor mRNA expression in the brain. This led us to investigate the mechanisms underlying the expression of ADHD-related behavioral and molecular phenotypes in the offspring. One plausible mechanism is epigenetic modification of the father's germ cell DNA. We found a significant increase in DNA methylation in the nicotine-exposed fathers' spermatozoa. Specifically, DNA methylation was significantly altered in the dopamine D2 receptor promoter region suggesting that epigenetic modification of father's germline is associated with ADHD related behavioral and molecular phenotypes in the offspring

The EBV LMP1 interactome contains ESCRT-dependent and -independent extracellular vesicle sorting proteins

Presenter: David Meckes Ph.D.
Biomedical Sciences, Florida State Univesity , Tallahassee, FL, 32306
The Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is an oncogene required for immortalizing resting B lymphocytes and also plays a key role in the transformation of non-lymphoid tissue. The discovery and characterization of LMP1 protein-protein interactions will likely generate new targets to treat EBV-associated cancers. Unfortunately, classical molecular tools for identifying protein associations are restrictive. In this study, we define the broader LMP1 interactome using the recently developed BioID method. We fused the bacterial biotin ligase (BirA) to LMP1 and harvested biotinylated proteins; the biotin "tag" indicated proteins with vicinal, transient, or stable associations with LMP1. Using mass spectrometry, we identified over 1000 proteins across seven experiments with direct or indirect relationships to LMP1. Pathway analysis suggested that a significant number of the proteins identified are involved in signal transduction and endosome trafficking. Interestingly, a large number of proteins thought to be important in exosome formation and protein targeting were recognized as probable LMP1 interacting partners, including CD63, syntenin-1, ALIX, TSG101, Hrs, CHMPs, and sorting nexins. It is likely that LMP1, which signals from endosomal membranes and is secreted from cells in exosomes, modifies protein trafficking throughout the cell. By way of manipulating the endosomal pathways, LMP1 may exert its oncogenic effects on the surrounding microenvironment.

Proteasomal ATPases Rpt3 and Rpt6 allosterically stabilize the proteasome through a conformation-specific salt bridge

Presenter: Antonia Nemec Ph.D.
Authors: Robert Tomko Jr
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The proteasome is a multisubunit ATP-dependent protease complex responsible for most protein degradation. It consists of lid, base, and core subcomplexes. Recent structural studies have unexpectedly shown that the proteasome oscillates between an inactive apo state, and an activated engaged state that is competent for degradation. The lid-base interface is the major site of remodeling between these conformations, but how remodeling is triggered is unknown. Using structure-guided mutagenesis, we investigated the roles of individual lid-base contacts in proteasome structural integrity. Disrupting the apo state contact between one lid subunit, Rpn5, and the base paradoxically destabilized the interaction between the base and

core. Notably, trapping the base subunit Rpt6 in an ATP-bound state exacerbated base-core instability, whereas trapping the base subunit Rpt3 suppressed it, suggesting that ATP binding by Rpt3 and Rpt6 drives conformational switching of the proteasome. We identified a conserved, apo state-specific salt bridge between Rpn5 and the core, and mutating these residues destabilized the core-base interaction similarly to the Rpn5 apo mutations. Together, these data reveal a stabilizing salt bridge between the lid and core that is controlled by Rpt3 and Rpt6. Formation of conformation-dependent stabilizing salt bridges has also been observed for the multisubunit chaperonin GroEL, and thus may underscore a common theme reinforcing dynamic molecular machines.

Epstein-Barr virus LMP1 extracellular vesicle sorting is mediated by the N-terminus and Transmembrane domains

Presenter: Dingani Nkosi Graduate Student
Biomedical Sciences, Florida State University, TALLAHASSEE, FL, 32304
The Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is released from latently infected tumor cells in small membrane-enclosed vesicles called exosomes. LMP1 has been shown to be a major driver of exosome content and functions. LMP1-modified exosomes have been shown to influence recipient cell growth, migration, and differentiation. Even though the importance of LMP1-modified extracellular vesicles (EVs) on the infected microenvironment is well recognized, very little is known about how LMP1 enters the host exosome pathway. In this study, LMP1 deletion mutants were generated to assess protein regions required for EV trafficking by immunoblot analysis. The results demonstrate that the N-terminus together with specific the transmembrane domains of LMP1 are required for efficient sorting into the EVs. A mutant lacking the N-terminus and transmembrane domains 1 through 4 (TM5-6) that fails to be packaged into EVs exhibited higher co-localization with endoplasmic reticulum and early endosome markers when compared to the wild-type protein. Other mutations within LMP1 resulted in enhanced levels of secretion. TM5-6 maintained the ability to co-localize and form a complex with the tetraspanin CD63, an exosome protein that is important for the incorporation of LMP1 into exosomes. These data suggest new functions of the N-terminus and transmembrane domains in the role of LMP1 intra- and extra-cellular trafficking that are likely downstream of an interaction with CD63.

Elucidating the molecular basis for sexually dimorphic behaviors in Drosophila

Presenter: Colleen Palmateer
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
A major remaining challenge in the field of neuroscience is determining how neural circuits are specified to direct behaviors. One fascinating question is: how is the potential for sex-specific behaviors established from a shared genome and shared neuronal substrate? The Drosophila courtship system provides an ideal model for studying sex-specific behaviors, as there is an existing knowledge of the genes and neurons that underlie both male and female reproductive behaviors. These reproductive behaviors are under the genetic control of the sex hierarchy genes doublesex (dsx) and fruitless (fru) that encode sex-specific transcription factors. Previous investigations have identified the chromatin modifiers Bonus, HDAC1, and HP1 as fru interacting partners, indicating that chromatin landscape may contribute to creating and maintaining the potential for sexually dimorphic behavior. Here, I focus on chromatin modifications in fruitless-expressing neurons in both males and females to gain insight into sex-differences in chromatin modifications that contribute to behavioral differences. We have developed a tool to examine chromatin modifications in a cell-type specific manner. Using these tools, we have examined several chromatin marks at three developmental stages. We find interesting sex and developmental differences that will be presented.

TGF-ß signaling controls formation of IFT complex

Presenter: Diana Perez Bachelor of Science
Biomedical Sciences, Florida State University, Tallahassee, FL, 32306
The cilium is a small organelle that emanates from the cell surface of most eukaryotes during the G0 stage, and is a key coordinator of signaling pathways throughout development. Various signaling pathways and molecules involved in the regulation of cilia formation have been reported, however, mechanisms involved in ciliary length control have remained enigmatic. There are two types of cilia; the primary cilia, which are immotile cilia, involved in sensing extracellular signals, and motile cilia, which produce coordinated, directional fluid flow. We previously reported that TGF-ß signaling pathway impairs structure and/or function of the transition zone and regulates motile cilia length. TGF-ß signaling impacts ciliogenesis of motile cilia in Xenopus gastrocoel roof plate (GRP), the neural tube, and the epidermis. Currently, we are examining how inactivation of the TGF-ß signaling pathway may affect the recruitment of intraflagellar transport (IFT) proteins to the ciliary base. The IFT system helps transport cargo into and out of the cilia, and it is composed of an IFT-A and IFT-B complex which are involved in retrograde and anterograde trafficking along microtubules, respectively. Here we report that TGF-ß signaling pathway is necessary for the recruitment of IFT-A complex peripheral subunits to the ciliary base, which in turn may affect the regulation of ciliary length.

This is SPrTAC: A New Method to Track the Assembly and Subunit Composition of Multisubunit Complexes In Vivo

Presenter: Anna Peterson BS
Authors: Robert Tomko Jr
Biomedical Sciences, Florida State University, Tallahassee, FL, 32308
Large multisubunit complexes conduct the vast majority of essential cellular processes in eukaryotes. These complexes are often constructed of dozens or even hundreds of highly similar but non-equivalent subunits. Further, the exact subunit composition of these complexes is frequently altered to fine-tune their biological activities. Thus, a difficult and pervasive challenge in deciphering the biology of multisubunit machines is distinguishing complexes with non-identical but highly similar compositions from one another in vivo. To address this important challenge, we have developed a new approach, called Split Protein-based Tracking of Assembly or Composition (SPrTAC). This method exploits split protein complementation to produce growth-based, or potentially, fluorescence-based readouts for particular subunit configurations, subcomplexes, or the presence of accessory factors within a given multisubunit complex in vivo. We demonstrate the utility of SPrTAC to detect a rare proteasome isoform containing two a4 subunits and whose production is driven by several known oncogenes in humans. Further, we use this approach to show that disruption of subunit expression stoichiometry compromises normal proteasome biogenesis in vivo. This methodology can be applied to any multisubunit complex for which a basic subunit architecture is known, and can be used to monitor the composition and abundance of such complexes with minimal perturbation to cell function.

Myelin Debris Generated in the Injured Spinal Cord Promotes Survival of Infiltrating Bone Marrow Derived Macrophages

Presenter: Alyssa Rolfe
Authors: Li Sun PhD, Xin Sun PhD, Yijie Chi PhD, Yi Ren PhD
Biomedical Sciences, Florida State University, Tallahassee, FL, 32309
In the United States alone, there are an average of 17,000 new spinal cord injury cases each year. Even with timely medical interventions, the primary injury is often exacerbated by a period of inflammation and pathological vascular changes that result in additional secondary injuries. During both the primary and secondary injuries, substantial quantities of myelin debris is generated from dying glia and neurons. While it is known that myelin debris clearance by professional phagocytes such as macrophages is a prerequisite for neuro-regeneration, it is unclear how to mitigate the deleterious inflammatory effects that accompany an increased macrophage presence in the lesion. Dr. Yi Ren's lab has previously demonstrated that bone marrow derived macrophages (BMDM), but not resident microglia, are the primary phagocytes in the injured spinal cord. Following recruitment from the blood, BMDM are retained in the lesion epicenter for protracted periods of time where they engulf myelin debris and become myelin laden macrophages which contribute to the secondary injury. We have also found that exposure to myelin debris supports long-term BMDM survival, prolonging their potential to induce damage. Thus, the therapeutic manipulation of infiltrating BMDM represents a means to limit secondary injuries and promote recovery.

The role of norovirus VP2 in suppressing maturation of antigen presenting cells

Presenter: Alexa Roth
Other, University of Florida, Gainesville, FL, 32601
The norovirus minor capsid protein VP2 is packaged into the virion interior at low copy number. Our lab has demonstrated that VP2 can globally suppress maturation of infected antigen presenting cells (APCs) in a virus strain-specific manner. The murine norovirus 1 (MNV-1) VP2 prevents upregulation of MHC and costimulatory molecules on macrophages, correlating with impaired induction of protective immunity. Conversely, the MNV-3 VP2 is unable to antagonize APC maturation of macrophages, correlating with more robust protection. Uncovering the mechanism by which VP2 acts will shed light on norovirus immune antagonism strategies. Other functions of VP2 include virion stabilization, predicted genome packaging, and polymerase suppression. Based on these findings by others in the field, we hypothesize that VP2 regulates APC maturation by influencing the viral entry process in a manner that affects host sensing and cytokine induction. Our data show that cytokine induction and virion thermostability are not regulated in a VP2-dependent manner and thus do not explain differential APC maturation but do not exclude the possibility that VP2 otherwise regulates cellular entry. Ongoing studies to dissect the molecular mechanism by which VP2 impairs APC maturation have enabled identification of seven putative key residues. We have engineered reciprocal single amino acid changes between parental MNV-1 and MNV-3 viruses and are currently testing these for activity in preventing APC maturation.

Sex- and dose-dependent abuse liability of repeated low-dose ketamine in rats

Presenter: Kristin Schoepfer B.S.
Authors: Samantha Saland MS, Mohamed Kabbaj PhD
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Subanesthetic ketamine (KET) yields rapid and robust antidepressant effects but is also recreationally abused, raising long-term treatment concerns. Women are likelier than men to seek depression treatment, escalate from casual to compulsive drug use, and are more sensitive to antidepressants, suggesting urgent optimization of sexually-stratified KET therapy protocols. Female rats need less KET than males to achieve rapid antidepressant-like effects (2.5 & 5 mg/kg i.p., respectively). Here, we assessed addictive-like behavioral and biochemical outcomes after six KET injections at three concentrations (2.5, 5, 10 mg/kg, i.p.) in both sexes. Low doses failed to evoke a conditioned place preference and only males positively responded to

10 mg/kg, suggesting sexually-divergent associative reward. Behavioral sensitization to KET's stimulatory effects was established at 5 or 10 mg/kg; females' sensitized response to 5 mg/kg was greater than males'. KET-induced hyperlocomotion positively correlated with deltaFosB protein expression in nucleus accumbens (NAc). Inhibiting NAc deltaFosB-mediated transcription with AAV-deltaJunD did not prevent sensitization to 10 mg/kg KET, suggesting non-canonical neural mechanisms. These data suggest repeated 2.5 mg/kg KET is free from abuse liability, and sensitization to 10 mg/kg KET is not mediated by deltaFosB in NAc. More research is needed to extrapolate these data to inform sex-dependent clinical strategies for long-term KET therapy.

Dual and mono axis robotic crystal imager

Presenter: Thayumanasamy Somasundaram Ph.D
Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, 32317
The poster will describe a simple robotic imager. It features a robot capable of scanning and storing of images of 96-reservoir-3-well or 96-reservoir-2-well ARI Intelliplate crystallization screens using our home built IMB designed Simple Robotic Imager (SRI). We have also developed mono axis version capable of scanning 24 well VDX plates. We will demonstrate both the dual and mono axis versions of the Simple Robotic Imager (SRI).

Selective Membrane Disruption Mechanism of an Antibacterial AApeptide

Presenter: Likai Song Ph.D.
Authors: Pavanjeet Kaur Dr., Yaqiong Li Dr., Jianfeng Cai Dr.
Other, Florida State University, Tallahassee, FL, 32310
?-AApeptides are a new class of antibacterial peptidomimetics that are not prone to antibiotic resistance and are highly resistant to protease degradation. How ?-AApeptides interact with bacterial membranes and alter lipid assembly and properties are unclear, but such information is essential in order to understand their antimicrobial activities. Using electron paramagnetic resonance (EPR) techniques, we characterized the membrane interaction and destabilizing activities of a lipo-cyclic-?-AApeptide. The analyses revealed that the ?-AApeptide binding increases the membrane permeability of POPC/POPG liposomes, which mimics negatively-charged bacterial membranes. Moreover, the ?-AApeptide interacts strongly with POPC/POPG liposomes, thereby inhibiting membrane fluidity and reducing solvent accessibility around the lipid head group region. Furthermore, binding of the ?-AApeptide induces significant lipid-lateral-ordering and membrane thinning. In contrast, minimal membrane property changes were observed upon the ?-AApeptide binding for liposomes mimicking mammalian cell membranes, consisting of neutral lipids and cholesterol. Our findings suggest that the ?-AApeptide interact and disrupt bacterial membranes through a "carpet-like" mechanism. The results illustrated that the intrinsic features of ?-AApeptides are important for their ability to selectively disrupt bacterial membranes, the implications of which extend to developing new antibacterial biomaterials.

Abuse potential of low-dose intermittent ketamine exposure in male and female rats

Presenter: Caroline Strong BS
Biomedical Sciences, Florida State University, Tallahassee , FL, 32308
Clinical evidence suggests superior antidepressant response over time with a repeated, intermittent ketamine treatment regimen as compared to a single infusion. However, ketamine is commonly abused. Therefore, the abuse potential of repeated ketamine injections at low doses needs to be investigated. In this study, we investigated the abuse potential of repeated exposure to either 0, 2.5, or 5 mg/kg ketamine. Locomotor activity and conditioned place preference were assayed to evaluate measures of behavioral sensitization to ketamine and its rewarding properties, respectively. Our results show that while neither males nor females developed CPP, males treated with 5 mg/kg and females treated with either 2.5 or 5 mg/kg ketamine behaviorally sensitized. Furthermore, dendritic spines were increased in the NAc of both males and females administered 5 mg/kg ketamine. Additionally, males administered 5 mg/kg ketamine displayed increased protein expression of ?fosB, CaMKIIa, and BDNF, an effect not observed in females administered either dose of ketamine. However, males and females administered 5 mg/kg ketamine displayed increased protein expression of GluA1. Taken together, low-dose ketamine, when administered intermittently, induces behavioral sensitization at a lower dose in females than males, accompanied by an increase in spine density in the NAc and protein expression changes in pathways commonly implicated in addiction.

A single aromatic core mutation converts a designed "primitive" protein from halophile to mesophile folding

Presenter: Connie Tenorio
Biomedical Sciences, Florida State University, Tallahassee, FL, 32304
Experiments in prebiotic protein design suggest that the origin of folded proteins may have favored halophile conditions. These results are consistent with salt induced peptide formation which shows that polymerization of amino acids is also promoted by high salt concentrations. As a result of various origin of life studies, a consensus on which amino acids likely populated early earth has emerged. These residues were synthesized by abiotic chemical and physical processes from

molecules present in the surrounding environment. The properties of the consensus set of common prebiotic amino acids (A,D,E,G,I,L,P,S,T,V) are compatible with known features of halophile proteins, meaning these proteins are only stable in the presence of high salt concentrations. The halophile environment, thus, has a number of compelling aspects with regard to the origin of structured polypeptides. Consequently, a proposed key step in evolution was, movement out of the halophile regime into a mesophile one commensurate with biosynthesis of “phase 2” amino acids – including the aromatic and basic amino acids. We tested the effects of aromatic residue addition to the core of a “primitive” designed protein enriched for the prebiotic amino acids (A, D, E, G, I, L, P, S, T, V) that required halophilic conditions for folding. The subsequent results show that the inclusion of just a single aromatic residue was sufficient for movement to a mesophile folding environment. Thus, the inclusion of aromatic residues into the codon table could have conferred key stability to early proteins enabling adaptive radiation outside of a halophile environment.

Dissecting Molecular Determinants of Proteasome Activation with Conformation-Specific Reporters

Presenter: Robert Tomko Jr. Ph.D.
Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, 32306
The proteasome is a multisubunit protease that conducts most regulated protein degradation in eukaryotes. Numerous human diseases are characterized by deregulated proteasome activity, and modulating proteasome activity to balance the proteolytic load is a proven therapeutic strategy. Recent structural analyses of the proteasome unexpectedly revealed that it undergoes a largescale conformational rearrangement from an autoinhibited “apo” state to an activated, “engaged” state. This transition aligns the catalytic sites and substrate channels to facilitate substrate degradation. Harnessing this conformational switching thus holds the potential to activate or inactivate the proteasome at will, but the signal(s) and mechanisms mediating this rearrangement is triggered remains unknown.
Structural approaches are effort-intensive and lack the throughput required for detailed mechanistic analyses. To fill this methodological gap, we developed rapid and simple conformation-specific reporters founded on engineered disulfide crosslinking. We use these reporters to demonstrate that nucleotide binding, but not engagement of the peptidase active sites, influences the proteasome’s conformation. Together, these results yield an experimental platform for rapid mechanistic analysis of proteasome activation and catalysis, validate recent high-resolution proteasome structures, and implicate nucleotide binding and hydrolysis as key regulators of proteasome conformational dynamics.

The impact of DNA topology on target selection by a cytosine-specific Cas9

Presenter: Tsz Kin Martin Tsui
Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, 32306
Cas9 is an RNA-guided DNA cleavage enzyme being actively developed for genome editing and gene regulation. To be cleaved by Cas9, a double stranded DNA, or the protospacer, must be complementary to the Cas9-bound guide RNA and adjacent to a short Cas9-specific element called Protospacer Adjacent Motif (PAM). Understanding the correct juxtaposition in time and space of the protospacer- and PAM-interaction with Cas9 will enable development of versatile and safe Cas9-based technology. We report identification and biochemical characterization of Cas9 from Acidothermus cellulolyticus (AceCas9). AceCas9 depends strictly on a 5’-NNNCC-3’ PAM and is more efficient in cleaving negative supercoils than relaxed DNA. We further showed that mismatches to the guide RNA on a supercoiled protospacer are tolerated by AceCas9, whereas the same mismatches on its relaxed form were not. The cytosine-specific and DNA topology-sensitive properties of the AceCas9 maybe explored for chromosome domain specific genome editing applications.

Reinforcing properties of an intermittent, low-dose of ketamine in male and female rats: effects of estrus cycle

Presenter: Katherine Wright
Authors: Naomi Brownstein PhD, Mohamed Kabbaj PhD
Biological Sciences, Florida State University, Tallahassee, FL, 32304
Repeated intermittent ketamine has rapid and long-lasting antidepressant effects, but its abuse potential has only been assessed at high doses. Furthermore, while females are more sensitive to ketamine’s antidepressant-like effects, its abuse potential in females is unknown. Therefore, we investigated the reinforcing properties of low-dose intermittent ketamine in both sexes and whether cycling gonadal hormones influence females’ response to ketamine. We also determined whether reinstatement to intermittent ketamine is comparable to intermittent cocaine in males. Male rats intravenously self-administered cocaine (0.75 mg/kg/infusion) or ketamine (0.05 and 0.1 mg/kg/infusion) once every fourth day, while intact cycling females self-administered ketamine during stages of their four-day estrus cycle when gonadal hormones are either high (proestrus) or low (diestrus). After self-administration, rats underwent extinction training followed by cue-primed and drug-primed reinstatement to assess drug-seeking behavior. Males and proestrus females reinstated to ketamine-paired cues, but diestrus females did not. Male rats reinstated to cocaine priming independent of cue presentation. Additionally, progressive ratio testing indicated that diestrus-trained females were more motivated at lower doses of ketamine than males or proestrus females. Therefore, both females and males respond to ketamine’s reinforcing effects under this treatment paradigm. Females’ response was cycle-dependent.

Ceramide- and CD63-dependent trafficking of Epstein-Barr virus LMP1 to extracellular vesicles

Presenter: Sara York
Authors: David G. Meckes Jr.
Biomedical Sciences, FSU, Tallahassee, FL, 32306

Epstein-Barr virus (EBV) is a human herpesvirus that is associated with a multitude of epithelial and lymphoid cancers. Latent membrane protein 1 (LMP1) encoded by EBV is expressed in most EBV-associated cancers and is believed to be the major viral oncogene. LMP1 is secreted from infected cancer cells in small membrane-enclosed extracellular vesicles (EVs). Despite the potential significance of extrace