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.

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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