813-Pos
Stress Generation by Actin Myosin Networks
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We perform a series of simulations to study the effects of myosin minifilaments on the stress distribution in a crosslinked actin network. Previous theoretical studies suggest that the maximum tension generated per myosin depends strongly on the length of the myosin minifilaments and the actin filaments (A. E. Carlsson, Phys. Rev. E74, 051912, 2006). We study both two- and three-dimensional actin networks. The two-dimensional actin network is modeled as a collection of randomly oriented rods connected at their edges to a square frame. Before including the myosin-induced stresses, we studied the elastic response of the network under affine and nonaffine strain. The network structures are obtained by minimization of an energy function including terms due to both stretching and bending of rods. Rods are allowed to rotate without any energy cost at a crosslink where it connects to another rod. After energy minimization following a strain, forces and torques exerted by the network on the walls and Young's modulus of the network are calculated. The myosin forces are included by treating each myosin minifilament as a force dipole whose components act on different actin filaments. Then evaluation of the forces on the walls allows us to calculate the tension induced per myosin. These calculations allow us to understand the myosin induced stress as a function of structure of the network, by varying properties like connectivity, filament length, the extent of branching, and the treadmilling rate.

814-Pos
G146V Mutant Actin is Defective in Conformational Changes, Accompanied by Impaired Motility with Skeletal Myosin
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In the lever arm model for actomyosin motility, the only roles of actin filaments are to stimulate Pi release from myosin and to provide foothold for tension generation. However, actin filaments undergo myosin-dependent cooperative conformational changes. To understand the possible functions of those changes in actomyosin motility, we hoped to obtain mutant actins with defective conformational changes, and consequently, impaired motility. Mutant actins of this class are presumably dominantly inhibitory by impairing functions of copolymerized wild type actin. Thus, we constructed a series of mutant genes in which Gly residues in actin were systematically substituted to Val, and identified 5 dominant negative mutant actins on the basis of growth inhibition when expressed in yeast. Of these, we chose G146V mutant for further analyses, because changing Gly146 at the hinge between the small and large domains of actin might impair relative conformational changes between the two domains. G146V actin polymerized more readily than wt, but gliding velocity and force production of G146V filaments on skeletal (sk) HMM surfaces decreased by ~80%. Kinetic analyses indicated that prolonged strongly-bound state is not the cause of the slow movement. In contrast, G146V filaments moved and produced force normally on myosin V. To probe structural changes of actin involving Gly146, we measured FRET efficiency between two fluorophores in the small and large domains (Thr41 and Ala235) of individual actin molecules in filaments. Control actin subunits take at least two different states, while most of the G146V actin subunits were in one state with a higher FRET efficiency. These results suggest the possibility that G146V actin take inappropriate conformation for motility of sk myosin. We are currently performing FRET experiments in the presence of sk HMM and myosin V.

815-Pos
Simultaneous Measurement of Actin Sliding Velocities and Actin-Myosin Dissociation Kinetics
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Muscles contract through actin-myosin interactions modulated by the ATPase reaction. To determine how muscle shortening is generated by actin-myosin biochemistry and kinetics, we are developing an in vitro motility assay that allows us to simultaneously measure actin sliding velocities, V, and actin-myosin attachment times, T_{on}. To measure T_{on} during a motility assay, we monitor changes in actin filament dynamics near a myosin binding site using nanometer tracking of actin filaments labeled with single qdots. Actin-myosin binding damps actin dynamics, and T_{on} is estimated from the average duration of these binding events. With this approach, we observe that the duration of actin-myosin binding events decreases linearly with increasing ATP concentrations, resulting in an estimated ATP induced dissociation rate constant of ~9 $\mu M^{-1} \cdot s^{-1}$, consistent with previous kinetic measurements. This technique allows us to determine the mechanochemistry of a single myosin head functioning within the context of many myosin molecules interacting with a single actin filament.

816-Pos
The Combined Effects of ADP, ATP, and Myosin Density on Cooperative Activation of Thin Filaments
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Cooperative activation of thin filaments has been achieved by decreasing [ATP], increasing [ADP], and increasing myosin densities; however, these observations have yet to be incorporated into a self-consistent description of thin filament regulation. It has been noted in previous skinned muscle fiber studies that contraction can be initiated with high [ADP] when Ca^{2+} is absent. Similarly, low [ATP] has been shown to activate thin filaments in Ca^{2+}-free motility assays. Based on these observations, we hypothesize that high concentrations of ADP can cause thin filament cooperative activation in the absence of Ca^{2+}. Using an *in vitro* motility assay, we determine the effects of ATP, ADP, and myosin density on the cooperative activation of myosin-based thin filament sliding, V. At 1mM [ATP], V is activated over an [ADP] range from ~2 to 8mM and becomes increasingly inhibited at [ADP] >8mM. This biphasic effect of [ADP] on V is similar to the effects of [ATP] on cooperative thin filament activation previously measured *in vitro*. The observed effects of [ADP] and [ATP] on V are consistent with a model in which increasing [ADP] or decreasing [ATP] increase the probability that a myosin head is bound within a thin filament regulatory unit, cooperatively activating that regulatory unit (and possible adjacent ones) and increasing V. At sufficiently high [ADP] or sufficiently low [ATP], the thin filament becomes fully activated, and a further increase in [ADP] or decrease in [ATP] slows V by inhibiting detachment kinetics.

817-Pos
Effect of Phosphomimetic Mutation of Caldesmon on the Migration Activity of Vascular Smooth Muscle Cells
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Migration of differentiated smooth muscle cells is usually pathogenic. It contributes to diseases such as atherosclerosis. The low molecular weight isoform of caldesmon (l-CaD) binds actin filaments in mammalian cells and modulates the assembly of the actin cytoskeleton. To determine the effect of phosphorylation of l-CaD on the mobility of cultured vascular smooth muscle cells, we have performed Transwell migration assays on A7r5 cells expressing l-CaD variants with mutations at the phosphorylation sites mediated by PAK (Ser452 and Ser482) and/or ERK (Ser497 and Ser527). The chemotactic migration activity of transfected cells compared to the normal, untransfected cells was evaluated. Among all constructs the A1234 mutant (Ser residues at all 4 positions changed to Ala) resulted in most hindered mobility. The relative migration activity for the A1234-transfected cells was about 13% of the vehicle-transfected cells. Transfection with wild-type l-CaD decreased the rate of cell migration to a lesser extent (~50% of the control cells). Cells transfected with l-CaD mutated only at the PAK sites (A1A2) or the ERK sites (A3A4) also migrated approximately 50% slower than those control cells. Apparently, both ERK and PAK contribute to the mobility of A7r5 cells and the effects are comparable and additive. Phosphorylation was indeed found at the ERK sites of ectopically expressed A1A2 and l-CaD, but not that of A3A4 and A1234. The mobility of cells transfected with D1234 (all 4 Ser changed to Asp) was higher than that of cells transfected with all other CaD variants, but still about 20% lower than the control cells. Taken together, these results suggest l-CaD plays a role in controlling the migration activity of smooth muscle cells, and reversible phosphorylation of l-CaD facilitates this activity. Supported by NIH (HL-92252).

818-Pos
Functional Changes of Actin-Binding Proteins for Human Umbilical CD-105 Positive Stromal Cell Proliferation and Differentiation
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Human umbilical Wharton's jelly cells (WJCs) possess the capacity of self-renewal and differentiation of mesenchymal stromal cells. In this study, human umbilical CD105-positive WJCs were cultured to investigate the functional roles of actin binding proteins in cell proliferation and adipogenic differentiation. Genistein, a tyrosine protein kinase inhibitor, lowered intracellular Ca^{2+} such as to attenuate cell proliferation and DNA synthesis through the β-catenin/cyclin D1 pathway in the cells. Immunofluorescence confocal scanning microscopy indicated that changes in the subcellular distribution of tropomyosin (Tm), in which the diffuse cytosolic staining was shifted to show colocalization of Tm with actin stress fibers. Genistein treatment of cells also induced increases in the colocalization of caldesmon (CaD) and stress fibers. In contrast,