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.

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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.

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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.

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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.

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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

cofilin and cofilin bound to the filament in our model is expressed as a function of the torsion angle on the cofilin-bound filament based on the Gibbs-Duhem relation. According to our analyses, binding and dissociation of cofilin from the filament are equivalent at a critical torsion angle. This critical angle is about -164[deg/2.75 nm] and is almost constant along the length of the filament, except in case of very short actin filaments. The chemical potential difference is negative above and positive below the critical angle. Therefore, untwisting the double helix of the cofilin-bound filament causes cofilin to dissociate from the filament. Furthermore, the double helical nature of the filament likely induces stretch-twist coupling. Thus, tensile forces induce changes in the torsion angle of the filament, causing a positive chemical potential difference leading to the dissociation of cofilin from actin.

632-Pos Board B511
Slow skeletal muscle actin
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It had been thought that vertebrates synthesize the same isoform of sarcomeric actin in all skeletal muscles. The present work demonstrates the existence of a unique variant which accounts for all of the sarcomeric actin in the slow skeletal trunk muscle of Atlantic herring. Residues 48–375 of the primary structure have been inferred from nucleotide sequencing (Acc# EF495203) and residues 48–61 confirmed by Edman based sequencing of a fragment generated by subtilisin cleavage. EF495203 differs from the same segment of slow skeletal muscle actin from salmonids (Mudalige et. al. FEBS J. (2007) 274, 3452-3461) by a single residue (# 353), but there are eleven and ten substitutions, respectively, between EF495203 and salmon fast skeletal actin and rabbit skeletal actin. At least half of these substitutions are of a non-conservative nature. Actins isolated from different skeletal muscles from herring and salmon, but not rabbit, chicken and frog, can be differentiated by electrophoretic mobility at alkaline pH in the presence of 8M urea; digestion with various proteases, including thrombin, subtilisin and V8, and resistance to induced-denaturation. The melting temperatures of various G-actins (Ca.ATP) are: ~45 (salmon slow skeletal muscle); ~50 (herring slow skeletal muscle) and ~55 degrees C (salmon, herring and rabbit fast skeletal muscle). Possible sources of the enhanced chain flexibility will be discussed. The demonstration of slow skeletal muscle actin in two unrelated teleosts indicates that it is not a lone occurrence.

633-Pos Board B512
Binding Studies Between Cofilin And Actin Using Fluorescence Resonance Energy Transfer And Molecular Modeling
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The actin cytoskeleton is a 3-dimensional network within all eukaryotic cells. The assembly of actin monomers (G-actin) to form filaments (F-actin) is regulated by a number of actin binding proteins (ABPs). Cofilin is a principle regulator of actin dynamics and is essential for cell division and changes in cell shape. A full understanding of the functional effects of cofilin on actin is not possible due to the lack of an atomic-resolution model of the actin-cofilin complex. To date, several predicted models have been proposed based on the putative cofilin-actin interface. The actin-binding surface of cofilin was identified from structural homology with other ABPs, mutagenesis and NMR. Recently a new model was proposed (Kamal JKA et al, 2007, Proc Nat Acad Sci USA 104:7910) which identified the cofilin-binding interface on actin using radiolytic footprinting. Additionally, we have generated 16 possible cofilin-actin complexes *ab initio* using RosettaDock software (http://www.rosettacommons.org) by inputting the atomic models of actin and cofilin.
In vitro mutagenesis within cofilin has been undertaken to provide sites suitable for modification with extrinsic fluorescent probes, while preserving the capacity of cofilin to bind to G-actin. Selective mutation of several amino acids in cofilin has allowed us to identify mutants that remain functional. To test the proposed actin-cofilin models, intermolecular fluorescence resonance energy transfer (FRET) spectroscopy has been performed to calculate distances between several sites on cofilin and actin. These FRET distances will be used as constraints to generate a molecular model of the cofilin-actin complex.

634-Pos Board B513
Starting Actin Filaments Anew - Adenomatous Polyposis Coli Is an Actin Nucleator
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Adenomatous polyposis coli (APC) protein has emerged as a complex, multifunctional regulator in the Wnt-signaling pathway and in controlling the actin and microtubule cytoskeleton during basic cellular events such as cell polarization, migration, adhesion, chromosome segregation, and apoptosis. Here we demonstrate that APC directly binds F-actin and also affects actin dynamics by acting as a potent actin nucleator regulated by the microtubule (+)-end tracking protein, EB1. In addition, we show APC mediates the formation of cytoskeletal networks by actin bundling and regulated crosslinking of actin filaments with microtubules (MTs). These newly-identified functions of APC suggest that APC may function at the intersection of microtubule (+) - ends and actin rich zones to direct cell polarity and motility, and that these cytoskeletal APC-based functions may be important for the initiation and progression of cancerous tumors.

635-Pos Board B514
Measuring Molecular Interaction between Actin Filament and Actin Binding Protein Governing Mechanical Properties of Cross-Linked F-Actin Network
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Actin binding proteins (ABPs) regulate the assembly of actin filaments (F-actin) into various structures that provide physical support for the cell and play important roles in numerous cellular processes. Although the mechanical properties of F-actin networks have been extensively studied, a full understanding of how molecular interactions between ABPs and actin filaments influence the network mechanical properties of F-actin network is lacking. Here, we study the molecular interaction between ABPs and actin filaments and the mechanical rupture of a cross-linked F-actin network. A single molecule assay was used to measure the rupture force of a complex formed by an ABP filamin linking two actin filaments by pulling one of the filaments with the optical tweezers. The rupture forces for filamin/F-actin complex were 88 ± 34 pN for loading rates of 1209 ± 119 pN/s. About 20% of the force-extension traces exhibited a sawtooth pattern with intervals ~30 nm comparable to length changes due to unfolding of individual immunoglobulin domains of filamin. We also prepared the F-actin gels and monitored their mechanical responses while an embedded bead was displaced at constant speed. Compared to similar experiments with an entangled F-actin solution, the force-extension curves for the filamin/F-actin network exhibited multiple transitions in the force of 37 ± 17 pN, suggestive of bond rupture. In addition, using the optical tweezers, active microrheology was used to measure the strain-dependent mechanical properties of the filamin/F-actin network. Results demonstrate the network to be highly nonlinear and irreversible. Support from the NIGMS (GM-076689) is gratefully acknowledged.

636-Pos Board B515
Interaction of CapZ with Actin: Molecular Mechanism and Regulation
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The heterodimeric actin-capping protein (CP) is a major capper of barbed ends of actin filaments in eukaryotes, which prevents the incorporation or loss of actin subunits. CP regulates actin-dependent events in cells, including controlling cell shape and movement. CP is regulated by CARMIL, which inhibits CP in vitro and proposed to be able to physically remove CP from actin filaments. Here, we have identified the residues on the surface of CP that are important for binding to actin and to CARMIL. Previous cryo EM studies and computational docking studies predicted the residues involved in the interaction of CP and actin filaments, and functional assays with site-directed mutants of CP confirmed the predictions. Using TIRF (total internal reflection fluorescence) microscopy, we observed that adding CARMIL rapidly changed capped actin filaments to grow, consistent with uncapping. Together, these results extend our understanding of how CP binds to the barbed end of the actin filament.

637-Pos Board B516
Structural Binding Model of Cofilin and F-actin
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The regulation of actin polymerization within the cell is critical for many cell functions. Cofilin plays an important part in this process since it binds and severs actin filaments leading to depolymerization as well as the creation of new barbed ends. Although the details of cofilin's interaction with G-actin have been elucidated through a range of experimental studies, the specific interactions with F-actin have remained more elusive. Here we present the results of a detailed computational study involving a combination of molecular dynamics simulations and protein-protein docking. The resulting structural model for the cofilin/F-actin complex matches very well with existing cryoEM and mutagenesis data. Further insight is gained from a sequence and secondary