The sole class I myosin in the fission yeast, Schizosaccharomyces pombe, Myo1 is required to promote polymerisation of cortical actin patches, as well as to regulate lipid organisation and endocytosis. We have identified the serine residue within the motor domain of Myo1, which corresponds to the TEDs site. Using a phosphospecific antibody we have established that this conserved serine is phosphorylated in vivo within fission yeast. Mutating this serine within the Myo1 protein to either alanine or aspartic acid has revealed that its normal phosphorylation plays a crucial role in regulating the protein's affinity for actin and ability to function within the cell. Live cell imaging of these strains indicate Myo1 TEDs site phosphorylation is required for Myo1 to recruit to dynamic non-motile foci at the cell surface. In addition and unregulated phosphorylation can lead to inappropriate association with actin filaments, which is normally inhibited by tropomyosin. We also present data which illustrate the important role this phosphorylation event plays in Myo1's functions during actin organisation, endocytosis and lipid raft distribution.

2803-Plat
How Do Myosin VI and Myosin Va Navigate Intersections And Cooperate On Actin Tracks While Transporting Cargo In Vitro?
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Myosin Va (myoVa) and myosin VI (myoVI) are processive molecular motors that transport cargo on actin tracks in opposite directions. We have shown that myoVa can effectively maneuver through an in vitro cytoskeletal model system composed of actin filament intersections and Arp2/3 branches (Ali et al. 2007). Here we challenge Quantum dot (Qdot)-labeled expressed myoVI with actin filament intersections and observed that myoVI maneuvers through intersections with the following statistics: 38% turned left or right with equal probability; 28% crossed over the intersecting actin filament; 34% terminated their run. The myoVI cross over probability is twice that of myoVa suggesting that the range of the myoVI leading head's diffusional search may be longer than myoVa. Similar to myoVa, myoVI has significant flexibility allowing it to turn at intersection angles up to 155°. When multiple myoVI were attached a Qdot, the turning probability increased to 53% whereas the cross over probability decreased to 15%. MyoVa and myoVI may be colocalized to the same cargo in vivo and to determine how these oppositely directed motors might interact during cargo transport, we attached both motors in a 1:1 ratio to a Qdot. We observed two types of movement associated with these myoVa/myoVI-labeled Qdots. A given Qdot would move in both the plus- or minus-end direction for periods of time at velocities appropriate for the specific motor, suggesting that myoVa and myoVI take turns transporting the Qdot. Other Qdots moved continuously but at velocities suggesting that both motors are simultaneous interacting with actin and undergoing an effective ''tug of war.'' These studies may help characterize how actin-based motors deliver their cargo through the complex actin network.

2804-Plat
Smy1p: An Orphan Kinesin Finds a Home
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Long distance cargo transport in budding yeast is carried out not by kinesin, but along actin cables by two non-processive class V myosins, Myo2p and Myo4p. Overexpression of Smy1p, a kinesin-related protein, rescues the temperature sensitive *myo2-66* mutant yeast strain, which is defective in Myo2p transport.[1] The mechanism by which a kinesin family protein rescues actin-based transport is unknown, but does not require microtubules.[2] To address this question, we expressed Smy1p and Myo2p in insect cells and characterized them *in vitro*. Smy1p does not move microtubules in an ensemble motility assay, and is not an active motor. Using total internal reflection fluorescence microscopy (TIRFM), we find that Smy1p does not bind strongly to microtubules, but diffuses along them in the presence or absence of ATP. Surprisingly, Smy1p also binds to and diffuses along actin-fascin bundles. This binding is ionic strength-dependent, indicating the interaction is electrostatic in nature. When a single Myo2p is attached to a quantum dot cargo, the complex does not move processively on actin bundles. However, when several Smy1p molecules are attached to the quantum dot in addition to a single Myo2p, the complex supports continuous, unidirectional movement. 46% of moving quantum dots run to the end of the actin bundle, with run lengths greater than 10 microns observed. We hypothesize that Smy1p acts as an electrostatic tether, keeping the quantum dot bound to actin after Myo2p undergoes its powerstroke. We propose that overexpression of Smy1p rescues the *myo2-66* mutant by enhancing the binding of cargo to actin. A similar mechanism likely contributes to transport in wild-type cells when both Smy1p and Myo2p are present on the same cargo.

1. SH Lillie and SS Brown (1992), *Nature* **356**, 358-61.
2. SH Lillie and SS Brown (1998), *JCB* **140**, 873-83.

2805-Plat
The molecular basis for bundle selectivity of myosin X
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Eukaryotic cells organize their contents through trafficking along cytoskeletal filaments. When presented with many apparently similar alternatives within the cortex, it is important to understand if and how myosin motors identify the few actin filaments that lead to their correct destinations. Recently we showed that myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. Myosin X, while poorly processive on single actin filaments, takes long processive runs on actin filaments tightly bundled by fascin. Such a fascin bundle is the precise actin structure to which myosin X motors localize in vivo. Using single molecule optical trapping experiments we have determined the step size of this motor to be 17 nm, which is nearly half of the 36 nm pseudo-helical actin repeat required for motors to be processive on single actin filaments. These results indicate that straddling two filaments within a bundle stimulates this motor's function. Our initial model attributed this motility to the short lever arms of myosin X, consisting of 3 IQ repeats rather then the six found in the processive myosin V. To test this, chimeras were constructed where the heads, the IQ domains and the post IQ sequence (containing the coiled-coil dimerization domains) of Myosin V and Myosin X were used to create six combinatorial constructs. Single molecule fluorescence studies of these constructs revealed that the post IQ region and not the short lever arm of this motor is the main contributor to its unique selectivity. This result provides remarkable insight into the ability of nature to fine-tune myosin motors to serve their specific functions in the cell.

2806-Plat
Watching 'ankle' action of myosin V
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Myosin Va is an actin-based linear molecular motor that 'walks' in discrete ~35-nm steps following a ''hand over hand'', alternating site mechanism in which the two feet ('heads' or 'motor domains') switch between leading and trailing positions along the actin. Previously, we have reported that the lifted foot has access to a next forward binding site by combination of its rotational Brownian motion through a flexible joint at the leg('neck' or 'lever arm')-leg junction and forward movement of the joint by lever action of the landed leg. Our purpose here is to understand how the lifted foot binds to the leading site on actin. To step successfully, the 'toe' of the lifted foot should point down to let the actin binding site of the foot be properly oriented relative to actin. Meanwhile, in two-foot binding posture, the trailing leg which is dissociated by ATP-binding corresponds to the toe-up position. Therefore, we characterize ankle action with ATP-binding in the absence of actin. To observe the ankle action under an optical microscope, we fixed the leg of monomeric myosin Va on a substrate, attached beads to the foot and visualized orientation of the duplex. When triggered by UV flash of caged ATP, the beads swing 60-100 degrees, maintain this position for tens of seconds, then relax back to the original angle position. This cycle is observed repeatedly and only by UV flash of caged ATP. Thus, we clearly showed that myosin Va adopts (at least) two stable angles depending on the nucleotide state, which suggests that the toe of the lifted foot points down before binding to actin. Lastly, the observed swing supports ''swinging lever arm'' model which is generally believed to be a common mechanochemical mechanism of a conserved catalytic motor domain of myosin family.

2807-Plat
A Branched Kinetic Pathway Facilitates Myosin Va Processivity
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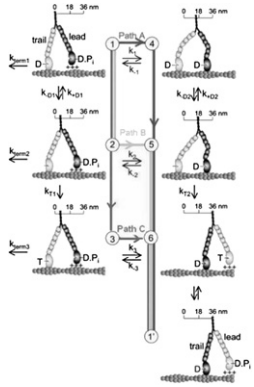
Myosin Va is a double-headed molecular motor capable of long distance cargo transport. How the heads coordinate their enzymatic and mechanical cycles during processive movement is still unclear. Previously, we reported that myosin Va utilizes two kinetic pathways (Baker et al., 2004; Kad et al., 2008). Here we challenge a Qdot-labeled myosin Va HMM in the TIRF assay under various substrate conditions. Increasing [ATP] >1mM, [ADP] >1mM, or [Pi]=40mM reduces run lengths. These run length and associated velocity data confined an analytical model of myosin Va's unloaded multipathway

ATPase cycle (see Figure). The reduced run lengths with increasing ATP, ADP, and Pi suggest that runs terminate from two distinct states; one with both heads weakly-bound (state 3) another with ADP in the trailing head while the leading head has yet to undergo its powerstroke (state 1). In addition, to strain dependent accelerated ADP-release from the trailing head (State 4), the model also predicts that strain accelerates ATP binding (state 5) two-fold. These data and model analysis suggest that myosin Va processivity involves a complex branched kinetic pathway, providing the motor versatility when meeting the physical challenges presented by the intracellular environment.



2808-Plat

Bidirectional Cooperative Motion Of Myosin-II Motors On Actin Tracks With Randomly Alternating Polarities

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The cooperative action of many molecular motors is essential for dynamic processes such as cell motility and mitosis. This action can be studied by using motility assays in which the motion of cytoskeletal filaments over a surface coated with motor proteins is tracked. In previous studies of actin-myosin II systems, fast directional motion was observed, reflecting the tendency of myosin II motors to propagate unidirectionally along actin filaments. Here, we present a motility assay with actin bundles consisting of short filamentous segments with randomly alternating polarities. These actin tracks exhibit bidirectional motion with macroscopically large time intervals (of the order of several seconds) between direction reversals. Analysis of this bidirectional motion reveals that the characteristic reversal time, τ_{rev}, does not depend on the size of the moving bundle or on the number of motors, *N*. This observation contradicts previous theoretical calculations based on a two-state ratchet model (Badoual *et al.* 2002. *Proc. Natl. Acad. Sci.* 99:6696-6701), predicting an exponential increase of τ_{rev} with *N*. We present a modified version of this model that takes into account the elastic energy due to the stretching of the actin track by the myosin II motors. The new model yields a very good quantitative agreement with the experimental results.

Platform AU: Protein-Ligand Interactions

2809-Plat

The Lysine At Position 13 Of Pten'S N-terminus Is Necessary For Its Preferred Interaction With Pi(4,5)p_2

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Phosphatase and tensin homologue deleted on chromosome 10, also known as PTEN, has been identified as the most important regulator of the PI3K pathway, mutation or deletion of one copy of this protein results in a tumorigenic state. PTEN has also been identified as the second most important and mutated tumor suppressor, rivaled only by p53. PTEN contains within its N-terminus a $PI(4,5)P_2$ binding domain which has been shown to bind preferentially to $PI(4,5)P_2$, and whose presence within the protein is necessary for binding and activity of the enzyme. Within the $PI(4,5)P_2$ binding domain resides a lysine which is frequently mutated in many types of cancer, one of the most important mutations being $PTEN_{K13E}$. Because this mutation results in a change in the overall charge of the $PI(4,5)P_2$ binding domain, we have studied the effects of not only this mutation on the interaction of PTEN and its N-terminally derived peptide with $PI(4,5)P_2$, but have also mutated this lysine to arginine to maintain the overall charge of the binding domain, as well as moving only the position of this lysine within the N-terminus. We have found that mutation of this lysine, even those that maintain charge and overall identity of the residues within the $PI(4,5)P_2$ binding domain result in decreased ability to bind to $PI(4,5)P_2$ containing membranes. Interestingly, the proteins which have mutations at this lysine also do not undergo any conformational changes upon interaction with membranes containing $PI(4,5)P_2$, in contrast what was observed for the wild type protein. The lack of binding of these mutated proteins and subsequent conformational changes give insight into the mechanism of these mutations in the development of a tumorigenic state.

2810-Plat

Structural and Biophysical Characterization of the GAF Domains from Phosphodiesterases 5 and 6

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Phosphodiesterases 5 and 6 control the intracellular levels of cGMP through hydrolysis. The catalytic domains of both proteins are regulated by allosteric binding of cGMP to the N-terminal GAF domain (GAF A) of a tandem pair. We present the atomically detailed structures of both cGMP-bound GAF A domains as determined by NMR (PDE5A) and x-ray crystallography (PDE6C). Each domain adopts a conserved overall fold with well-defined cGMP binding pockets. However, the nucleotide coordination is distinct with a series of altered binding contacts. Nucleotide binding specificity is provided in each by the orientation of an Asp/Asn residue that is within hydrogen bond distance of the guanine ring. In PDE5A, a D196A mutation disrupts cGMP binding and increases cAMP affinity causing an altered cAMP-bound structural conformation in constructs containing only GAF A. NMR studies reveal that both GAF domains undergo significant cGMP-dependent conformational changes. In PDE5A, GAF B stabilizes the highly dynamic multi-state apo GAF A domain, presumably via direct interaction. In contrast, cGMP-free GAF A from PDE6C is more defined and in a single ''open'' state with flexible elements. Biophysical characterization of the GAF domains by Circular Dichroism and Analytical Ultracentrifugation further underlines the difference between the two PDEs. The structural features of the GAF domains from PDE5 and PDE6 revealed here provide a basis for future investigations of the regulatory mechanism of both PDEs and the design of GAF-specific small molecule inhibitors of PDE function.

2811-Plat

Mechanism Of Interaction Between The Volatile Anesthetic Halothane And A Model Ion Channel Protein: Fluorescence And Infrared Spectroscopy Employing A Cyano-phenylalanine Probe And Molecular Dynamics Simulation

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We demonstrate that cyano-phenylalanine (Phe_{CN}) can be utilized to probe the binding of the inhalational anesthetic halothane to a model ion channel protein hbAP-Phe_{CN} possessing a designed binding cavity. The Trp to Phe_{CN} mutation adjacent the cavity alters neither the α-helical conformation nor the 4-helix bundle structure. The halothane binding properties of hbAP-Phe_{CN}, based on fluorescence quenching, are consistent with those of the Trp-prototype, hbAP1. The dependence of fluorescence lifetime on halothane concentration implies a one-dimensional diffusion of halothane along the nonpolar core of the protein bundle. Consequently, the fluorescence quenching is dynamic at lower halothane concentrations, becoming static at higher concentrations. The 4-helix bundle structure present in aqueous detergent solution and at the air-water interface, is preserved in multilayer films of hbAP-Phe_{CN}, enabling vibrational spectroscopy of both the protein backbone and its nitrile label (-CN). The -CN stretching vibration exhibits a largely reversible blue-shift upon halothane binding.

The complexity of this 4-helix bundle protein, where four Phe_{CN} probes are present adjacent to the designed binding site within each bundle, all contributing to the infrared absorption, requires molecular dynamics simulation to interpret the infrared results. Decomposition of the forces acting on the nitrile probes indicates that -CN's blue shift arises from the halothane induced changes in the probes' electrostatic protein environment averaged over the four probe oscillators. Although halothane remains localized within the binding cavity, it undergoes significant translational and rotational motion, modulated by the interaction of halothane's $-CF_3$ group with backbone hydrogen atoms of residues forming the cavity. This halothane-backbone interaction strongly outweighs the halothane-probe interaction, making -CN a good ''spectator'' probe of the halothane-protein interaction.

2812-Plat

Binding Kinetics of Two Hyperactive Antifreeze Proteins are Revealed by Using Novel Microfluidic Devices

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Antifreeze proteins (AFPs) are produced by some cold-adapted organisms and function against freezing by arresting the ice crystal growth and preventing ice recrystalization. The questions regarding the binding kinetics of antifreeze proteins to ice surfaces are still a matter of debate and experimental data evaluating