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

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Experiments in prebiotic protein design suggest that the origin of folded proteins may have favored halophilic 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.

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

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Epstein-Barr virus (EBV) is a human herpesvirus that is associated with a multitude of cancers in immunocompromised or genetically susceptible individuals including lymphomas, gastric carcinoma, and nasopharyngeal carcinoma. Latent membrane protein 1 (LMP1) of EBV is expressed in most EBV-associated cancers and is thought to be the major viral oncogene as it is required for B-cell immortalization and can transform cells in vitro. LMP1 has been found to be secreted from infected cancer cells in small membrane enclosed extracellular vesicles. Extracellular vesicles (EVs) represent a new means of cell to cell communication through the transfer of biologically active lipids, proteins, and RNAs. LMP1 containing EVs have been shown to inhibit immune cell function and enhance cell growth and migration. Therefore, LMP1-modified EVs may represent an important mechanism through which EBV manipulates the tumor microenvironment to enhance tumor growth and progression. Despite the potential significance of exosomal LMP1, very little is known about how this viral protein traffics to EVs. Recently, the tetraspanin protein CD63, has been found to form a complex with LMP1 and knock-down of CD63 resulted in a
reduction of exosomal LMP1. In certain cell lines, CD63 is trafficked to EVs through a ceramide-dependent mechanism. Therefore, we hypothesize that interaction with CD63 in lipid-rich microdomains drives the trafficking and incorporation of LMP1 into EVs. To analyze the effects of CD63 and ceramide inhibition on exosome secretion and protein trafficking, a stable CD63 knockout/down patient-derived lymphoblastoid cell line (LCL) was created using CRISPR Cas-9 and the chemical GW4869, was used to inhibit ceramide production.

Nanoparticle tracking analysis (NTA) of extracellular vesicles demonstrated a significant decrease in relative particle secretion in the CD63 CRISPR cell line and GW4869 treated cells when compared with controls. Additionally, immunoblotting and quantification of protein levels revealed a reduction of exosomal LMP1 from the CD63 CRISPR and GW4869 treated cells. Further characterization of the CD63 CRISPR and control extracellular vesicle preparations with electron microscopy verified the presence of concave-shaped vesicles of a variety of sizes less than 150 nm. IFA and confocal microscopy images suggest that LMP1 localization may be altered in the CD63 CRISPR cells when compared with the controls.

Altogether, these data indicate that LMP1 trafficking may be disrupted in the CD63 CRISPR cells, supporting a role for CD63 in LMP1 vesicle secretion. In addition, it appears that ceramide inhibition may affect LMP1 protein trafficking, but this will need further confirmation. Furthermore, it will be important to determine whether inhibiting LMP1 secretion affects tumor development or progression.

**Phagocytosis and autophagic clearance of myelin debris by microvascular endothelial cells promotes angiogenesis and inflammation after spinal cord injury**

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Spinal cord injury provokes chronic inflammation and secondary tissue damage, which contributes to progressive neurodegeneration. Demyelination, or myelin breakdown following spinal cord injury is one of the adverse factors, and clearance of myelin debris has been shown critical for axon recovery and tissue homeostasis. However, it remains unclear how the myelin debris are removed in injured spinal cord to facilitate functional recovery. Our current study shows an unappreciated role of microvascular endothelial cells, a cell type of blood-spinal cord barrier system in phagocytosis and clearance of myelin debris after spinal cord injury in mice. Engulfment of myelin debris by these endothelial cells in turn remarkably promotes the growth of microvessels and induces inflammatory responses. In vitro evidences from isolated brain endothelial cells and a cell line further revealed that endothelial cells have strong but inferior phagocytosis capacity to macrophages, known phagocytes to efficiently clear myelin debris. We then showed the phagocytic myelin debris in endothelial cells is delivered through autophagy pathway, not endosomal route to lysosomal degradative system in endothelial cells. Through autophagy-lysosome pathway, myelin debris is degraded into variety of lipid components in endothelial cells as analyzed by thin-layer chromatography. Consistent with the increased angiogenesis and inflammation after spinal cord injury in mice, our in vitro study shows that myelin debris promotes endothelial cells proliferation and migration as well as inflammatory responses. Taken together, our study demonstrates a novel role of
microvascular endothelial cells in clearing myelin debris, which in turn induces secondary injury by promoting endothelial angiogenesis and inflammation.

A group of Inner Kinetochore Proteins Regulates the Spindle Assembly Checkpoint

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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 ybp2Δ 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.

Elucidating the molecular basis for species differences in behavior in Drosophila

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A major remaining challenge in the field of neuroscience is determining how neural circuits are specified to direct innate behaviors and how the potential for different behaviors observed in closely related species have evolved. The Drosophila courtship system provides an ideal method for studying complex and sex-specific behaviors as males exhibit sequential, innate, and species-specific courtship steps. These behaviors are under the genetic control of the sex hierarchy genes doublesex (dsx) and fruitless (fru) which encode sex-specific transcription factors. Here, I focus on the male-specific transcription factors encoded by fru (FruM), as these male-specific transcription factors are both necessary and are largely sufficient to direct the potential for courtship behavior. Previous investigations have attempted to determine the molecular basis for the evolution of courtship behavior using transgenic approaches and found that heterospecific fru and dsx loci were sufficient to rescue courtship. However, these genes were unable to establish the species-specific courtship behaviors from their species of origin, suggesting that changes in the genes regulated by fru and dsx drive courtship evolution. In order to address this, I’ve proposed to identify the species differences in FruM binding and also the genes actively translated in FruM expressing neurons in two Drosophila species; D. melanogaster, D. simulans, and melanogaster-simulan hybrids.
Centrosome proteins are required for autophagy to maintain neural health

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The centrosome is the major microtubule-organizing center in animals. Mutations in centrosomal genes cause primary microcephaly (MCPH), a neurological disorder characterized by smaller brain size, highlighting the importance of centrosome proteins in maintaining neural health. Yet the etiological basis for MCPH pathogenesis remains unclear. To explore the etiological basis of MCPH, we performed 2-D difference gel electrophoresis in combination with mass spectrometry to identify molecular signatures of centrosomin (cnn) mutant brains. Proteomic analysis led us to discover a novel role of centrosomal proteins-regulation of autophagy. Autophagy is a major means by which cells clear misfolded or aggregated proteins and damaged organelles that contribute to the decline of neuronal health. We show that mutations in at least two essential centrosome proteins in Drosophila, cnn and sas-4, impair starvation-induced autophagy, which could be associated with poor locomotor function of cnn mutant flies. EM data showed that Cnn regulates early steps in the assembly of autophagosomes, the double-membrane structures that deliver cargos for lysosomal degradation. Mechanistically, we showed that Cnn could act through a novel partner protein, an E3 ubiquitin ligase, to regulate autophagy. Overexpression of this E3 ligase induces robust autophagy, reduces cell size and causes fat body degeneration dependent on its E3 ligase activity of RING domain. These findings reveal a novel role of centrosomal proteins and implicate defective autophagy as a novel etiological basis for MCPH.

Activation of the centrosome by the Zika virus (ZIKV)

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Recent outbreaks of the Zika virus (ZIKV) in the Americas has resulted in a public health crisis due to its links to ZIKV-induced 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. In the inherited forms of microcephaly, a large percentage of mutations affect centrosomal proteins. 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 activation of 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 accumulation of the centrosomal proteins Ninein and Polo-like kinase 1 (PLK1) at the the centrosomes of ZIKV infected cells compared to
uninfected cells. Plk1 has multiple roles in regulating cell division. Activation of Plk1 through phosphorylation of Thr210 results in centrosome maturation in G2 phase. The functional role of PLK1’s activation through a second phosphorylation site, Ser137, is not as well understood. Phosphorylation at Ser 137 does occur in late mitosis but it is not required for early PLK1 activation. In ZIKV infection, we observe an elevation in pPlk1-Ser137 but not in pPLK1-Thr210 levels. We propose that PLK1 these proteins is are modified at Ser137 and recruited along with Ninein to the centrosome by in response to the Zika virus, and that they are required for centrosomal activation maturation during viral infection. We further propose that ZIKV-mediated centrosome activation is required for ZIKV proliferation and that PLK1 activation may be a feasible pharmacological target to acutely block ZIKV.

Histone variant H3.3 plays an evolutionarily conserved role in DNA repair that can be targeted for treating H3.3 mutant tumors

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The majority of histone proteins involved in the packaging of DNA inside the tiny nuclei of eukaryotic cells are the replication dependent canonical histones. Histone variants differ from their canonical counterparts in their primary sequence at just a few amino acids and are expressed throughout the cell cycle at lower levels. Remarkably, several of the variant histones appear to have evolved unique roles in chromatin structure and function. Histone H3.3 is one such variant that is enriched at transcriptionally active loci among others and is believed to facilitate transcription. More importantly, mutations in H3.3 were shown to drive a majority of lethal childhood glioblastoma tumors, as well as nearly all of the non-lethal chondroblastomas and large cell tumors of the bone primarily in children and young adults. Not surprisingly, these H3.3 mutations were found to alter important posttranslational epigenetic modifications, leading to aberrant transcription which in turn is believed to drive carcinogenesis. Here we show that histone H3.3 also plays a crucial role in surviving DNA damage independent of its role in transcription in several evolutionarily distant species and thereby contributes to genomic stability. Histone H3.3 is rapidly recruited to sites of DNA damage while the cancer associated H3.3 mutants are deficient in this response. Further, H3.3 knockdown cells as well as cells as carrying cancer related mutations in H3.3 accumulate spontaneous DNA damage and are hypersensitive to exogenous DNA damaging agents. Together, these data suggest that histone H3.3 plays an evolutionarily conserved role in DNA repair. We have now identified the specific DNA repair pathways regulated by histone H3.3 and we have used this knowledge to devise a potential therapeutic strategy for eradicating H3.3 mutant tumor cells by targeting specific DNA repair pathways. Our initial results suggest that targeting specific DNA repair pathways may be a promising avenue for treating H3.3 mutant tumors in the future.
Transmembrane Domains Mediate Intra- and Extra-cellular Trafficking of Epstein-Barr Virus LMP1

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The Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is released from latently infected tumor cells in small membrane-enclosed vesicles called exosomes. These LMP1-modified extracellular vesicles can be taken up by cells resulting in the activation of cellular signal transduction pathways through paracrine or autocrine mechanisms. Accumulating evidence suggests that LMP1 is a major driver of exosome content and functions. For example, LMP1-modified exosomes have been shown to influence cell growth, migration, differentiation, and immune cell function. Despite the importance of exosomal LMP1 on the infected microenvironment, very little is known about how this viral protein enters or manipulates the host exosome pathway.

Results: In this study, we analyzed the ability of LMP1 deletion mutants to traffic to exosomes. Our results demonstrate that specific domains within the transmembrane regions of LMP1 are required for efficient sorting into the exosome pathway. Consistent with these findings, a mutant lacking transmembrane domains 1 through 4 (TM5-6) 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, alluding to potential positive and negative regulation mechanisms for LMP1 exosome sorting. Surprisingly, TM5-6 maintained the ability to co-localize and form a complex with CD63, an abundant exosome protein that is important for the incorporation of LMP1 into exosomes. These data suggest new functions of the transmembrane domains in LMP1 exosome trafficking that are likely downstream of its interaction with CD63.

Adiponectin is necessary for exercise training-induced muscular hypertrophy and vascular adaptation

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Adiponectin has been reported to be produced by skeletal muscle fibers and to influence muscle phenotype. We tested the hypothesis that adiponectin is necessary for exercise training-induced muscular hypertrophy and alterations of the resistance vasculature in skeletal muscle. Adult C57BL/6 wild-type (WT) or homozygous adiponectin knockout (Adipo KO) mice were obtained at 10 weeks of age and underwent treadmill exercise training (12 m/min, 5° incline, 1 hr/day, 5 days/wk for 8 wks) or remained sedentary in cages. Body mass was recorded weekly during the training/cage confinement period. At the end of the training/cage confinement period, the gastrocnemius-plantaris-soleus complex was isolated, and soleus muscle feed arteries were isolated for study of vascular responses to increasing intraluminal pressure (the myogenic response). The body mass of WT mice increased significantly over the training/cage confinement period, by 11.7% in sedentary mice and by 13.9% in exercise trained mice.
In Adipo KO mice, body mass increased by 15.1% in sedentary mice (P<0.01 wk 8 vs wk 1), but increased by only 7.2% in exercise trained mice (P=0.07). In WT mice, the masses of both the soleus muscle (20.4±2.3 mg vs. 12.2mg; exercise trained vs. sedentary; P<0.01) and the gastrocnemius muscle (248.8 mg vs. 178.5 mg; exercise trained vs. sedentary; p<0.01) were greater in exercise trained mice as compared to sedentary mice. When expressed relative to body mass (muscle weight:body weight), these increases in mass persisted in the muscles of the exercise trained mice. In Adipo KO mice, exercise training did not increase the mass of either the soleus (12.5±1.5 mg vs. 12.1±1.5 mg; exercise trained vs. sedentary; P=0.62) or gastrocnemius muscle (195.2±11.2 mg vs. 182.7±18.9 mg; exercise trained vs. sedentary; P=0.12), whether expressed as absolute muscle mass or relative to body mass. Exercise training increased myogenic responsiveness of soleus muscle feed arteries significantly in WT mice (P<0.01, WT exercise trained vs. WT sedentary), but did not alter myogenic responsiveness of soleus muscle feed arteries in Adipo KO mice (P=0.87, Adipo KO exercise trained vs. Adipo KO sedentary). These results indicate that exercise training-induced hypertrophy of skeletal muscle and associated improvement of vascular function does not occur in mice deficient in adiponectin. These results suggest that adiponectin is vital to the vascular and metabolic adaptations that occur in skeletal muscle in response to aerobic exercise training.

**Physiological consequences of prenatal exposure to valproic acid in prairie voles**

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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.
Transcriptomic Changes in Murine Bone Marrow Derived Macrophages Following Phagocytosis of Myelin Debris

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Macrophages are mature phagocytic leukocytes that have roles in both physiological and pathological processes. They can be activated through numerous stimuli, such as injury, infection, and inflammation. The activation state of macrophages is often defined as either classically activated M1 (pro-inflammatory), or alternatively activated M2 (anti-inflammatory). While this dichotomy can prove useful in a general context, current research points to the fact that there are many states between the two extremes.

Spinal cord injury (SCI) results in massive axon death which produces large amounts of myelin debris. There is a large degree of bone marrow derived macrophage infiltration at the primary injury site. Upon engulfment of myelin debris, macrophages take on an M1-like phenotype which contributes to the secondary injury and prevents functional recovery. Little is known about the transcript level changes that myelin debris phagocytosis induces in macrophages, or how this contributes to their pathological role in the injured spinal cord. We have implemented mRNA microarray analysis of murine bone marrow derived macrophage transcripts. Using this method we have identified several related gene clusters that are correlated with known phenotypic changes. Among the clusters with known correlation are immune response, taxis, and G-protein coupled signaling. Additionally, mapping to previously unreported epigenetic methylation pathways was identified using this method.

Dissecting Molecular Determinants of Proteasome Activation with Conformation-Specific Reporters

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The proteasome is an ATP-dependent multisubunit protease that conducts most regulated and quality control protein degradation in eukaryotes. Numerous human diseases are characterized by excessive or insufficient proteasome activity, and modulating proteasome activity to balance the proteolytic load is a validated therapeutic strategy. Recent high resolution structures of the proteasome have unexpectedly revealed that at least two major conformations exist: an autoinhibited “apo” state in which the major catalytic sites and substrate channels are blocked; and an active “engaged” state in which these sites are aligned and poised for substrate degradation. Harnessing this conformational switching thus holds the potential to activate or inactivate the proteasome at will.

Although structural approaches have been instrumental in revealing these conformations, they are effort-intensive and lack the flexibility and throughput required for detailed mechanistic analyses. To fill this critical methodological gap, we developed rapid and simple conformation-specific reporters founded on engineered disulfide crosslinking. Using these reporters, we demonstrate that the proteasome freely samples both the apo and engaged conformations in solution. Further, engagement of the peptidase
active sites does not influence the conformation of the proteasome. However, the proteasome becomes locked in the engaged state upon saturation with non-hydrolyzable ATP analogs. 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.

A role for the conserved sorting nexin SNX4 in the autophagic clearance of proteasomes and other multisubunit complexes

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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 the model eukaryote, baker’s yeast, 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.

Tyrosine Phosphorylation of the Transcription Factor Yin Yang 1

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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. Our previous work has shown the modulation of the function of YY1 through phosphorylation at amino acid residues serine and threonine. However, little is known about the molecular mechanisms by which tyrosine phosphorylation regulates YY1’s function. We have developed two rabbit poly-clonal phosphospecific antibodies against tyrosine residues 251 and 254; (α-pY251) and (α-pY254), respectively. We have utilized high-throughput proteomic and computational data in combination with biochemical assays and our phosphospecific antibodies to identify several protein tyrosine kinases that phosphorylate YY1. Our findings will have great importance for understanding cellular signaling pathways that regulate this multifunctional protein.

This is SPrTAC: A New Method to Distinguish Multisubunit Complexes with Highly Similar Compositions In Vivo

Anna Peterson and Robert J. Tomko Jr.

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. For example, changes to the subunit composition of the proteasome promote antigen processing, suppress self-recognition by immune cells, and lead to chemotherapy resistance in some cancers. 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 α4 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.

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

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The proteasome is a multisubunit ATP-dependent protease complex responsible for degrading damaged or unneeded proteins. It consists of three major subcomplexes, the lid, base, and core, which coordinate to bind, process, unfold, and cleave substrate proteins. Recent structural studies have unexpectedly shown that the proteasome oscillates between an autoinhibited, inactive apo state, and an activated engaged state competent for degradation. The interface between the lid and base is the major site of remodeling between these conformations, although how this is mediated remains unknown.

Using a structure-guided approach, we systematically mutated conformation-specific contacts between the lid and base. Disrupting contact between one lid subunit, Rpn5, and the base in the apo state paradoxically destabilized the interaction between the base and core rather than base and lid. No such effect was observed with engaged state mutations. 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 the engaged and apo states, respectively.

Careful examination of the proteasome structure revealed extensive salt bridging between highly conserved residues in Rpn5 and the core present only in the apo state, and mutating these residues destabilized the core-base interaction similarly to the Rpn5 apo mutations. Together, these data reveal a conformation-specific stabilizing salt bridge between the lid and core that is controlled by ATP binding within the base. 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.

Neurobehavioral and Neuroanatomical Consequences of Cell-type specific Inactivation of Dopamine D2 Receptors in the Mouse Brain

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Neuropsychiatric disorders including depression, schizophrenia, and attention deficit disorders have a developmental basis linked with disruptions in dopamine (DA) homeostasis within the forebrain. We and others have previously demonstrated that loss of DA receptors, in particular the D2 receptor, can alter neuronal migration, cerebral cortical interneuron expression, and behavioral outputs. Amongst diverse cell types in multiple brain regions that express dopamine D2 receptors, we are interested in identifying which neuronal subpopulations are responsible for D2 receptor-mediated effects. In our model, we deleted D2 receptor (Drd2<sup>flox/flox</sup> mice) from either forebrain glutamatergic neurons (Emx1<sup>tm1(cre)Krj</sup>) targeting cortical and hippocampal pyramidal neurons or select GABAergic neurons (Nkx2-1<sup>cre2Sand</sup>) targeting inhibitory interneurons. Conditional knockouts from both lines (Drd2<sup>flox/flox</sup>; Nkx2.1<sup>Cre+</sup> or Drd2<sup>flox/flox</sup>; Emx1<sup>Cre+</sup>) exhibited no differences in tests of anxiety, depression-like behavior as well as working and spatial memory relative to controls (Flox controls: Drd2<sup>flox/flox</sup>; Cre- and Cre controls: Drd2<sup>+/+</sup>; Cre+). Both deletion of GABAergic and glutamatergic KO lines performed similarly to controls in open field, spontaneous locomotor behavior as well as in rotarod task indicating no observable motor deficit or initial motor coordination concomitant. In the cellular level, there were also no significant changes in glutamic
acid decarboxylase+ or parvalbumin+ interneurons in the anterior cingulate cortex of D2f, Nkx2.1 KO mice. Additional assays to determine potential ectopic compensation as well as dysfunction in other social behavioral or cognitive domains are underway. By delving into alterations due to deletion of D2 receptor in our model, we can specifically identify developmental, cellular and behavioral roles of D2 receptor within various cell types of the telencephalon and how dysfunction of D2 receptor contributes to the development and pathophysiology of neuropsychiatric disorders.

**Dopamine D1 receptor activation and neuronal migration**

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Neurotransmitters play unique growth-promoting roles in the developing nervous system, which are distinct from their canonical role at the synapse in the mature nervous system. We have shown that the neurotransmitter dopamine (DA) and its receptors not only appear in the fetal brain during early development but also play critical roles in developmental phenomena such as cell proliferation, neuronal migration, differentiation and circuit formation. Perturbation of these developmental events is believed to be associated with a number of neurological and psychiatric disorders with developmental onset. Many of these developmental disorders are also associated with impairment of inhibitory GABA circuits. GABA neurons originate in the dopamine-rich medial ganglionic eminence (MGE) of the embryonic basal forebrain and migrate to regions of the dorsal forebrain. These migrating GABA neurons express dopamine receptors. Therefore, we hypothesized that dopamine receptor activation influences migration of the GABA neurons. Using live cell imaging techniques, we examined the migratory behavior of these GABA neurons following application of the DA type 1 receptor (D1R) agonist SKF 81297. We used a co-culture system in which MGE explants were plated on dissociated cortical neurons; both obtained from embryonic day 13 mice. Following 24 hours in culture, the D1R agonist (1 µM) was applied and live cell imaging was performed over a 7 hr period. We found the migration velocity of treated GABA neurons to be significantly decreased by 28% compared to those treated with a vehicle control. Migrating GABA neurons typically display a saltatory behavior characterized by the alternation between a nuclear jump-like movement and a nuclear resting phase. We found that D1R agonist application significantly increased the average time that GABA neurons spent in the resting phase by 35%. Moreover, D1R agonist application significantly decreased the frequency of medium jumps (characterized by an instantaneous migration velocity between 1.2 and 2.4 µm/min) by 30% and the frequency of large jumps (characterized by an instantaneous migration velocity greater than 2.4 µm/min) by 36%. Collectively, these results demonstrate that D1R activation can alter the migratory behavior of GABA neurons during early brain development suggesting a role for impaired D1R activity in the etiology of developmental disorders associated with dopamine imbalance.
TGFβ regulates ciliogenesis in *Xenopus*

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Primary cilia serve as the medium for critical developmental pathways and intercellular communication, so proper cilia development is essential for eukaryotes. A broad range of developmental disorders termed ciliopathies, arise from compromised ciliary structure and function, resulting in completely or partially impaired cellular signaling pathways, such as canonical sonic hedgehog signaling. We have previously shown transforming growth factor beta (TGFβ) mediates cilia formation in Xenopus embryos by influencing transition zone assembly. An RNA-seq analysis revealed a series of ciliary genes whose transcription is upregulated by TGFβ expression, we are using both loss of function and rescue experiments on these candidate genes in Xenopus tissue to determine their possible roles in ciliogenesis.

FMRP in dendritic development and axonal guidance of developing auditory brainstem

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Fragile X syndrome (FXS) is the leading cause of inherited mental retardation and autism, characterized by a constellation of visual, auditory and motor dysfunction. FXS results from the absence of fragile X mental retardation protein (FMRP), an mRNA-binding protein that pleiotropically regulates protein translation. It is known that FMRP is important for proper wiring and function of neuronal circuits. However, it is not known exactly how FMRP regulates auditory development and how its loss leads to auditory dysfunction in FXS.

In this study, we characterize the function of FMRP in the developing auditory brainstem of birds. The chicken nucleus magnocellularis (NM) and nucleus laminaris (NL), analogues to the mammalian anteroventral cochlear nucleus (AVCN) and medial superior olive (MSO), are important for temporal processing and binaural hearing, two functions that are compromised in FXS.

Western blotting on micro-dissected tissue samples from NM and NL, reveals significantly stronger expression of FMRP during development than in mature systems. Importantly, immunocytochemical studies further revealed an accumulation of FMRP at dendritic branch points, implying a role of FMRP in dendritic branching. We next examined the effect of knocking down FMRP expression on the development of NM/NL circuit by introducing shRNAs into unilateral neural tube via in ovo electroporation. Consistent with previous studies, non-transfected NM neurons display dendrites before embryonic day 15 (E15) with progressive loss of dendrites afterwards. In contrast, transfected neurons with reduced FMRP protein level exhibit extensive dendrites at E15 with persistence of dendrites in some neurons until E19, indicating delayed dendritic pruning. Moreover, transfected NM neurons display abnormal axonal projection. Normally, NM axons projecting to the contralateral NL are restricted to the ventral dendrites of NL. Axons
of transfected NM neurons terminate in both dendritic layer as well as the somatic layer of contralateral non-transfected NL, suggesting presynaptic desensitization to repulsive signal in NL.

These data demonstrate that normal FMRP expression is required for correct dendritic development and axon guidance of NM neurons. To start exploring the underlying mechanisms, we examined the effect of FMRP knock-down on the protein level of calretinin. Calretinin is a FMRP targeted calcium binding protein, deletion of which leads to increased neuronal excitability. We found significantly lower calretinin immunoreactivity in transfected NM neurons as compared to neighboring non-transfected neurons. Ongoing studies are investigating how FMRP knockdown caused structural and protein expression deficits affect the biophysics and function of NM neurons in temporal processing and binaural hearing.

Reinforcing properties of intermittent, low-dose ketamine in males and female Sprague-Dawley rats

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Repeated intermittent exposure to ketamine has rapid and long-lasting antidepressant effects, but the abuse potential of ketamine has only been assessed at high doses. Furthermore, while females are more susceptible to depression and more sensitive to ketamine’s antidepressant-like effects, its abuse potential in females is unknown. Therefore, the objective of this research is to determine the reinforcing properties of low-dose intermittent ketamine in both sexes and whether cycling gonadal hormones influence females’ response to ketamine. In male rats, we also aimed to determine whether reinstatement to intermittent ketamine is comparable to intermittent cocaine. 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 female rats self-administered ketamine only during stages of their four-day estrus cycle, when gonadal hormones are either high (proestrus) or low (diestrus). After acquiring self-administration, rats underwent daily 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, due to lack of acquisition. Ketamine-primed reinstatement was dependent on simultaneous cue presentation. Male rats reinstated to cocaine priming independent of cue presentation. Additionally, progressive ratio was tested to assess the motivational salience of ketamine at different doses. Therefore, both females and males respond to ketamine’s reinforcing effects under this treatment paradigm. Female’s response was cycle-dependent.

An Mcm10 mutant defective in ssDNA binding shows defects in DNA replication initiation

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Mcm10 is an essential protein that functions to initiate DNA replication after the formation of the replication fork helicase. In this report, we identified a budding yeast Mcm10 mutant (Mcm10-m2,3,4)
that is defective in DNA binding in vitro. Moreover, this Mcm10-m2,3,4 mutant does not stimulate the phosphorylation of Mcm2 by DDK in vitro. When we expressed wild-type levels of mcm10-m2,3,4 in budding yeast cells, we observed a severe growth defect and substantially decreased DNA replication. We also observed a substantially reduced RPA-ChIP signal at origins of replication, reduced levels of DDK-phosphorylated-Mcm2 and diminished GINS association with Mcm2-7 in vivo. mcm5-bob1 bypasses the growth defect conferred by DDK-phosphodead-Mcm2 in budding yeast. However, the growth defect observed by expressing mcm10-m2,3,4 is not bypassed by the mcm5-bob1 mutation. Furthermore, origin melting and GINS association with Mcm2-7 are substantially decreased for cells expressing mcm10-m2,3,4 in the mcm5-bob1 background. Thus, the origin-melting and GINS-Mcm2-7-interaction defects we observed for mcm10-m2,3,4 are not explained by decreased Mcm2 phosphorylation by DDK, since the defects persist in an mcm5-bob1 background. These data suggest that DNA binding by Mcm10 is essential for the initiation of DNA replication.

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

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

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Low-dose ketamine is gaining popularity as an alternative treatment for people suffering from major depressive disorder whose symptoms are not alleviated by typical SSRIs. Among this subpopulation with treatment resistant depression, a single infusion of low dose ketamine can alleviate suicidal ideation and other depressive symptoms within one hour, and can have long-lasting effects up to two weeks. However, ketamine in higher doses is a well-known club drug that is commonly abused. For this reason, and because addiction and depression are often comorbid, the abuse potential of ketamine at low doses needs to be investigated. Our lab previously showed that female rats respond to a lower dose than males, rescuing depressive-like behaviors at 2.5 mg/kg and 5 mg/kg (i.p.), respectively. In this study, we investigated the abuse potential of low doses of ketamine (2.5 and 5 mg/kg) administered once weekly in both males and females to mimic the treatment timeline used in the clinic. Locomotor activity and conditioned place preference (CPP) assays were used to assess measures of sensitization and drug-liking, and brains were collected to examine protein expression of molecular markers for addiction in the nucleus accumbens (NAc) as well as dendritic spine density in the core and shell of the NAc. While neither males nor females formed a place preference in the CPP paradigm, males treated with 5 mg/kg and females treated with both 2.5 and 5 mg/kg sensitized to ketamine. Additionally, dendritic spines were increased in the NAc shell in males and females at both doses. Expression of deltaFosB, CaMKIIα, and BDNF was increased in males, while GluA1 expression was increased in females. Taken together, we show that low dose ketamine, when administered intermittently, induces behavioral sensitization, accompanied by an increase in spine density and the NAc and protein expression changes in pathways commonly implicated in addiction.

Repeated low-dose ketamine treatment elicits a sex-differential effect on behavioral sensitization and reward state

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Clinical studies have revealed rapid and robust antidepressant effects following treatment with a low subanesthetic dose of the noncompetitive NMDA receptor antagonist ketamine (KET) in treatment-resistant patients. Further, KET’s therapeutic effects are maintained over a longer time course when delivered repeatedly. However, at higher doses, KET is a known recreational drug of abuse. As compared to males, females are at an elevated risk for both developing major depression and escalating from casual to compulsive drug use. Thus, investigating the long-term safety of repeated low-dose KET infusions in both sexes is crucial to its viability as a sound treatment option for depression. We recently reported that
female rats are more sensitive than males to KET’s antidepressant-like effects, responding to an acute dose 2.5 and 5 mg/kg (i.p.), respectively. Here, we aimed to determine whether there are also sex differences in addictive properties of repeated KET treatment in adult rats at these doses, as well as a higher concentration (10 mg/kg, i.p.) previously shown to elicit addictive behaviors in male rats. Using the conditioned place preference test to gauge pharmacological reward, we found that while all rats failed to develop a preference for 2.5 or 5 mg/kg KET, males formed a preference for 10 mg/kg KET. This suggests that repeated KET treatment at therapeutically-relevant doses is not innately rewarding. Behavioral sensitization to KET’s locomotor-activating effects was also assessed: Males and females both sensitized to repeated 5 or 10 mg/kg, but females’ response to 5 mg/kg KET was amplified relative to males’. Sensitization to KET in both sexes was tied to increased ΔFosB expression in the nucleus accumbens (NAcc). However, unlike cocaine, viral-mediated ΔJunD overexpression in NAcc failed to block behavioral sensitization to repeated KET treatments. Taken together, these findings suggest that repeated low-dose KET induces behavioral and physiological changes similar to those of other drugs of abuse and high doses of KET, but that the transcriptional machinery underlying low-dose KET’s behavioral phenotype may be unique. Therefore, more studies are needed to establish the safety of repeated KET treatment for treatment-resistant depression.

Reversal of Hypertrophic Cardiomyopathy Phenotype via Conditional Ablation of Cardiac Myosin Light Chain Kinase

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Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in young adults, affecting approximately 1 in 500 individuals, and it exhibits a wide range of symptoms including diastolic dysfunction, fibrotic tissue formation, impaired contractile properties, and, most characteristically, increased myofilament Ca2+ sensitivity and left ventricle hypertrophy (LVH). On the other hand, dilated cardiomyopathy (DCM) exhibits systolic dysfunction, decreased Ca2+ sensitivity, and, many times, ventricular dilation. The mechanisms of disease for HCM or DCM are unknown, but data points toward sarcomeric mutations as major triggers of the disease phenotypes. Mutations in cardiac troponin C (cTnC), the Ca2+ sensor in contraction, seem to be responsible for some of the maladaptive compensatory mechanisms triggering HCM and DCM phenotypes, as seen in previously published work. Our lab recently reported that the A8V (Alanine8 → Valine) mutation of cTnC in mice, previously found as a common mutation in HCM patient screenings, causes LVH and increased Ca2+ sensitivity, thus exhibiting the HCM phenotype. Recently, cardiac myosin light chain kinase (cMLCK) knock-out mice were shown to recapitulate the hemodynamic, morphological and histological phenotypes seen in humans with DCM, characterized by a growth in cardiac chambers. Preliminary data of “in utero” studies from our lab have also shown that an HCM phenotype mutant mouse bred with a DCM phenotype mouse spawn mice with chamber size, sarcomere length, and Ca2+ sensitivity normalized toward wild type (WT) values. This
preliminary data from our lab confirmed a proof of concept that a DCM phenotypic mouse and an HCM phenotypic mouse can be bred to produce pups that are phenotypically similar to WT mice. However, realistically, patients would already exhibit the disease. To mimic this scenario, we created a mouse model that would allow us to test the rescue of an advanced phenotype of HCM. Here, we show preliminary data pointing to the successful breeding of an HCM cTnC-A8V mouse with a cMLCK genetically-floxed mouse to spawn progeny with both genotypes. We show that the tamoxifen induced conditional cMLCK knock-out in a post-HCM-symptomatic mutant mouse can alter its phenotype toward WT values, showing improvement in overall contractile phenotypes. These parameters were analyzed through ECHO, histopathology, cardiomyocyte measurements, as well as Western blots for Ca2+ handling proteins and RLC phosphorylation. These preliminary data suggest that cMLCK activity should be considered as a primary therapeutic target in hypertrophic cardiomyopathy treatment. HCM is a dangerous and often fatal disease, thus finding an effective treatment is of principal concern for the field of cardiology.