its >6000 anti-human polyclonal, affinity-purified antibodies of which 49 were identified by MS and 79 were novel. HPA antibodies were available for 82 the 142 proteins but only 43 reacted positively with the ICDs based on immunohistochemistry. We supplement these approaches with 14 proteins identified using conventional 2-DE. Combining all techniques. Only 14 proteins were common to the MS data, HPA antibodies and the literature. We can categorize all 273 identified ICD proteins according to their known functions and demonstrate their functional interactions in a single inter-active relationship according to their functions: (i) adhesion, anchoring and binding (88); (ii) enzymes (46); (iii) proteins that maintain the structure and function of the ICDs (35); (iv) myofibrillar (34); (v) channels (32); (vi) ligands and their receptors (18); (vii) cytoplasm proteins (6); and (viii) mechanoreceptors (4). We will now extend these analyses to ICD proteins that change as a result of human heart failure.

3933-Plat

Quantitative Analysis of MyBP-C Phosphorylation in Human Heart using Phosphate Affinity SDS-Page

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Phosphorylation sites in Cardiac MyBP-C have been predicted at Ser273,282 and 302 but studies in intact tissue have identified 5 phosphorylted sites and suggested up to 4.6molsPi/mol MyBP-C is present in human heart.

We analysed MyBP-C phosphospecies in human heart myofibrils by phosphate affinity SDS-PAGE using a non-specific antibody raised against the MyBP-C peptide 2-14. We observed six bands corresponding to 0, 1P, 2P, 3P, 4P, 5P phospho-species. Control experiments with pure MyBP-C indicated that the antibody labelled all phosphospecies equally. The assigned phosphorylation levels were confirmed by staining western blots with PhosTools phosphoprotein stain. This separation permits direct quantitative determination of MyBP-C phosphospecies without need for calibration.

In donor heart myofibrils the highly phosphorylated species predominated: 0, $7 \pm 3\%$: 1P, $1 \pm 1\%$: 2P, $23 \pm 7\%$: 3P, $41 \pm 2\%$: 4P, $20 \pm 8\%$ (n=4) from which total phosphorylation of MyBP-C was calculated to be 3.4molsPi/mol. In failing heart unphosphorylated MyBP-C predominated (0, $48 \pm 4\%$: 1P, $4 \pm 4\%$: 2P, $27 \pm 5\%$: $1 \pm 1\%$: 3P, $17 \pm 4\%$: 4P, $4 \pm 2\%$, n=4) and calculated total phosphorylation was 1.5 molsPi/mol. Total phosphorylation in failing heart myofibrils was 44% of donor and in myectomy samples from HCM patients it was 29% of donor, compared with 45 and 40% respectively determined in previous assays.

We conclude that MyBP-C is highly phosphorylated in vivo with significant phosphorylation of at least 5 sites and that phosphorylation is dynamic, being greatly reduced in pathological muscle. Initial tests using antibodies specific to Ser 273, 282 and 302 show distinct patterns on phosphate affinity SDS-PAGE indicating varying preferences for the highly phosphorylated species of MyBP-C in normal and pathological muscle.

3934-Plat

Identification of Amino Acid Residues in the Cardiac Myosin Binding Protein-C Motif Important for Actin Binding

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N-terminal domains of cardiac myosin binding protein-C (cMyBP-C) can activate actomyosin interactions in the absence of Ca^{2+} and bind to actin in a phosphorylation dependent manner. We have previously shown that two N-terminal domains, C1 and the MyBP-C motif (''M'') domain, bind specifically to actin and to thin filaments; however, the sequences or residues that mediate actin binding have not been identified. The goal of this study was to identify residues in the M-domain that contribute to actin binding and to investigate whether interactions between the M-domain and actin mediate the activating properties of cMyBP-C. We therefore used alanine-scanning mutagenesis to target candidate actin binding sites in the M-domain that bear homology to the actin binding motifs in other known actin binding proteins and to assess the effects of mutations on the ability of recombinant proteins to bind actin and activate actomyosin interactions in motility assays. Results demonstrate that mutation of select positively-charged amino acids in the M-domain that are homologous to binding motifs in known actin binding proteins reduced binding of cMyBP-C to actin. The mutations also reduced or eliminated the activating properties of recombinant cMyBP-C in *in vitro* motility assays. However, mutation of other positively-charged amino acids did not affect actin binding or protein functional properties. These results indicate that specific residues within the M-domain confer actin binding and that interactions with actin contribute to the functional effects of recombinant cMyBP-C N-terminal proteins. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

3935-Plat

N-Terminal Fragments of Cardiac Myosin Binding Protein-C Inhibit Actomyosin Motility by Tethering Actin

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Cardiac myosin binding protein-C (cMyBP-C) mutations are a leading cause of hypertrophic cardiomyopathy. cMyBP-C has 11 domains, C0 through C10, that bind sarcomeric proteins, including myosin and actin. A 29 kD N-terminal fragment (C0C1f) of cMyBP-C containing the first two domains C0 and C1 and the first 15 residues of the conserved MyBP-C motif is cleaved from cMyBP-C following ischemic-reperfusion injury (Sadayappan et al., JMCC 44:S44, 2008). Expressed C0C1f fragments inhibit actin velocities in the motility assay at a 2:1 molar ratio to myosin, similar to other N-terminal fragments: C0C3, C0C2, and C1C2. Interestingly, fragments containing only the C0C1 domains do not alter velocity, suggesting the additional 15 residues in C0C1f are necessary for inhibition. Adding C0C1 to the motility assay can partially reverse the C0C3-mediated inhibition of velocity, suggesting C0C1 may compete with C0C3 for actin binding. cMyBP-C fragments may affect motility by creating a tether between actin and the flowcell surface. To test this, motility experiments were performed under high ionic strength, saturating MgATP, and in the absence of methylcellulose, conditions in which most actin filaments diffuse away from the surface due to weak interactions with myosin. In the presence of C0C2, many actin filaments bound and translocated on the surface, confirming this fragment's tethering capacity. Additionally, in the laser trap we adhered C0C3 fragments to a bead in the absence of myosin and observed C0C3 transiently binding to a single actin filament with an ~100 ms attached lifetime. We also saw evidence that C0C3 may partially unfold under load. These experiments strongly suggest that N-terminal domains of cMyBP-C containing the MyBP-C motif tether actin filaments and provide one mechanism for modulating actomyosin motion generation, i.e. by imposing an effective viscous load within the sarcomere.

3936-Plat

PKC Phosphorylation of Titin's PEVK Element–A Novel and Conserved Pathway for Modulating Myocardial Stiffness

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Protein Kinase C (PKC) regulates contractility of cardiac muscle cells by phosphorylating multiple thin- and thick- filament based proteins, and plays key roles in development of cardiovascular pathologies. Myocardial sarcomeres also contain a third myofilament, titin, which we demonstrate here to also be phosphorylated by PKC. Titin phosphorylation was observed in skinned myocardial tissues following incubation with PKCα and this effect was exacerbated ~5 fold in the mouse and ~2.5 fold in the pig by preincubation with Protein Phosphatase 1 (PP1). In vitro phosphorylation of recombinant protein representing titin's spring elements shows that PKCα targets the PEVK spring element. Mass spectrometry in combination with site-directed mutagenesis identified two highly conserved sites in the PEVK region that are phosphorylated by PKCα (S11878 and S12022); when these two sites are mutated to alanine, phosphorylation is effectively abolished. Mechanical experiments with murine and porcine skinned LV myocardium revealed that PKCα significantly increases titin-based passive tension in a sarcomere length (SL)-dependent fashion. Single molecule force-extension curves show that PKCα decreases the PEVK persistence length (from 1.20 nm to 0.55 nm), without altering the contour length, and using a serially-linked wormlike chain (WLC) model we show that this results in an ~20% increase in titin-based passive force with a SL dependence that is similar to that measured in skinned myocardium following PKCα phosphorylation. We conclude that PKC phosphorylation of titin is a novel and conserved pathway that links myocardial signaling and myocardial stiffness.

3937-Plat

Titin Strucure and Extensibility in Healthy and Failing Hearts

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The giant sarcomeric protein, titin is the primary determinant of myocardial passive stiffness. In failing human hearts however, it has been proposed that reduced myofilament passive tension is due to an altered titin isoform expression profile[1, 2]. In this study we set out to directly quantify the tensile strength of titin molecules isolated from healthy and diseased myocardium.

Heart failure was induced in adult male ferrets by ascending aortic coarctation. All procedures accorded with The United Kingdom Animals (Scientific Procedures) Act, 1986. Protein extracts from sham and failing hearts were separated by 2% SDS PAGE. The mean ratio of the major titin isoforms N2BA:N2B increased from 0.3 in control to 0.5 in failing hearts (n=6, $p<0.01$).
Titin molecules were isolated from left ventricle, aligned and stretched by a receding liquid meniscus (which applied a tensile force of ~60pN)[3] and visualised by atomic force microscopy. Combed titin molecules exhibited a straightened and beaded appearance. The mean molecular diameter of titin decreased in failing hearts compared to control (0.26 ± 0.001nm *vs* 0.33 ± 0.001nm, $p<0.001$, n=104-130 molecules, 3 animals per group). This difference was more pronounced in the shorter molecules (<3.5 μm). The mean distance between beads was increased in failing hearts (49.3 ± 1.5nm *vs* 126.8 ± 4.5nm, $p<0.001$, n=370-429, 3 animals per group). The decreased titin molecular diameter combined with an increased inter-bead distance suggests that titin from failing hearts is less resistant to tensile forces when compared to control, and may help to explain the decreased titin-based passive tension observed in diseased hearts.
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3938-Plat
Insulin Signaling Regulates Cardiac Titin Isoform Composition in Development and Diabetic Cardiomyopathy
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Isoform switching of the giant elastic protein titin is a main mechanism for adjusting passive myocardial stiffness in perinatal heart development and chronic heart disease. Previous evidence suggested that thyroid hormone (T3) signaling converging onto the phosphoinositol-3-kinase (PI3K)/AKT pathway is an important determinant of the cardiac titin-isoform pattern in developing cardiomyocytes. We hypothesized that other activators of PI3K/AKT, particularly insulin, may similarly alter the titin-isoform composition, thereby modifying titin-based stiffness. Embryonic rat cardiomyocytes were cultured in medium containing 0.5% hormone-reduced serum and were treated with 175 nmol/L insulin for seven days. Analysis of titin-isoform expression by 2% SDS-PAGE showed a significant increase in the mean proportion of the stiff N2B titin isoform (3,000 kDa), from 53% in control cells to 64% in insulin-treated cells, the remainder being the more compliant N2BA isoform (>3,200 kDa). This insulin-dependent titin-isoform shift was blocked in the presence of PI3K-inhibitor, LY294002, suggesting that insulin regulates the cardiac titin-isoform pattern by activating the PI3K/AKT pathway. Whether this mechanism operates in vivo was studied by testing the effect of insulin deficiency on titin-isoform expression in streptozotozin-treated (STZ) rats as a model for diabetes mellitus (type 1). Within four months, STZ rats developed cardiac hypertrophy and mild left ventricular (LV) fibrosis, concomitant with elevated glucose levels. The mean proportion of N2B-titin was significantly decreased from 86% in control LV to 78% in LV of STZ rats. Wormlike chain modeling of titin elasticity suggested that such a change reduces titin-based passive stiffness by ~6%. Results of mechanical measurements on skinned cardiac fiber bundles confirmed minor passive stiffness modifications in STZ myocardium. We conclude that insulin signaling regulates titin-isoform composition in cardiac development and could also contribute to altered diastolic function in diabetic cardiomyopathy.

3939-Plat
The Effect of Stiffness and Beta-Adrenergic Stimulation on Neonatal Cardiomyocyte Calcium-Mediated Contractile Force Dynamics
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Embryonic or induced pluripotent stem cells have great potential to treat multiple cardiopathologies. Current limitations include a lack of understanding how contractility of immature cardiomyocytes is affected by microenvironment mechanical properties and beta-adrenergic stimulation. Inability to apply traditional force assessment techniques to immature cardiomyocytes led us to utilize 6 micrometer spaced arrays of elastomer-based microfabricated post force sensors. Posts act as cantilever springs with tunable constants (kp (nN/micrometer)), deflecting linearly in response to cultured cell's acto-myosin contraction transmitted through focal adhesions formed at their tips. We combined this method with an IonOptix system for real-time post displacement and intracellular Ca++ flux monitoring. We found for neonatal rat cardiomyocytes (NRCs) exposed to nanomolar concentrations of the β-adrenergic stimulant isoproterenol for ~2 minutes Ca++ flux decreased with little effect on flux rates. Maximum contractile force increased by as much as 90 % with 100 nM isoproterenol along with a significant increase in relaxation rate (kr). NRCs cultured on post arrays with an effective modulus (calculated based upon the kp) greater than normal cardiac extracellular matrix (~29.3 kPa vs. 10-20 kPa, respectively) generated the largest force per post (56.0 ± 9.8*! nN) and the fastest kr ($0.44 \pm 0.08 \cong \exists$ nN/ms) compared to post with Ep~10.6 kPa (16.1 ± 2.7!% nN, and 0.16 ± 0.030# nN/ms) and Ep~23.0 kPa (30.6 ± 4.5*% $\cong$ nN, 0.14 ± 0.023#$\exists$ nN/ms). Interestingly, kr for lower Ep values remained unchanged (#pvalue=0.349) while force increased with stiffness. Immunofluorescence staining revealed that myofibril actin and z-disc associated vinculin were more organized into parallel, longer fibers on stiffer post arrays. Increased contractility on stiffer posts also correlated with increased isoproterenol effects. The results indicate stiffness of the microenvironment at NRC's focal adhesiveness play a critical role in determining contractility and β-adrenergic responsiveness. (*,!,%,$\cong$pvalue<0.05, $\pm$SEM) HL061683(MR); NSF CAREER and HL097284(NS)

Platform BG: Nano-Materials

3940-Plat
Bionanoelectronic Devices Based on 1d-Lipid Bilayers on Nanotube and Nanowire Templates
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Biological molecules perform sophisticated functions in living systems with complexity often far exceeding most of man-made devices and objects. Direct integration of biological components with electronic circuits could drastically increase their efficiency, complexity, and capabilities and result in novel sensing and signaling architectures. Yet, one of the obstacles for this vision of a bionanoelectronic circuit is the absence of a versatile interface that facilitates communication between biomolecules and electronic materials. We have been building platforms that integrates membrane proteins with one-dimensional inorganic materials such as carbon nanotubes and silicon nanowires. In our devices, a nanotube of nanowire is covered by a lipid bilayer that serves both as a universal membrane protein matrix and an insulating shield. I will discuss the fabrication and properties of these ''shielded'' nanowires and of their use in bionanoelectronic devices that incorporate working membrane proteins in an electronic circuit.

3941-Plat
Molecular Dynamics Study of CNT Nanopores Embedded in Lipid Bilayers
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There is considerable interest in the use of carbon nanotubes (CNTs) as 'nanosyringes'that span membranes. These nanosyringes form biomimetic pores capable of drug delivery or of the selective transport of ions and water in biosensor devices. To date, a number of different types of simulation system have been used to explore the transport properties of CNT nanopores. These range from isolated CNTs in water, through to CNTs in a bilayer-mimicking 'slab'. However, it has been shown that what lies outside a nanopore may have an important effect on its transport properties, arguing for more realistic membrane models to be used. Up to now, few studies have addressed the transport properties of CNT nanopores embedded in a phospholipid bilayer. This more complex system may capture important effects of the membrane environment on the functional behaviour of the nanopore. Here we use molecular dynamics to simulate CNT nanopores that are embedded in a lipid bilayer. We explore how the size of the nanopore influences both its interactions with the lipid bilayer and the transport properties through the pore.

3942-Plat
Single-Step Coating and Bifunctionalization of Gold Nanoparticles
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Metal nanostructures are attracting increasing attention in bio-sciences owing to their versatility and the peculiarities of their optical properties1,2. Their exploitation, however, often demands stable and biocompatible multifunctional surface coating.
We shall present a single-step method to coat and functionalize gold nanoparticles (NPs) with two distinct reactive groups by a properly designed peptide. NPs were prepared by reducing tetrachloro auric acid in water. The peptide we employed bonds to the NP by the N-Cysteine aminoacid and terminates with a C-terminal Lysine. In this way we can produce stable nanospheres