resolution on the order of seconds. To explore different mechanisms used by bacteria, we moved exponentially growing cells from LB medium to an iso-osmotic buffered medium and allowed the cells to adapt. We subsequently challenged the cells with varying levels of sucrose (as an external osmolyte) and potassium or proline. We measured the dependence of the adaptation time and adaptation level on different amounts of extracellular potassium or proline and the magnitude of the osmotic shock. This type of measurement allows us to uncouple the different adaptation pathways and to study them individually and in small groups to quantify their function and interactions.

2886-Pos

Nck Function in Tyrosine Kinase Signaling to the Actin Cytoskeleton

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Tyrosine kinase signaling leads to the post-translational modification of proteins and their binding partners. These modifications lead to the membrane recruitment of signaling proteins, promoting an increase in their local concentration, which results in a cellular response to the phosphorylation of tyrosine residues. Nck, an SH2/SH3 adaptor protein, functions in tyrosine kinase signaling by linking tyrosine phosphorylation on the membrane with binding partners, such as N-WASp, that function in facilitating actin nucleation and polymerization. However, quantitative and mechanistic aspects of signaling through Nck remain poorly understood. To explore the linkage of Nck to the actin cytoskeleton, our lab developed a system in which Nck SH3 domains can be aggregated on the plasma membrane following antibody application. Aggregation of Nck SH3 domains results in localized actin polymerization in the form of actin comet tails. Using the Virtual Cell, we have built a comprehensive, quantitative actin cycle model. With this model, we have produced predicted results that have been confirmed in vivo. This model predicts experimental comet tail length, actin distribution within the comet tail, and maximum actin concentration in the tail based on the number of molecules in the aggregate and the speed at which the aggregate is moving across the cell surface. We have also adapted the model to test the implications of the recent findings that binding of two N-WASp molecules to the Arp2/3 complex enhances actin nucleation and polymerization when compared with single N-WASp activation of the Arp2/3 complex. The combination of modeling and precise experimental manipulation provides unique insights into the relationship between increased local concentration of Nck and resulting localized actin polymerization.

2887-Pos

Probing the Protein-Protein Signaling Mechanism in Intact Archaeal Cells Using Time-Resolved FTIR Difference Spectroscopy

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Elucidation of the molecular mechanisms of protein-protein interaction and signal transduction remains an important goal in biophysics. Fourier Transform Infrared (FTIR) difference spectroscopy allows the study of protein structural changes at atomic resolution, however most FTIR studies are currently performed on purified proteins removed from their original environment. Here, we present evidence that FTIR spectroscopic methods can be successfully applied to detect conformational changes of individual proteins and protein complexes in the native cell membranes as well as in intact cells. The FTIR spectra of haloarchaeal blue-light phototaxis receptor sensory rhodopsin II (SRII), a seven-helical membrane protein, linked to its full-length cognate transducer HtrII was studied in isolated cell membranes. The SRII-HtrII complex exhibited a greater extent of conformational changes assigned to receptor-transducer interactions compared to earlier studies of heterologously expressed, detergent purified and reconstituted SRII-HtrII complex which is truncated in the membrane-proximal region. The difference spectra of full-length complex also reveal conformational changes which are likely to occur in the distant cytoplasmic region of the transducer that functions as a binding site for histidine kinases. Moreover, for the first time, time-resolved spectra of the SRII-HtrII complex were recorded in intact halobacterial cells (Halobacterium salinarum) using visible light to trigger the SRII signaling pathway, which ultimately controls the cell's motility. The ability to monitor time-resolved protein structural changes that occur inside living cells has the potential to significantly expand the scope of biological FTIR spectroscopy. For example, the effects of transmembrane potential and interaction of proteins downstream of the receptor-transducer complex can be investigated in a native environment.

2888-Pos

Crystal Structure and Mutational Analysis of the Periplasmic Flagellar Protein FlgA

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Bacterial motility is achieved by rotation of flagella. The bacterial flagellum is a macromolecular complex that allows bacterial cells to swim in liquid environment. The complex is composed of the helical filament, the flexible hook and the basal body embedded in bacterial inner and outer membranes penetrating peptidoglycan layer. The basal body is divided into three sub-structures, the LP-ring, the MS-ring and the rod. The LP-ring, as molecular bushing, spans between bacterial outer membrane and peptidoglycan layer. FlgH and FlgI are the subunit proteins of the LP-ring and a periplasmic flagellar protein FlgA is involved in the P-ring assembly. Previous biochemical studies indicated that FlgA might associate with FlgI after secretion into the periplasm and act as a key protein for the flagellar P-ring assembly. The atomic structure of Salmonella FlgA has been determined at 2.1 Å resolution. The over-all structure revealed that FlgA comprised of two distinct domains as previously shown by limited-proteolysis experiments. The beta-clip fold in the FlgA structure could be involve in binding to peptidoglycan. FlgA mediates the assembly of the flagellar P-ring by means of its interactions with the carbohydrate moieties of peptidoglycan. Site-directed mutagenesis to residues at the putative FlgI binding site of FlgA caused reduced ability to complement in the FlgA-deficient Salmonella strain, indicating that FlgI required the constitutive interaction with FlgA in the flagellar P-ring assembly. We discuss about fundamental functions of FlgA and propose the molecular mechanism of the flagellar P-ring assembly.

Unconventional Myosins I

2889-Pos

Cymobase - the Reference Database for Cytoskeletal and Motor Proteins

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Motor proteins are involved in processes like cellular transport, muscle contraction, and cell division. Three motors are myosin, dynein, and kinesin. They convert chemical energy (ATP) into mechanical work (movement). Protein sequences are the bases for many biochemical and cell biological experiments, as well as bioinformatical analyses.
We implemented a web application (CyMoBase) to represent all sequence related information. Since the fist publication of Pfarao (www.cymobase.org), many changes have been integrated in the database scheme and the web application. Now, it is also possible to derive information about structures and genes. Over the years, the number of data has increased considerable. Today, there are 37 proteins, 16500 sequences, 132 domains, 819 species, 599 publications and 1392 projects, with a total sequence length of over 16 million amino acids. The web application has got a new central search page including nine search modules (species names, protein classes, taxonomy, species groups, domains, sequence meta data, publications, sequence names, and genes) and eleven result tabs (sequences, publications, downloadable FASTA files, alignment viewer, phylogenetic trees, sequence stats, domain composition, complex inventory, protein inventory, molecular weights, and class composition). All search modules can be combined to filter the results.
Furthermore, we provide the gene structure of all sequences as computed by WebScipio. An other viewing option is the ''Complex Inventory'', which presents the existence or absence of sequence homologs of certain complexes. The aligned protein sequences are available via the ''FASTA Files'' result tab.
The size of the database, the kind of annotation, the possibility to use and combining different search modules, and the number of information and options offered by the web interface makes Pfarao the reference database number one for cytoskeletal and motor proteins.

2890-Pos

Regulation of Myosin Motility by D-Loop of Actin

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Subdomain 2 of actin, which contains the DNase I binding loop (D-loop, residues 38-52), slightly changes its conformation during actin polymerization and interacts with the C-terminus of the adjacent subunit in actin filament. This region is suggested to be important for actin-myosin interaction: it was found that

binding of myosin II induces conformational changes in subdomain 2 and the proteolytic digestion of the D-loop disturbs the motor function of myosin II. However, although as many as 24 classes of myosin have already been found and their *in vivo* roles are completely different, the contribution of the D-loop to actin-myosin interaction has so far been studied only for myosin II.
In this study, to determine whether the D-loop contributes to the interaction with myosin V and if so, in what way it affects its motor function, we prepared actins modified in the D-loop and analyzed the effects of modifications on the motile properties of myosins II and V. We found that the D-loop modifications, namely, the proteolytic digestion with subtilisin and the M47A point mutation, significantly decreased the gliding velocity on myosin II-HMM in an *in vitro* motility assay, due to a weaker generated force. On the other hand, single molecules of myosin V "walked" with the same velocity on both the wild-type and modified actins; however, the run lengths decreased sharply, correlating with a lower affinity of myosin for actin due to the D-loop modifications.These results show that the D-loop strongly modulates the force generation by myosin II and the processivity of myosin V, presumably affecting actin-myosin interaction in the A.M.ADP.P_i state of both myosins. Our findings are important to understand the principles how an actin molecule may regulate diverse *in vivo* functions of various myosin isoforms.

2891-Pos
Spontaneous Oscillations of a Minimal Acto-Myosin System Under Elastic Loading
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Spontaneous mechanical oscillations occur in various types of biological systems where groups of motor molecules are elastically coupled to their environment. By using an optical trap to oppose the gliding motion of a single bead-tailed actin filament over a substrate densely coated with heavy meromyosin molecules, we mimicked this condition in vitro. We show that this minimal acto-myosin system can oscillate spontaneously. Our finding accords quantitatively with a general theoretical framework where oscillatory instabilities emerge generically from the collective dynamics of molecular motors under load.

2892-Pos
Kinetic Characterization of Non-Muscle Myosin IIB Single-Headed Heavy Meromyosin on Single Molecule Level with Optical Tweezers
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Non-muscle myosin IIB (NMIIB) is a cytoplasmic conventional myosin, which plays an important role in development of the brain and heart, and in directed growth cone motility by maintaining cortical tension in motile cells. It forms short bipolar filaments with ~14 myosin molecules on each side of the bare zone. NMIIB is a very slow myosin both in terms of actin-activated ATPase activity and actin translocation capability. Our previous studies showed that the NMIIB is a moderately high duty ratio (at least 20-25%) motor. The ADP release step (~0.35 s-1), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 $\pm$ 0.01 s-1). Because of its slow ADP off-rate, acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily ($<$0.15 μM). To examine the mechanics and kinetics of NMIIB at the single-molecule level we used a dual-beam optical tweezer to perform the "three-bead" assay. The surface-immobilized bead was coated with recombinantly engineered single-headed heavy meromyosin-like (NMIIB-SH-HMM) molecules. We measured the lifetimes of unitary actomyosin interactions and determined the actin-detachment kinetics with varying ATP concentrations. Results showed that at physiological ATP concentration (1 mM), the rate of detachment of acto-NMIIB-SH-HMM interactions was ~0.51 s-1, similar to the ADP release rate and steady-state ATPase rate reported from solution kinetic studies. Decreasing the ATP concentration to 1 μM did not alter this rate of detachment (~0.47 s-1). Also, our results showed that the power-stroke of NMIIB-SH-HMM was ~8 nm. We will discuss our single-molecule results from the perspective of the essential cellular functions of NMIIB in cell locomotion, tension generation and maintenance.

2893-Pos
Myosin-1 Function Extends to Microtubule-Dependent Processes
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Class 1 myosins are motor proteins found on various organelles and at defined structures at the cell periphery, where they play important roles in organelle translocation, intracellular transport, and cytoskeleton organization. Here, we investigated the cellular function of *Dictyostelium* myosin-1C, a long-tailed, monomeric motor with three tail homology domains called TH1, TH2, and TH3. We identified that in addition to its actin-dependent function in endocytotic processes during interphase, in *Dictyostelium* cells the motor co-localizes with centrosomes and spindle microtubules (MT) during mitosis. *In vitro* TIRF microscopy experiments and MT-cosedimentation assays using truncated myosin-1C tail constructs revealed a direct binding of myosin-1C tail to MTs. Only constructs containing TH1 and TH2 bound efficiently to MTs. Moreover, these two domains were sufficient to prevent MT depolymerization at low nanomolar concentrations of myosin-1C tail, while MT formation, i.e. nucleation and elongation, was unaffected. Additionally, we observed myosin-1C tail mediated cross-linking of MTs to F-actin. In cells, myosin-1C constructs lacking the motor domain did not associate with the spindle. This demonstrates that actin-dependent motor function is required for the cell cycle-dependent relocalization of myosin-1C from actin-rich structures at the cell periphery to MT-associated mitotic structures. Cells producing a hydrolysis deficient full-length myosin-1C mutant exhibited reduced growth rates with increased size of nuclei due to defects in spindle alignment and prolonged mitosis. Single kinesin molecule motility assays showed that MT-bound myosin-1C reduced the attachment rate of kinesin-1 to MTs without affecting its velocity and run-length. From this we propose that myosin-1C may regulate MT-dependent motility and MT dynamics. Our cell biological and functional characterization of a long-tailed class 1 myosin shows that myosin function is not limited to the F-actin network but extends to MT associated processes.

2894-Pos
Myo1e Binds Anionic Phospholipids with High Affinity
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Myo1e is a single-headed motor protein that has been shown to play roles in clathrin-mediated endocytosis in HeLa cells (Krendel *et al.* 2007. *FEBS Letters*. 581:644-650) and podocyte function in the kidney (Krendel *et al.* 2009. *J. Am. Soc. Nephrol.* 20:86-94). The myo1e C-terminal tail domain includes a basic region that is required for localization to clathrin-coated vesicles and is homologous to regions of other myosin-1 proteins that have been shown to bind phospholipids. However, the phospholipid binding properties of myo1e have not been examined. We used sedimentation assays and stopped-flow fluorescence to determine the membrane binding affinities and kinetics of a fluorescently labeled recombinant myo1e-tail construct. We found that the myo1e-tail binds tightly ($K_{eff}^{lipid} < 5$ μM) to large unilamellar vesicles (LUVs) containing physiological concentrations of the anionic phospholipids phosphatidylinositol (4,5)-bisphosphate (PIP_2) and phosphatidylserine (PS). Unlike myo1c, myo1e can also bind to physiological concentrations of PS in the absence of PIP_2. While myo1e has a slightly higher affinity for PIP_2 over PS, this selectivity is much less than observed with myo1c, which contains a putative pleckstrin-homology (PH) domain and shows strong specificity for phosphoinositides. Soluble inositol phosphate headgroups, such as inositol (1,4,5)-trisphosphate, can compete with PIP_2 for binding, but the apparent affinity for the soluble inositol phosphate is substantially lower than that for PIP_2. The rate of myo1e attachment to LUVs is similar to that of myo1c, but the rate of detachment from LUVs is slower than that found for myo1c. The high affinity of the myo1e-tail for phospholipids suggests that, *in vivo*, myo1e is strongly attached to membranes where it plays a role in endocytosis and other physiological processes.

2895-Pos
Control of Myosin-I Force Sensing by Alternative Splicing
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Tension sensing by myosin motors is important for numerous cellular processes, including control of force and energy utilization in contracting muscles, transport of cellular cargos, detection of auditory stimuli, and control of cell shape. Myosins have evolved different tension sensitivities tuned for these diverse cellular tasks, thus it is important to determine the mechanisms and regulation of force sensing within the myosin superfamily. In this study, we examined force sensing by the widely expressed myosin-I isoform, myo1b, which is alternatively spliced in its light chain binding domain (LCBD), yielding proteins with lever-arms of different lengths. We found that the step sizes of the myo1b proteins are not linearly related to the number of IQ motifs in the LCBD, suggesting that splicing introduces a structural feature into the LCBD that affects the lever arm size. We also found the actin-detachment kinetics of the splice isoforms to be extraordinarily tension sensitive, with the magnitude of tension sensitivity linearly related to lever arm length. Thus, in addition to regulating step-size, motility rates, and myosin activation, the LCBD is a regulator of force sensing. Finally, we found that myo1b is substantially more