ventricular elastance during systole. Therefore it is paramount to identify specific sites of PTMs. In-gel digestion has classically been used for PTM identification, however this approach is limited by protein size, pI, and difficulties in peptide extraction. We report a solution-based workflow for purifying endogenous sarcomeric proteins designed to enrich for peptides containing low-abundance PTMs. We focus particular attention on regulatory light chain (RLC), which was shown first by W.T. Perrie and S.V. Perry to be phosphorylated *in vivo*, but the specific sites have been unclear. Simplification of our sample with sub-cellular fractionation followed by OFFGEL electrophoresis (OGE) resulted in discriminatory purification of thick filament proteins including regulatory and essential light chains, myosin heavy chain, and myosin binding protein-C. Digestion and HPLC profiling of OGE-separated charge variants identified unique peptides suggestive of protein modifications, thus effectively enriching for endogenous PTMs which are low in abundance and have been historically difficult to identify with mass spectrometry. In addition, UV detection provided an additional unbiased quantitative analysis of peptides without having to explore more time-intensive quantitative MS methods. Using LC/MS/MS we unequivocally identified three distinct endogenous charge variants of cardiac RLC in unique OGE fractions, thus providing explanation for isoelectric point shifts observed, both in OGE and 2D-PAGE. The singly- versus doubly-phosphorylated RLC may evoke unique conformational states and thus may be functionally distinct in regulating cardiac contraction.

2589-Pos Board B559

High Resolution Top-Down MS/MS Reveals Single Amino Acid Sequence Polymorphisms in Rat Cardiac Troponin

Raquel Sanchos Solis[1], Ying Ge[1], Jeffery W. Walker[2].
[1]University of Wisconsin, Madison, WI, USA, [2]University of Arizona, Tucson, AZ, USA.

Heterotrimeric cardiac troponin (cTn) is a critical component of the thin filament regulatory complex. Two of the three subunits, cTnI and cTnT, are expressed only in cardiac muscle and are widely used in the clinic as serum biomarkers of cardiac injury. cTnI and cTnT are subject to extensive post-translational modification such as proteolysis and phosphorylation, but linking modification patterns to function remains a major challenge. In order to obtain a global view of the state of post-translational modification of cTn, we are performing high resolution top-down mass spectrometry on cTn subunits isolated from native tissues. Whole cTn complexes affinity purified from a single rat heart were analyzed in a 7 Tesla Thermo LTQ-FT-ICR mass spectrometer equipped with an ESI source. High resolution MS spectra of cTn from healthy adult rats showed molecular ions for intact cTnT and cTnI as well as phosphorylation and acetylation patterns similar to human cTnI (Zabrouskov et al., 2008 *Mol Cell Proteomics*, in press). 'Shadow peaks' of similar intensity to parent peaks were detected exhibiting masses of cTnI + 16 Da and cTnT + 128 Da, suggestive of single amino acid polymorphisms. Tandem mass spectrometry (MS/MS) analysis by ECD and CAD fragmentation of intact and protease-digested cTn subunits localized an Ala/Ser polymorphism at residue 7 of cTnI, and an additional Gln within a 3 residue alternative splice site beginning at residue 192 of cTnT. High resolution top-down MS/MS has revealed intriguing heterogeneity not only in the extent of phosphorylation, but also in amino acid sequences of cTnI and cTnT even within a single rat heart. Supported by NIH, AHA, UW-CVRC & Wisconsin Partnership for a Healthy Future.

2590-Pos Board B560

Cytotoxicity of non-myofilament-incorporated troponin T fragments

Euy-Myoung Jeong, M. Moazzem Hossain, J.-P. Jin.
NorthShore University Health System and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.

Troponin T (TnT) is a striated muscle-specific protein and an abundant component in skeletal and cardiac myofilaments. Forced expression of TnT protein in non-muscle cells or undifferentiated myoblasts in the absence of myofibrils indicated cytotoxicity (Wang *et al., J. Biol. Chem.* 280:13241-9, 2005). To investigate the cytotoxic effect of non-myofibril-incorporated TnT, we constructed non-fusion co-expression vectors encoding green fluorescence protein tracer and different regions of the TnT polypeptide chain. Transient transfection in culture was studied in HEK293 non-muscle cells and undifferentiated C_2C_{12} myoblasts. Cytotoxicity of the TnT fragments was examined by the viability of the transfected cells. The results revealed distinct toxic effects of different regions of TnT. The evolutionarily conserved middle and C-terminal segments of TnT were highly toxic to cells whereas the N-terminal variable region was not. The cytotoxicity of the middle and C-terminal regions of TnT was associated with apoptotic cell death. Although muscle cells have high capacity of proteolysis to rapidly remove non-myofilament incorporated TnT protein, peak releases of TnT or TnT fragments from myofibrils may occur in the events of myocardial ischemia reperfusion and skeletal muscle fatigue or injuries. When the level of non-myofilament-associated TnT and TnT fragments exceeds the protective capacity of proteolytic removal in the muscle cell, they may impose cytotoxic effect and cause cell death. Therefore, the activity of non-myofilament-associated TnT or TnT fragments in inducing apoptosis and cell death is a potential pathogenic factor, particularly important in adult cardiac myocytes that lack the ability of regeneration.

2591-Pos Board B561

Proximity mapping of troponin T and troponin I in cardiac troponin using molecular dynamics simulations

Jayant J. Jayasundar[1], Jun Xing[2], John M. Robinson[2], Herbert C. Cheung[2], Wen-Ji Dong[1].
[1]The School of Chemical Engineering and Bioengineering and The Department of Veterinary and Comparative Anatomy Pharmacology and Physiology, Washington state university, Pullman, WA, USA, [2]The Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA.

The intersite distances from cTnT residues 240, 276, and 288 to cTnI N-terminal residues 5, 17, 27, 40, and to the cTnI C-terminal residues 131, 145, 152, 160, and 167 in reconstituted cTn were determined in the presence of Mg^{2+} and Mg^{2+} plus Ca^{2+} using FRET. The distances from cTnT residues 276 and 288 to cTnI residues 5, 17, 27 were also determined in the presence of Ca^{2+} with cTnI bisphosphorylated at Ser^{23} and Ser^{24}. The results showed that the transition of troponin from the Mg^{2+} state to the Ca^{2+} state was accompanied by small to moderate changes in distances, suggesting small global conformational changes. The distance changes were accompanied by changes in the half-width of the distributions of the distances. To clarify the structural basis for population broadening, we performed MD simulations with explicit solvent. The published NMR structure of the N-terminal region of cTnI was docked and integrated into cTn. The average ensemble structure showed interactions of the cTnI N-terminal region with cTnC. In the Mg^{2+} state, the cTnI N-terminal segment interacted with the defunct Ca^{2+}-binding site I and the functional site II in cTnC. In the Ca^{2+}state, non-phosphorylated cTnI interacted with the helix A and site I of cTnC. These interactions stabilized the open hydrophobic pocket in the N-domain of cTnC, and the cTnI regulatory region was constrained within the hydrophobic pocket. The bisphosphorylated segment of cTnI was bent, interrupting its interaction with Ca^{2+} site I of cTnC. This loss of interaction resulted in depressed opening of the cTnC N-domain, forcing the regulatory region of cTnI to move out from the hydrophobic pocket.

2592-Pos Board B562

The Rate Of Calcium Dissociation From The Cardiac Thin Filament Is Affected By Multiple Modulatory Factors

Bin Liu[1], Sean C. Little[1], Ryan S. Lee[1], Kristopher P. Kline[1], Darl R. Swartz[2], Svetlana B. Tikunova[3], Jonathan P. Davis[1].
[1]The Ohio State University, Columbus, OH, USA, [2]Purdue University, West Lafayette, IN, USA, [3]University of Houston, Houston, TX, USA.

The rate of cardiac muscle relaxation is dynamically modulated. We hypothesize that the rate of Ca2+ dissociation from the thin filament is at least one of the factors that can strongly influence the rate of relaxation. Thus, we would expect the various physiological and pathological stimuli that affect the kinetics of cardiac muscle relaxation to likewise affect the rate of Ca2+ dissociation from the thin filament. In this study, we investigated various modulators of the Ca2+ exchange kinetics in the physiologically relevant biochemical model systems of reconstituted thin filaments and rabbit ventricular myofibrils such as: 1) PKA phosphorylation of TnI, 2) ischemia-reperfusion associated truncation of TnI, 3) familial cardiomyopathy related mutations of TnI and TnT, 4) the calcium sensitizing compound bepridil, 5) rationally engineered TnC mutations, and 6) tropomyosin isoforms. Consistent with the effects of PKA on accelerating relaxation, the rate of Ca2+ dissociation from the thin filament was accelerated by TnI mutations (S23D,S24D) mimicking PKA phosphorylation. Additionally, the rate of Ca2+ dissociation was slowed by truncation of TnI (residues 1-192), consistent with ischemia-reperfusion slowing the rate of cardiac relaxation. The hypertrophic and restrictive cardiomyopathy mutations (TnIS166F and TnIR192H) slowed the rate of Ca2+ dissociation from

the thin filament, whereas the dilated cardiomyopathy mutations (TnTR141W and TnTdeltaK210) accelerated the rate of Ca2+ dissociation from the thin filament. The Ca2+ sensitizing compound bepridil had no effect on the rate of Ca2+ dissociation in either thin filaments or myofibrils, but engineered mutations of TnC can accelerate or slow the rate. Finally, the alpha and beta isoforms of tropomyosin slowed and accelerated the rate of Ca2+ dissociation from the thin filament, respectively. Thus, multiple factors can modulate the rate of Ca2+ dissociation from the thin filament.

2593-Pos Board B563

Kinetics of Ca^{2+} Dissociation-Induced Structural Transitions of Cardiac Thin Filament

Jun Xing[1], Jayant j. Jayasundar[2], Yexin Ouyang[2], **Wenji Dong[2]**.
[1]University of Alabama at Birmingham, Birmingham, AL, USA,
[2]Washington State University, Pullman, WA, USA.

Ca^{2+} induces structural transitions within the cardiac thin filament. The structural kinetics at the troponin-actin interface was investigated by Förster Resonance Energy Transfer (FRET) to understand the molecular basis underlying thin filament regulation. The kinetics of the Ca^{2+}-induced conformational changes at the cTnC N-domain, the cTnC-cTnI and the cTnI-actin interfaces were studied. The structural transition of the cTnC N-domain was examined by monitoring FRET between a donor (AEDANS) attached to one cysteine and an acceptor (DDPM) attached the other cysteine mutant of cTnC(13C/51C). The cTnC-cTnI interactions were investigated by monitoring the distance changes from cTnC(89C) to cTnI(151C) and cTnI(167C). Both cTnI(151C) and cTnC(167C) were labeled with AEDANS as FRET donor and cTnC(89C) was labeled with DDPM as the FRET acceptor. These two labeled cTnI mutants were also used to monitor Ca^{2+}-induced distance changes from cTnI residues 151 and 167 to the cysteine residue 374 of actin labeled with DABM as the FRET acceptor. Results from FRET Ca^{2+} titrations and stopped-flow kinetic measurements demonstrated that different structural transitions have different Ca^{2+} sensitivities and different Ca^{2+} dissociation-induced kinetics. Structural transitions involving the regulatory region and the mobile domain of cTnI occurred at fast kinetic rates, while the structural transitions involving transversal movement of the cTnI inhibitory region occurred at slow kinetic rates. Our results suggest a two-step deactivation of the thin filament upon Ca^{2+} dissociation. The first step may involve rapid binding of the mobile domain of cTnI to actin, which was kinetically coupled with the conformational change of cTnC N-domain and dissociation of the regulatory region of cTnI from the cTnC hydrophobic pocket. The second step involved the inhibitory region of cTnI switching its interacting from cTnC to actin. The latter processes may participate in regulating crossbridge kinetics.

2594-Pos Board B564

An Internal Domain of Beta Tropomyosin Increases Myofilament Calcium Sensitivity

Ganapathy Jagatheesan[1], Emily M. Schulz[1], Rafeeq PH Ahmed[1], Natalia Petrashevskaya[2], Arnold Schwartz[1], Gregory P. Boivin[1], Grace M. Arteaga[3], Stephen B. Liggett[2], R. John Solaro[3], David F. Wieczorek[1].
[1]University of Cincinnati College of Medicine, Cincinnati, OH, USA,
[2]University of Maryland, Baltimore, MD, USA, [3]University of Illinois at Chicago, Chicago, IL, USA.

Table of Contents

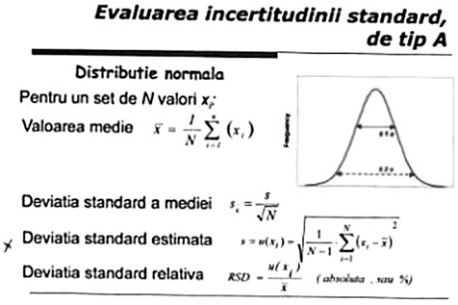
Tropomyosin (TM) is involved in calcium mediated muscle contraction and relaxation in the heart. Striated muscle alpha TM is the major isoform expressed in the heart. Expression of striated muscle beta TM in murine myocardium results in a decreased rate of relaxation and increased myofilament calcium sensitivity. Replacing the carboxyl terminus (amino acids 258-284) of alpha TM for beta TM (a troponin-T [TnT] binding region) results in decreased rates of contraction and relaxation in the heart and decreased myofilament calcium sensitivity. We hypothesized that the putative internal TnT binding domain (amino acids 175-190) of beta TM may be responsible for the increased myofilament calcium sensitivity observed when the entire beta TM is expressed in the heart. To test this hypothesis, we generated transgenic mice that express a chimeric TM containing beta TM amino acids 175-190 in the backbone of alpha TM (amino acids 1-174 and 191-284). These mice express 16% - 57% chimeric TM, and they do not develop cardiac hypertrophy or any other morphological changes. Physiological analysis shows these hearts exhibit systolic and diastolic dysfunction and a positive response to isoproterenol. Skinned fiber bundle analyses show a significant increase in myofilament calcium sensitivity. Biophysical studies demonstrate that the exchanged amino acids do not influence the flexibility of TM. This is the first study to demonstrate that a specific domain within TM can increase calcium sensitivity of the thin filament. Further, these results enhance the understanding of why TM mutations associated with familial hypertrophic cardiomyopathy also demonstrate increased myofilament sensitivity to calcium.

2595-Pos Board B565

The Assessment of Uncertainty in Measurement of Cholesterol: A Model of Calculation

Aurelian Udristioiu.
COUNTY HOSPITAL TARGU JIU, TARGU JIU, Romania.

The objective of this work was to identify all the components of uncertainty in measurement of Cholesterol, undergoing a reasonable estimation of results in the acceptable method.
Material and Method:
For identification of the uncertainty in measurement of Cholesterol in laboratory, running Hitachi 912 Roche Analyzer, was necessary the Standard Cholesterol (ST).
Results:
Was calculated, in function of Standard Deviation (SD) and Student Factor (t), by estimation (SD * t), Compound Uncertainty of ST, assembling Uncertainty of A type and Uncertainty of B type, in value of 11 mg%.
Conclusion:
Budget of Uncertainty, in assessment of Cholesterol was established to a permitted error of 11% in normal range and under cut-off.



Microtubules & Microtubule-associated Proteins

2596-Pos Board B566

Tau Directly Inhibits The On-rate Of Kinesin, For Microtubules, During Transport

Derrick P. McVicker, Christopher Berger.
University of Vermont, Burlington, VT, USA.

Kinesin belongs to a class of microtubule (MT) based molecular motors, which can facilitate the intracellular transport of vesicle bound cargo and organelles throughout the cell. This vital function is especially pronounced in the neuron, where transport down exceedingly long processes, such as axons and dendrites, must be efficiently accomplished and cannot be explained be diffusion alone. Like most cellular processes, regulation is a fundamental aspect of kinesin mediated intracellular trafficking and to date many modes to regulate kinesin have been elicited. Recently the microtubule associated protein (MAP), tau, has been implicated in playing a central role in the regulation of kinesin mediated transport in the neuron. Tau has previously been shown to reduce the procesivity and attachment frequency of kinesin motors on MTs. Although it has been demonstrated that tau has a dramatic affect on kinesin based transport, the mechanism by which this occurs is presently unknown. Using stopped-flow rapid kinetics, we demonstrate that tau directly affects the on-rate of kinesin for MTs causing the motor to dissociate from the MT track. Because kinesin releases ADP at a greatly accelerated rate in the presence of MTs, we can effectively monitor the on-rate of kinesin for MTs by following the release of the fluorescent ADP analog mantADP. We demonstrate the on-rate of kinesin, for MTs, is reduced by tau in both a concentration and isoform specific manner.

2597-Pos Board B567

Synchrotron X-ray Scattering Study of the Effects of Microtubule-associated-protein (MAP) Tau on Interprotofilament and Intermicrotubule Interactions

Myung Chul Choi[1,2], Uri Raviv[1,3], Herbert Miller[1], Michelle Gaylord[1], Erkan Kiris[1], Donovan Ventimiglia[1], Daniel J. Needleman[1,4], Mahn Won Kim[2], Leslie Wilson[1], Stuart Feinstein[1], Cyrus R. Safinya[1].