which produces polarized actin bundles with 13 nm filament spacing. We applied a modified particle tracking program, which allowed us to analyze thousands of simultaneous myosin tracks and determine the run lengths and velocities typical of processive movement on the bundled networks. Myosin V moved processively on all types of in vitro actin structures. Myosin X moved well on polarized fascin cross-linked bundles, but movement was impaired or nonexistent on non-polarized alpha-actinin bundles. We hypothesize that forward runs of myosin X on alpha-actinin cross-linked bundles are inhibited because myosin X might makes ''sidesteps" to a neighboring filament, which stalls the run. The presence of an SAH domain in the lever arm of myosin X could increase the working stroke or flexibility of the lever arm allowing it to more easily sidestep across the larger alpha-actinin filament spacing.

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Cargo-mediated dimerization of Myosin VI

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Myosin VI is one of 18 known classes of the molecular motor superfamily called myosin (1,2). All myosins rapidly bind and hydrolyze ATP in the presence or absence of actin. Until recently it was thought that all myosins moved toward the barbed (+) end of the actin filament. Myosin VI is the exception to that rule and may be unique among the myosin family members in that it moves toward the pointed (-) end of the actin filament (3).

Our working model for myosin VI in a cell is that the full-length protein exists as a monomer if not bound to cargo. Binding of myosin VI monomers to cargo alters the conformation of the molecule, possibly exposing the high probability coiled-coil region (dimerization domain). Once dimerized, the myosin VI can move a vesicle processively toward the minus-end of an actin filament. GiPC and optineurin, two of the known myosin VI binding partners can dimerize, and thus potentially can initiate the dimerization of myosin VI when it binds. Both GiPC and optineurin has been expressed in insect Sf9 cells. Surface plasmon resonance (SPR) analysis showed that both GiPC and optineurin interact with full-length myosin VI within the nanomolar range. Both GiPC and optineurin when incubated with full-length myosin VI initiated its dimerization showed by ATPase assays, EM and TIRF microscopy.

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Characterization of drosophila myosin 7a mechanics

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Myosin 7a is an unconventional myosin which participates in the sensory cell functions of numerous organisms, including humans, zebra fish, and flies. In drosophila, myosin 7a (DmM7a) appears responsible for bristle morphology, including the antennae involved in auditory transduction. The composition of motifs within the molecule is as follows: a motor head, containing sub-domains broadly typical of the myosin super-family, which connects to 5 IQ's, followed by the tail region. Within the tail are a putative coiled-coil followed by two tandem MyTH4-FERM domains separated by an SH3 domain. Here, data obtained using the optical trap three bead assay - the practice of using photon force to manipulate micrometer-scale beads to observe single molecule events - are presented for DmM7a. A truncated DmM7a construct (DmM7aTD1), cropped after the tail SH3 domain, was observed to interact with an actin filament at low ionic strength (50 mM KCl). Under the same conditions no interactions were seen with the full length version (DmM7aFL), however, at high ionic strength (200 mM) DmM7aFL became active. These findings are in agreement with recent studies demonstrating that the tail performs an internal regulatory function which is electrostatic in nature. The actin detachment rates (Kdet), calculated from dwell times, were similar for DmM7aTD1 and DmM7aFL at 10 μM ATP, approximately 0.2 s^{-1}. The Kdet for DmM7aFL was dependent on ATP concentration, and was increased at 1 mM ATP. These data support previous studies showing M7a to be a high duty motor with slow ATPase activity. Attempts to dimerise DmM7a on actin were unsuccessful based on the absence of ''stepping'' events which are a hallmark of processivity. This supports the case for DmM7a having a role in tension maintenance.

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Prefoldin 4 (PFD4): A putative new partner of myosin Va (MyoVa) in melanosome transport

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Recruitment of MyoVa and the proper transport of melanosomes during pigment dispersion requires the central region of melanophilin (Mlph, 90 kDa) to bind MyoVa and the N-terminal region bound to the melanosome membrane via Rab27a. The interaction among these proteins is the key to melanosome transport. Previously, we identified and mapped for the first time the interaction between PFD4 (~14kDa) and Mlph using the yeast 2-hybrid system (in vivo) and a biochemical assay (in vitro). PFD4 is a subunit of prefoldin (PFD, ~87kDa), a chaperone that delivers unfolded proteins to a chaperonin for correct folding. Our in-vivo results suggest that PFD4 interacts with Mlph at the same MyoVa binding site. Here we confirm that interaction using pull-down assays and fluorescence spectroscopy; PFD4 competes with MyoVa for the Mlph binding site and residues 400-590 (putative coiled coil) of Mlph are crucial for PFD4 binding. In-vitro fluorescence anisotropy reveals interaction of fluorescein-labeled full-length Mlph with MyoVa tail or PFD4, by an increase in anisotropy and polarization values. Neither mutated A453P full-length Mlph nor the 400-590 segment caused a significant change in anisotropy when incubated with MyoVa; thus these constructs do not bind Mlph. Full-length Mlph also did not bind muscle myosin II. The MyoVa binding domain for Mlph and fragments 150-400, 300-433, 400-590, and Mlph A453P seems to be intrinsically unstructured. When we pre-incubated Mlph with PFD4 or MyoVa the circular dichroism spectrum showed that binding Mlph 150-400 and 150-590 with PFD4 and MyoVa tail possibly causes an increase in α-helix content. Support: CNPq, FAPERJ, PRONEX, CAPES (Brazil); Wellcome Trust (UK)

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The Mechanism of Filament Rotation in Gliding Assays with Non-Processive Myosin Motors

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We present a model study of gliding assays in which actin filaments are moved by non-processive myosin motors. We show that even if the power stroke of the motor protein has no lateral asymmetry, the filaments will move in a helical, rather than straight fashion. Notably, the handedness of this twirling motion is the opposite from that of the actin filaments. It stems from the fact that the gliding actin filament has "target zones" where its subunits are oriented towards the surface and are therefore more accessible for myosin heads. Because each myosin head has a higher binding probability before it reaches the center of the target zone than afterwards, this results in a left-handed helical motion of the actin filament. We present a stochastic simulation and an approximative analytical solution to study this effect. We show that the pitch of the helix depends on the filament velocity, which in turn depends on the ATP concentration. It reaches about 400nm for slow gliding and increases with higher speeds. These values are in good agreement with recent experiments.

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Non-muscle Myosin IIb Is A Processive Actin-based Motor

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Proper tension maintainence in the cytoskeleton is essential for regulated cell polarity, cell motility and division. Non-muscle myosin IIB (NmIIB) generates tension in the actin cortex of non-muscle cells. Recent biochemical studies show that both heads of a NmIIB dimer can interact with a single actin filament and that this conformation demonstrates load dependent release of ADP. Using a three bead optical trapping assay we recorded NmIIB interactions with actin filaments to determine if a NmIIB dimer cycles along an actin filament in a processive manner. Our results show for the first time that NmIIB is the first myosin II to exhibit evidence of processive stepping behavior. Analysis of this data reveals a forward displacement of ~5 nm. Surprisingly, NmIIB can and does take frequent backward steps of ~5 nm. The short step size of NmIIB suggests that this motor twists actin. Actin twisting could facilitate the removal of actin crosslinking proteins from the cytoskeleton. Our data supports a model in which NmIIB takes processive forward steps to generate additional tension and also takes backwards steps to relieve tension in the actin cytoskeleton, suggesting that NmIIb is a general regulator of cytoskeleton tension.

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Lever Arm Length Determines The Azimuthal But Not The Axial Orientation Of Myosin V During Processive Motility

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