than in mGluR7/7 and agonist efficacy is even higher than in mGluR2/2, making mGluR7/2 a super-receptor. mGluR7 and mGluR2 have overlapping expression patterns introducing the possibility that the mGluR7/2 heterodimer may provide presynaptic nerve terminals with uniquely high efficacy glutamate sensitivity.

2810-Pos Board B18

Towards the Structure of DNase1L3

Jon J. McCord[1], Faraz Harsini[1], Sukanyalakshmi Chebrolu[1], Peter Keyel[2], Roger Bryan Sutton[1].
[1]Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, TX, USA, [2]Biological Sciences, Texas Tech University, Lubbock, TX, USA.

Systemic Lupus Erythematosus is a debilitating autoimmune disease that afflicts about 1.5 million Americans today. Although SLE is a multifactorial disease, SLE can arise with disruptions in two genes: Dnase1 and Dnase1L3. While the structure is known for DNase1, the structure for Dnase1L3 is unknown. To study the structure and function of the Dnase1L3 enzyme, we designed a heterologous expression system and purification protocol for mouse DNase1L3 in E. coli. This system will allow us to characterize the enzymatic activity of the wild-type enzyme. Towards the purpose of solving its 3D structure with X-Ray crystallography we proceeded to the crystallization step of Dnase1L3. A variety of culturing conditions, purification methods, and characterization techniques were performed on purified DNase1L3. After testing a few different methods, we successfully expressed DNase1-L3 in E. coli using Rosetta-gami cells as a fusion protein to Maltose Binding Protein. The fusion enzyme was isolated via amylose affinity resin. After elution from the affinity column, the enzyme was cleaved with TEV protease and further purified on S-resin as a defined peak. Finally, Dnase1L3 was purified using size exclusion chromatography. The activity of purified DNase1-L3 was assessed through digestion of plasmid DNA. The identify of Dnase1L3 was confirmed by Western blot. Now that we have obtained an efficient purification method, verified the activity, and confirmed identity with specific antibodies, we are interrogating the structural aspects of the enzyme through X-Ray crystallography. The structural composition of this enzyme will be invaluable as pertaining to possible use in treating Systemic Lupus Erythematosus.

2811-Pos Board B19

Proteolytically Resistant Cellular Prion Protein Construct Retains Metal Driven *CIS*-Interaction while Generating Toxicity in Cells

Graham P. Roseman[1], Alex J. McDonald[2], Bei Wu[2], David A. Harris[2], Glenn L. Millhauser[1].
[1]Universtiy of California Santa Cruz, Universtiy of California Santa Cruz, Santa Cruz, CA, USA, [2]Biochemistry, Boston University School of Medicine, Boston, MA, USA.

Transmissible Spongiform Encephalopathies, or prion disorders, are caused by the misfolding and aggregation of the cellular prion protein (PrP^C). The precise physiological role of the properly folded 209 amino acid GPI anchored PrP^C is unknown. PrP^C deletions have been used to study the role of certain domains within the protein. Interestingly, one of these mutations, ΔCR (Δ105-125 PrP), which deletes 21 amino acids in the highly conserved central region of the protein, causes neonatal fatality in mice and massive neurodegeneration. Furthermore, the toxicity that is caused by ΔCR is similar to that of prion disorders except without the aggregation of PrP^C. Biophysical studies showed that ΔCR causes two main changes: weakening of the metal driven *cis*-interaction between the N- and C- terminal domains and deletion of the region of the protein where regulatory proteolysis occurs. What is not known is if ΔCR neurotoxicity is a result of a weakened *cis* interaction or elimination of proteolytic sites. In this study, we test ΔCR's toxicity source by replacing the central region with an uncleavable flexible glycine-serine (GS) rich linker that retains the *cis* interaction of wild type PrP^C while blocking the cleavability of this region. NMR studies show the *cis* interaction is preserved, cell and *in vitro* based cleavage assay's show that canonical cleavage is blocked, and electrophysiological measurements show that these uncleavable linkers generate spontaneous currents, which correlate to toxicity that ΔCR exhibits. Therefore, these results show that the cleavability of PrP^C is a crucial regulatory function and that blocking it generates toxicity.

2812-Pos Board B20

Structural and Biochemical Assay of Dynamin-Like GTPases

Andrew Kehr[1], Leopold Kong[1], Huaibin Wang[1], Shunming Fang[1], Matt Martin[2], Jenny Hinshaw[1].
[1]NIDDK, National Institutes of Health, Bethesda, MD, USA, [2]University of Maryland, College Park, MD, USA.

Dynamins are a class of GTPase enzymes responsible for the fusion, fission, and vesiculation of cellular lipid membranes throughout the cell. The dynamin-like proteins Optic Atrophy 1 (Opa1) and Mitofusin (Mfn) 1 and 2 are responsible for the fusion of the mitochondrial inner and outer membranes, respectively. Atlastin mediates fusion of homotypic three-way junctions in the endoplasmic reticulum (ER). Dynamin 1 (Dyn1) is responsible for fission of endocytic clathrin-coated vesicles from the plasma membrane. Mutations in any of these proteins can lead to neuropathies including Dominant Optic Atrophy, Hereditary Spastic Paraplegia, and Charcot-Marie-Tooth, among others. Currently, structural and biochemical data is limiting for Opa1 and the Mfns, particularly in a lipid environment. We have developed a protocol for expressing and purifying biologically relevant and biochemically active shortened isoforms ($Opa1_{GG}$ and $Mfn1_{GG}$) in sufficient quantity to begin cryo-EM studies. We have shown, using analytical ultracentrifugation (AUC) and size exclusion chromatography (SEC) that an N-terminal heptad repeat of Opa1 is required and sufficient for dimerization of the proteolytically processed short form, Opa1S. $Opa1_{GG}$, lacking this N-terminal region, migrates differently under multiple nucleotide conditions by SEC. In addition, to verify work performed by x-ray crystallography, we have begun probing the GTPase cycle of Atlastin using single particle cryo-EM methods. We have recently calculated a 4.3 angstrom resolution structure of the constricted state of Dyn polymers on lipid by cryo-EM.

2813-Pos Board B21

Clinical and Biophysical Characterization of a Mutation in the N-Helix Region of Cardiac Troponin C: Evidence for an Allosteric Mechanism of Contractile Dysfunction

Jamie R. Johnston[1], Mayra de A. Marques[2], David Gonzalez-Martinez[1], Guilherme A.P. de Oliveira[2], Einat Birk[3], Nili Zucker[3], Maicon Landim-Vieira[1], Adolfo H. Moraes[4], P. Bryant Chase[5], Jerson L. Silva[2], Yael Wilnai[6], Jose R. Pinto[1].
[1]Biomedical Sciences, Florida State University, Tallahassee, FL, USA, [2]Instituto de Bioquímica Médica Leopoldo de Meis, Instituto Nacional de Biologia Estrutural e Bioimag, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3]Cardiology, Schneider Children's Medical Center, Tel Aviv University, Petah Tikva, Israel, [4]Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [5]Biological Science, Florida State University, Tallahassee, FL, USA, [6]Genetics Unit, Schneider Children's Medical Center, Tel Aviv University, Petah Tikva, Israel.

Troponin is a heterotrimeric, Ca^{2+}-binding protein responsible for regulating muscle contraction in the heart. Missense mutations in genes encoding the subunits of the cardiac troponin complex are associated with inherited dilated cardiomyopathy (DCM). However, the underlying molecular mechanisms of disease pathogenesis are not fully understood. Here, we report a clinical case of a 1-year-old female who presented with severe DCM and hypotonia. Whole exome sequencing revealed a previously unreported *de novo* heterozygous variant in exon 1 of the *TNNC1* gene (c.12C>G), resulting in an Ile4Met mutation located in the N-Helix region of cardiac troponin C (cTnC). We utilized various biophysical and biochemical techniques to examine the molecular basis of this pathogenic variant and provide evidence for allosteric communication within the Ca^{2+}-binding subunit of the troponin complex. *In vitro* extraction of native cTnC and reconstitution with either recombinant WT or I4M cTnC in cardiac muscle preparations revealed decreased Ca^{2+} sensitivity of isometric force development and slower kinetics of tension redevelopment (k_{tr}) for I4M compared to WT cTnC. Steady-state fluorescence measurements on isolated cTnC using Bis-ANS indicated enhanced Ca^{2+} binding at the C-terminal domain of I4M compared to WT. Furthermore, cTnC-I4M displayed a smaller magnitude of Ca^{2+}-induced hydrophobic exposure compared to WT. Finally, IANBD fluorescence (singly labeled at cysteine 84) titration studies revealed tighter binding of cardiac TnI to I4M compared to WT. Altogether, these results suggest that perturbed contractile kinetics and altered Ca^{2+}-binding, perhaps by an allosteric mechanism, likely contribute to the contractile dysfunction observed in this proband. Experiments using solution NMR spectroscopy are currently underway to determine how a pathogenic mutation in the N-helix affects the overall structure and dynamics of cTnC. NIH-HL128683.

2814-Pos Board B22

Computational and Experimental Studies of Divergent Clinical Effects in Proximate Thin Filament Mutations

Anthony Baldo[1], Salwa Abdullah[2], Andrea Deranek[3], Melissa Lynn[4], Michael Williams[1], Jil C. Tardiff[4], Steven D. Schwartz[1].
[1]Dept. of Chemistry and Biochemistry, The University of Arizona, Tucson, AZ, USA, [2]Dept. of Cellular and Molecular Medicine, The University of Arizona, Tucson, AZ, USA, [3]Dept. of Biomedical Engineering, The University of Arizona, Tucson, AZ, USA, [4]Dept. of Medicine, The University of Arizona, Tucson, AZ, USA.

The cardiac thin filament controls the contraction and relaxation of cardiac muscle. Mutations in this molecular machine have varying effects on the regulatory function of the cardiac sarcomere, and have been linked to both