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 IP3-dependent calcium signals including 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 calcium release in intracellular calcium chelator BAPTA-AM resulted in a complete inhibition of Hsp70 expression. Depolarization performed in the presence of the Cav1.2 subunit of the L-type Ca\(^{2+}\) channel and that release of intracellular Ca\(^{2+}\) via RyR1 depends on the presence of extracellular Ca\(^{2+}\) 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.

**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 ADP/ATP-cotinin 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. We measured the effect of bending on branching, bioinylated 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 < 1.5μm) 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 change the inter-monomer spacing that occurs for the experimentally probed range of filament curvatures. We conclude that mother filament bend strain is sufficient to alter actin branch inhibition 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 eukaryotic cells largely originate from the cytoskeleton. The predominant cytoskeletal constituent is the bio-polymer 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 microscopy studies performed 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 net-
works 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
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 net-
work—alize actin cytoskeleton in living cells with the nanometer-scale spatial resolu-
tion. In addition, we observed dynamics of actin cytoskeleton in lamellae of living fibroblasts. By
using time-lapse scanning probe microscopy (SPM), we established the method to viss-
alize actin cytoskeleton in living cells with the nanometer-scale spatial resolu-
tion 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 net-
works. Inhibitory studies showed that acto-nemysin and Rho-kinase, both essential for stress fiber formation, regulated the movement of the particles. Immunoflu-
orescent 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 direc-
tion determination of stress fiber formation induced by the particles.

628-Pos Board B507
Pericellular Organization 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 re-
modeling. These processes involve polymerization, depolymerization, sever-
ing, capping, and branching of actin filaments through the interaction with a vast array of actin binding proteins. Cytosplasmic 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 chroma-
tography using a carboxy-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 bound to the N-terminal of F-actin and the His-actin mixture was expressed in Escherichia coli and purified by 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, and vinculin, markers of dense bodies and dense plaques in dVSMC. Protein, which is known to associate with monomeric G-actin and VASP, facilitates actin filament elongation and also localizes 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 re-
sponse to stimuli. The EVH1 domain of VASP, made as a chimeric protein with the TAT transduction tag, acted as a decoy to inhibit stimulus-induced increases in actin polymeriza-
tion. In contrast, introduction of the EVH1 mutant F785S, 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 HL66565.

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 ini-
tially 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 sat-
uration each S1 binds only one actin and its cleft is closed while at partial sat-
uration 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 coil contraction severing longer than filaments not subjected to force. This actin-cofilin binding mechanosensitivity leads to the selective formation and maintenance of cellular stress fibers; how-
ever, the mechanism behind this mechanosensitivity remains elusive. Because the actin-cofilin binding increases the torsion angle applied to the double heli-
cal filament, the actin-cofilin affinity correlates with the degree of torsion ap-
plicated to the filament. We propose a thermodynamic model describing the mechanosensitivity of actin-cofilin binding. The chemical potential difference between the cytosolic