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