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