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,

genistein increased accumulation of the actin-nucleating protein formin-2 (FMN-2) and profilin in the peri-nuclear area. Silencing of FMN-2 by siRNA raised intracellular Ca^{2+} and rendered genistein resistance in decreasing intracellular Ca^{2+} in the cells. To define how actin filament assembly is regulated in the adipogenic differentiation, we determined functional changes in gene expression of actin binding proteins associated with morphological transformation in adipogenesis-induced WJCs. Adipogenic differentiation, as indicated by elevating expression of PPAR-γ mRNA, caused changes in β-actin mRNA expression and protein level. Gelsolin, an actin filament severing protein, also displayed a biphasic change of mRNA expression and protein level in the differentiation. During adiopogenesis mRNA expression levels for FMN-2 and Tm-1 were declined significantly, but no changes for Tm-2 and Tm-4. Taken together, our study resulted in the novel finding that actin-binding proteins act by modulating actin filament assembly for the proliferation and differentiation in human WJCs.

819-Pos

Analysis of De Novo Cell Cortex Assembly in Blebs as a Novel Assay for Probing Cortical Dynamics and Regulation

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The contractile actin cortex drives many cellular processes, such as cell migration and cytokinesis, but little is known about the proteins that regulate its assembly.

To address this question, we developed an assay for assessing the involvement of candidate proteins in the recruitment of the main cortical components, actin and myosin, during cortex build-up. One situation where cortex assembly can easily be studied is during the growth of blebs. Blebs are membrane protrusions that are initially devoid of cortical proteins and that subsequently reassemble a cortical layer prior to bleb retraction. They therefore constitute an ideal system for the study of de novo cortex assembly under physiological conditions. In the developed assay, we use laser ablation of the cell cortex to induce bleb growth in a controlled manner and subsequently quantitatively monitor the recruitment of fluorescently labelled actin and myosin during the bleb life cycle. This allows for the extraction of a range of dynamic parameters of cortical assembly that can be compared between control cells and cells with varying levels of candidate proteins. Preliminary data, obtained with this assay, show that proteins typically involved in actin polymerization, such as profilin and cofilin, influence the rates of cortex assembly in HeLa cells. Additionally, the assay allowed us to precisely characterize the dynamics of cortex assembly in control cells, providing new insights into the mechanisms of bleb growth and retraction. From these first tests, we conclude that the developed assay provides a highly sensitive tool for the study of cortex assembly.

820-Pos

The Actin Cytoskeleton Dynamically Associates with T-Cell Receptor Clusters

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The actin cytoskeleton is a key determinant of cell surface protein organization in many eukaryotic cells. In mammalian T-cells undergoing antigen-mediated activation, the cytoskeleton drives the development of a set of macroscale protein domains, collectively termed the supramolecular activation cluster. These protein domains, composed of T-cell receptors surrounded by adhesion molecules and their ligands, are highly characteristic of T-cell activation and are thought to play an important role in modulating receptor signaling intensity.

Though significant research has been undertaken to elucidate the interactions among various receptors involved in T-cell activation, the nature of the interactions between these receptors and actin remains poorly established. We have used live-cell fluorescence microscopy to image the actin cytoskeleton as it interacts with T-cell receptors in real time. Our results support recent work from our lab that had shown that T-cell receptors are likely to be friction-coupled to the cytoskeleton. Actin density tracking has also extended that work by demonstrating that cytoskeletal velocity may be affected by mobility-limited T-cell receptor clusters, and thus that T-cell receptors may have the capacity to regulate actin flow.

In addition, we have evaluated the time dynamics of the T-cell receptor-actin interaction and found that actin periodically accumulates and dissipates at T-cell receptor clusters. By applying an autocorrelation function to our image stacks, we found that the half-decay time ($Tau_{1/2}$) of the actin fluorescence at regions corresponding to T-cell receptor clusters was significantly increased compared to background, indicating a greater persistence of actin in those regions. Thus we have developed a novel method of analyzing actin kinetics and shown that the actin cytoskeleton dynamically associates with T-cell receptor clusters.

Cell & Bacterial Mechanics & Motility I

821-Pos

Response of the Bacterial Flagellar Motor to Controlled Temperature Change

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The bacterial flagellar motor is a rotary molecular motor capable of rotating at up to 700Hz. To resolve the individual steps that represent the discrete torque-generating step of the motor requires a low load marker and either high time resolution or a slowing of the motor. Previously the motor's speed has been reduced by decreasing the ion-motive force available to the motor[1]. Here we demonstrate two novel generic methods of microscope temperature control capable of slowing the motor while retaining nanometer resolution on the microscope. The first method involves a Peltier-cooled collar acting directly on the objective, and the second uses a chamber of fluid directly on top of the objective. These devices were used to probe the speed and function of the Bacterial Flagellar Motor across 0 °C - 40 °C. We confirmed that at slowing due to cooling was much greater at low loads than at high load, and extended previous chimera torque-speed curves to single-stator, low induction measurements. At high temperature we observed the motor stopping and subsequent resurrection-like behaviour as the motor was cooled. We investigated the membrane voltage response with temperature using the voltage sensitive dye TMR on cells treated with EDTA to allow the dye to penetrate the cell membrane[2]. From this we were able to investigate the cause of these stops at high temperature as a function of ion-motive-force.

[1] Y. Sowa, et. al, *Nature*, vol. 437, Oct. 2005, pp. 916-919.
[2] C. Lo, et. al, *Biophysical Journal*, vol. 93, Jul. 2007, pp. 294-302.

822-Pos

Conceptual Model for a Synthetic Bipedal Stepping Motor

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Biomolecular nanomotors have provided the inspiration for the design and construction of artificial nanoscale motors and machines based on several types of molecule including DNA. However, no synthetic nano-motors have yet been constructed from building blocks of protein-based material even though biomotors themselves are proteins. The HFSP smotor group (1) are in the process of developing a bottom-up approach to the understanding of biomotors by designing and constructing synthetic protein motors and numerically simulating their kinetic properties. One such concept is the ''tripedal tumbleweed'' motor, which is described in (1). In this context we present the results of numerical simulations for a bipedal motor with two connected peptide legs and with some of the properties of the tumbleweed motor. This motor walks on a one-dimensional track of periodically arranged binding sites. The two ''feet'' at the end of the legs represent different ligand-gated binding proteins which can only bind to their specific binding sites on the track when the related ligands are themselves bound to the binding proteins. The sequence of binding sites on the track is AB-AB-AB…. and the motor is powered by a temporally periodic sequence of composite washes which modulate the ligand concentrations and the leg angles. The washes cause the motor to undergo directed motion by a hand-over-hand mechanism on a track with asymmetric spacing between the AB and the BA binding sites. We will show simulation results for both two-dimensional and three-dimensional motor action of our bipedal motor which will include stepping diagrams, stall forces and first passage times for a range of parameters. This motor has the following properties observed for biomolecular motors: binding, power stroke and diffusional search. Extensions of the model will also be discussed. 1. E. Bromley et al. HFSP Journal (2009)

823-Pos

Tug of War: Dynamics of Bacterial Flagellar Motor with Multiple Stators

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In a single flagellar motor, there are multiple stator units that drive the rotation of the flagellar filaments. Here, we introduce a ''tug-of-war'' model for the flagellar motor where each individual stator can generate either positive or negative torque depending on its relative mechano-chemical state with respect to the rotor. The key ingredient of our model is that the instantaneous chemical switching (stepping) rate of a stator depends on the torque it generates: stators that generate negative torque switch faster. We find that the dynamics can be characterized by the waiting and moving time scales of the motor. We show that the experimentally observed torque-speed relationship can be explained