The aim of this work was to investigate calcium involvement in Hsp70 expression in both depolarized and IL-6 treated skeletal muscle cells.
We observed that electrical stimulation of myotubes increases Hsp70 mRNA level and protein expression. Depolarization performed in the presence of the intracellular calcium chelator BAPTA-AM resulted in a complete inhibition of Hsp70 induced expression. Inhibitors of IP_3-dependent calcium signals like 2-aminoethoxydiphenyl borate (2-APB) and LY294002, decreased Hsp70 mRNA induction and the protein expression in depolarized cells. In addition we determined that inhibitors of calcium dependent PKC abolished Hsp70 mRNA induction.
We established that IL-6 treatment of myotubes induced changes in intracellular calcium and promoted the increase of Hsp70 mRNA levels. Observed calcium transients could be associated to early events of IL-6-mediated Hsp70 expression.
Our results provide evidence for the involvement of slow calcium transients and PKC in the activation of Hsp70 expression in skeletal muscle cells and suggest that intracellular calcium signals also participate in IL-6 induced Hsp70 expression.
FONDAP 15010006

622-Pos Board B501
Ryanodine receptor 1 signaling in dendritic cells
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Dendritic cells express the skeletal muscle ryanodine receptor (RyR1), yet little is known concerning its physiological role and activation mechanism. In the present report, we provide evidence that dendritic cells also express the Cav1.2 subunit of the L-type Ca2+ channel and that release of intracellular Ca2+ via RyR1 depends on the presence of extracellular Ca2+ and is sensitive to ryanodine and nifedipine. Interestingly, RyR1 activation causes a very rapid increase in expression of MHC II molecules on the surface of dendritic cells, an effect which is also observed upon incubation of mouse BM12 dendritic cells with transgenic T cells whose T cell receptor is specific for the I-Abm12 protein. Based on the present results, we suggest that activation of the RyR1 signaling cascade may be important in the early stages of infection, providing the immune system with a rapid mechanism to initiate an early response, facilitating the presentation of antigens to T cells by dendritic cells prior to their full maturation.

623-Pos Board B502
Understanding Interval-Force Relations in the Rat Ventricular Myocytes with a Computational Model
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We have developed a model of cardiac excitation-contraction (EC) coupling for the rat ventricular myocytes based upon the published experimental literature and our previous modeling work in the guinea pig ventricular myocyte. Due to the rapid rate of Ca2+ cycling in the rat (resulting from the high heart rate) there are fundamental differences from the guinea pig myocyte data which was modeled previously. This requires the reformulation of Ca2+-handling mechanisms. The rat has a shorter action potential with different morphology than the guinea pig. This is thought to be due to the properties of the potassium channels. We reformulate three potassium currents (Ito, Iss, IK1), which give strong characteristics of the cardiac AP in rat ventricular myocytes (no plateau phase and shorter APD) and the Na+ current, based on the experimental literature. This new formulation simulates the negative force-frequency relation observed in experiments on rat ventricular myocytes with the appropriate changes in action potential duration and different pacing frequencies. The SR Ca2+ load decreases with increased frequency in contrast to the guinea pig which does the opposite and has a dome shaped force-frequency relation. Furthemore, the action potential duration increases with pacing rate opposite the guinea pig. The new rat model also simulated mechanical restitution and low levels post-extrasystolic potentiation levels seen in experiments with rat.

624-Pos Board B503
Ca++T LAB - A Computational Model for Intracellular Calcium Signaling In T Lymphocytes
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Calcium signaling is crucial for T cell activation. T cell receptor activation leads to the down-stream generation of IP3 which opens IP3 receptors on the membrane of the endoplasmic reticulum (ER), resulting in an initial small rise in cytoplasmic Ca^{2+}. T cells do not have sufficiently large Ca^{2+} stores to sustain Ca^{2+} elevation and therefore require additional Ca^{2+} influx through the voltage-independent Ca^{2+} channel CRAC, which is encoded by the ORAI1 or CRACM1 gene and is activated by STIM1, which ''senses'' decrease in ER Ca^{2+} content. However, CRAC can only bring in Ca^{2+} at negative membrane potentials. This potential is generated by the potassium channels Kv1.3 and KCa3.1 which drive the membrane potential back to -60 mV and facilitate Ca^{2+} entry for the duration of T cell activation ultimately leading to cytokine production and T cell proliferation. While there have been tremendous strides acquiring biophysical data on the implicated ion channels this data has not been integrated into a dynamic model. A computer model has been developed that integrates the available data on calcium signaling in T lymphocytes and that can be utilized to illustrate and theoretically probe T cell activation. The design is implemented in LabVIEW and can be run on a PC. The program allows free reign over the cell's environment and channel characteristics. The interface provides visualization of IP3 production, membrane potential, charge flow and open probability of the aforementioned channels. We suggest this program as a tool for identifying pharmaceutical targets for intervening with immune cell activation and as a teaching aid for immunology, physiology and cell signaling.

Actin & Actin-binding Proteins

625-Pos Board B504
Actin Branching Is Affected by Local Bending of the Mother Filament
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Actin filaments serve as structural elements of the cytoskeleton subject to mechanical forces and provide binding sites for actin-binding proteins (ABPs). Structural studies have established that actin filaments can adopt several different twist structures that are stabilized by ABPs such as ADF/cofilin or scruin. We sought to investigate whether strain on the actin filament due to bending affects the binding or function of ABPs and chose the Arp2/3 complex, which is involved in the assembly of force-generating actin networks, as our initial target. The Arp2/3 complex nucleates the growth of actin branches from pre-existing filaments, making it easy to detect where on the mother filament it has bound. To test the effect of bending on branching, biotinylated and rhodamine-phalloidin-stabilized actin filaments were bound to a streptavidin-coated glass surface, immobilized in a distribution of bent conformations. These tethered mother filaments were then incubated with actin, Arp2/3, and its activator. Growth of branches was stabilized with green fluorescent phalloidin. Branches originating from highly curved sections (radius of curvature < 1.5um) of actin filaments were more likely to grow from the extended side of the filament (positive strain) than from the compressed side (negative strain), with a statistically significant ($P < 0.05$) difference. To elucidate structural distortions that may give rise to this effect, we used Monte Carlo simulations based on a coarse-grained model of the actin filament to estimate the changes in inter-monomer spacing that occur for the experimentally probed range of filament curvatures. We conclude that mother filament bend strain is sufficient to alter actin branching and may play an important role in the organization of actin networks growing under load. Similar bend-induced effects may be important for other ABPs and provide a mechanism for mechanotransduction in cells.

626-Pos Board B505
Mechanics of Biophysical Networks with Flexible Cross-links
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Various mechanical properties and functions of eucharistic cells largely originate from the cytoskeleton. The predominant cytoskeletal constituent is the biopolymer filamentous actin (F-actin). In the presence of various cross-linking proteins, F-actins can comprise two rather different structures: isotropic orthogonal networks or bundled fibers. Actin bundles are formed mostly by short and stiff cross-linking proteins (like α-actinin and scruin), while large and flexible cross-linkers, such as filamin, lead to an orthogonal network. Orthogonal networks can also be formed at lower concentrations of short cross-linking proteins, but rheological experiments of in vitro F-actin networks showed that the mechanical response of such networks is different from that of networks cross-linked with filamin. Moreover, atomic force microscope stretching experiments on filamin demonstrated the possibility of force-induced domain unfolding, characterized by a sawtooth-like pattern in the force-displacement curve. Here we present a 3D discrete model of F-actin networks that extends our previous, rigidly cross-linked network model by incorporating a flexible cross-linking model for human filamin A (hsFLNa). The implemented hsFLNa element has a highly nonlinear response to stretching, incorporating the transition to a softer response that characterizes filamin domain unfolding. Simple shear

simulations of F-actin/hsFLNa networks show that the response of such networks is dominated by the behavior of the hsFLNa cross-linkers, while F-actin behaves almost rigid. We observe that force-induced unfolding of the hsFLNa relaxes the stresses in actin filaments, thus allowing for large network strains. By contrast, the shearing of F-actin networks with rigid cross-links leads to a large number of actin filaments stressed well beyond their breaking force. An increase in actin concentration increases the initial shear modulus, while the maximum network stiffening depends on the hsFLNa axial stiffness. The calculated initial modulus of F-actin/hsFLNa networks is found to be comparable with experimental measurements.

627-Pos Board B506

Intracellular Particles Involved in Stress Fiber Formation through Remodeling of Actin Filament Networks

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Formation of stress fibers, the actin filament (F-actin) bundles that align in a highly-ordered manner, is crucial for cell migration. The migrating cells retract their tails by contraction of stress fibers. The alignment angle of stress fibers controls the direction of cell migration. In the process of cell migration, stress fibers formed in lamellae, compensating for the contracted stress fibers. Although the alignment angle of the newly forming stress fibers is important for the regulation of cell migration, the mechanism that determines the direction of stress fiber formation is poorly understood.

To elucidate the mechanism of direction determination of stress fiber formation, we observed dynamics of actin cytoskeleton in lamellae of living fibroblasts. By using the scanning probe microscopy (SPM), we established the method to visualize actin cytoskeleton in living cells with the nanometer-scale spatial resolution and the second-scale temporal resolution. As a result of the time-lapse SPM observation, we found the submicron-size particles included in the mesh-like F-actin networks were replaced by the newly-forming stress fibers. The particles moved in the opposite direction of stress fiber formation. Further observation revealed that the new stress fibers formed in the region between the pre-existing stress fibers and the particles. We also revealed that the stress fiber formation resulted from the remodeling of the pre-existing F-actin networks. Inhibitory studies showed that actomyosin and Rho-kinase, both essential for stress fiber formation, regulated the movement of the particles. Immunofluorescent studies showed that vinculin, a focal adhesion protein, and F-actin were localized at some particles. From these results, we propose a model for the direction determination of stress fiber formation induced by the particles.

628-Pos Board B507

Purification Of Cytosolic Actin By Affinity Chromatography Using C-terminal Half Of Gelsolin

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Actin filaments in living cells undergo continuous dynamic turnover and remodeling. These processes involve polymerization, depolymerization, severing, capping, and branching of actin filaments through the interaction with a vast array of actin binding proteins. Cytoplasmic actin had previously been purified by the affinity chromatography using the immobilized DNase-I. which binds to G-actin with high affinity ($K(d) = 0.05$ nM). After being eluted from a DNase-I column, actin had to be exposed to high concentrations of a denaturant, such as 10 M formamide or 3 M guanidine-hydrochloride. We introduced a new method of the cytosolic actin purification, based on the affinity chromatography using a carboxyl-terminal half of gelsolin (G4-G6), which is an actin filament severing and capping protein, without the use of a denaturant. G4-G6 strongly binds to G-actin ($K(d) = 30$ nM) and has the actin-nucleating activity. His-tagged G4-G6 (His-G4-G6) was expressed in Escherichia coli and purified by Ni-affinity chromatography. When His-G4-G6 was added to a lysate of HeLa cells or insect cells infected with a baculovirus, expressing the beta-actin, in the presence of calcium and incubated overnight at 4 degrees centigrade, His-G4-G6 bound to actin with a 1:1 stoichiometry. His-G4-G6-actin complex was purified with Ni-agarose resin, and only actin was eluted from Ni-column by calcium chelation. To examine whether the purified actins were functional, we measured the polymerizability of actins and the velocity of actin filaments in an in vitro motility assay on myosin V. At this meeting, we report the properties of purified actins.

629-Pos Board B508

Actin Polymerization In Differentiated Vascular Smooth Muscle Cells Requires Vasodilator-Stimulated Phosphoprotein (VASP)

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Our group has shown that alpha agonists and phorbol esters increase net actin polymerization in differentiated vascular smooth muscle cells (dVSMC) and that actin polymerization is linked to contractility. However, the underlying mechanisms are still largely unknown. Inhibition of actin filament elongation by cytochalasin-D treatment decreases contractility without changing the level of myosin light chain phosphorylation in this tissue, suggesting that actin filament elongation processes are necessary for smooth muscle contraction. The enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) family of proteins is associated with actin filament elongation in non-muscle systems. In this study, we evaluated the possible functions of Ena/VASP in dVSMC. Among Ena/VASP proteins, only VASP is highly expressed in ferret aorta. High resolution 3-D deconvolved fluorescent images of immunostained freshly dissociated aorta cells show that VASP partially colocalizes with both alpha-actinin and vinculin, markers of dense bodies and dense plaques in dVSMC. Profilin, which is known to associate with monomeric G-actin and VASP to facilitate actin filament elongation also colocalizes with both alpha-actinin and vinculin, potentially identifying both the dense bodies and the dense plaques as hot spots of actin polymerization. Differential centrifugation and imaging data indicate that VASP may undergo subtle conformational or/and positional changes in response to stimuli. The EVH1 domain of VASP is known to be responsible for targeting VASP to its sites of action. Introduction of an expressed EVH1 domain of Ena/VASP, made as a chimeric protein with the TAT transduction tag, acted as a decoy to inhibit stimulus-induced increases in actin polymerization. In contrast, introduction of the EVH1 mutant F78S, which does not bind target poly-Pro sequences, had no effect. Thus, VASP may be involved in actin filament assembly at dense bodies and dense plaques in dVSMC. Support: NIH P01 HL86655.

630-Pos Board B509

Actin - Myosin Interaction

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Muscle contraction is resulted from the interaction of myosin with actin and ATP. The study of kinetics of binding of myosin subfragment 1 (S1) to F-actin revealed the two step binding, which were modeled by initial binding of S1 to one actin monomer (state 1) and then to the second neighboring monomer (state 2). The results of time-resolved cross-linking of S1 and F-actin upon their rapid mixing in stopped flow apparatus directly demonstrated that myosin head initially binds through the loop 635–647 to the N-terminus of one actin and then through the loop 567–574 to the N-terminus of the second actin (Andreev & Reshetnyak, 2007, J. Mol. Biol. 365(3), 551–554). The computational docking of S1 with F-actin demonstrated that both actin monomers are located in the same strand of F-actin with the first and second actins being close to the pointed and barbed ends of F-actin, respectively. The closing of the main cleft in 50 kDa of S1 might prevent binding of S1 with two actins since the distance between loops 635–647 and 567–574 became too short to interact with N-termini of two actins simultaneously. Depending on degree of saturation of F-actin with S1s there are two structurally different complexes are formed: at complete saturation each S1 binds only one actin and its cleft is closed while at partial saturation S1 interacts with two actins and its cleft is opened. The transition between one- and two-actin binding states of myosin accompanying with opening the cleft in central domain of S1 might be associated with force generation. The formation of actin-myosin interface would be associated with the energy release that might be used in part for the generation of force in muscle.

631-Pos Board B510

A Thermodynamic Model Describing the Mechanosensitivity of Actin-cofilin Binding

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Tensile forces cause actin filaments to resist cofilin severing longer than those filaments not subjected to force. This actin-cofilin binding mechanosensitivity leads to the selective formation and maintenance of cellular stress fibers; however, the mechanism behind this mechanosensitivity remains elusive. Because the actin-cofilin binding increases the torsion angle applied to the double helical filament, the actin-cofilin affinity correlates with the degree of torsion applied to the filament.

We propose a thermodynamic model describing the mechanosensitivity of actin-cofilin binding. The chemical potential difference between the cytosolic