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 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

Actin & Actin-binding Proteins

Actin Branching Is Affected by Local Bending of the Mother Filament

Viviana Risca1, Ovijit Chaudhuri1, JiaJun Chia2, Daniel A. Fletcher2.
1University of California, Berkeley, Berkeley, CA, USA, 2The Harker School, San Jose, CA, USA.

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 or cofilin. 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. Since the effect of bending on branching, bio- and submicroscopic 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 change the inter-monomer spacing. Similar bend-induced effects may be important for other ABPs and provide a mechanism for mechanotransduction in cells.

Mechanics of Biophysical Networks with Flexible Cross-links

Goran Zagar, Patrick R. Onck, Erik Van der Giessen.
University of Groningen, Groningen, Netherlands.

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 filamin), 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 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

Actin Branching Is Affected by Local Bending of the Mother Filament

Viviana Risca1, Ovijit Chaudhuri1, JiaJun Chia2, Daniel A. Fletcher2.
1University of California, Berkeley, Berkeley, CA, USA, 2The Harker School, San Jose, CA, USA.

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 or cofilin. 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. Since the effect of bending on branching, bio- and submicroscopic 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 change the inter-monomer spacing. Similar bend-induced effects may be important for other ABPs and provide a mechanism for mechanotransduction in cells.

Mechanics of Biophysical Networks with Flexible Cross-links

Goran Zagar, Patrick R. Onck, Erik Van der Giessen.
University of Groningen, Groningen, Netherlands.

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 filamin), 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 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 net-
works is dominated by the behavior of the hsFLNa cross-links, 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 compara-
tible with experimental measurements.

627-Pos Board B506
Intraacellular Particles Involved in Stress Fiber Formation through Remodeling of Actin Filament Networks
Kazushi Tamura, Takanori Mizutani, Hiashi Haga, Kazushige Kawabata.
Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan.
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 re-
tract their tails by contraction of stress fibers. The alignment angle of stress fi-
bers 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 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 visual-
ize 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 actomyosin 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
Perfusion Of Cytosolic Actin By Affinity Chromatography Using C-terminal Half Of Gelsolin
Takashi Ohki, Kowshik Ohyama, Shin’ichi Ishiwata.
Waseda University, tokyo, Japan.
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. 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 chroma-
tography 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 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, we measured the polymerizability of actin and the velocity of actin filament in an in vitro motility assay on myosin V. At this meeting, we report the prop-
erties of purified actins.

629-Pos Board B508
Actin Polymerization In Differentiated Vascular Smooth Muscle Cells Requires Vasodilator-Stimulated Phosphoprotein (VASP)
HakRim Kim, Francois Ferron, Malgorzata Boczkowska, Philip Graceffa, Cynthia Gallant, Paul Leavis, Roberto Dominguez, Kathleen G. Morgan.

1Boston University, Boston, MA, USA, 2University of Pennsylvania School of Medicine, Philadelphia, PA, USA, 3Boston Biomedical Research Institute, Watertown, MA, USA.

Our group has shown that alpha agonists and phosphodiesters 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 fila-
ment 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 reso-
lution 3-D deconvolved fluorescent images of immunostained freshly associ-
ated 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 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
well to 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 HL6655.

630-Pos Board B509
A Thermal Model Describing the Mechanosensitivity of Actin-myosin Interaction
Cynthia N. Prudence, Jim Segala, Yana Reshetnyak, Oleg Andreev.
1University of Rhode Island, Kingston, RI, USA, 2UT Southwestern Medical Center, Dallas, TX, USA.

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 satu-
ration each S1 binds only one actin and its cleft is closed while at partial satu-
ration S1 interacts with two actins and its cleft is opened. The transition between one- and two-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.