1189-Plat
Calcium Regulation of myo1b Tension Sensing
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We recently demonstrated that the widely expressed myosin-I isoform, myo1b, is exquisitely sensitive to tension (Laakso et al. 2008. Science. 321:133-6), where it transitions from a low duty-ratio to a high duty-ratio motor at very low opposing forces (< 1 pN). These forces are transmitted to the motor through the IQ-motif-containing light-chain-binding-domain (LCBD), which is structurally stabilized by calmodulin molecules. Calcium binding to these calmodulins affects the ATPase and motile properties of myo1b (Coluccio & Geeves. 1999. J. Biol. Chem. 274:21575-80). Using stopped-flow fluorescence, we confirmed that calcium accelerates the biochemical rates of phosphate and ADP release by 2 - 5 fold. We performed single molecule optical-trap experiments in the presence of 25 μM ATP and 0, 1 or 9 μM free calcium. At low forces in the presence of calcium, we found an acceleration of the actin detachment kinetics, which is consistent with stopped-flow measurements. We also found that the average displacement of the myo1b step decreases to ~ 0 nm. The decoupling of the LCBD displacement from the motor-domain kinetics prompted us to test how calcium impacts the force sensitivity of actin detachment kinetics. Using an isometric clamp, the addition of 9 μM calcium resulted in a 5-fold decrease in the distance parameter that describes force sensitivity. Finally, we measured the kinetics of calcium binding to myo1b and determined that it occurs in two steps. The first step is very fast and calcium dependent, while the second step is significantly slower and independent of calcium concentration. These results show clearly that calcium regulates the ability of myo1b to sense and sustain tension.

1190-Plat
Myosin 5A Walking Mechanism: The Structural Basis of Slow ADP Dissociatin from the Lead Head
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Using electron microscopy and image averaging, we have observed myosin 5a walking along actin filaments in the presence of low concentrations of ATP. Most molecules are attached with 13 actin subunits between heads but ~10% are bound with 11 or 15 subunit spacings. Most lead heads are in the pre-powerstroke conformation, but some post-powerstroke lead heads are observed, especially at smaller separations where there is less strain in the myosin. Post-powerstroke lead heads have the converter at the front of the motor domain with its lever bent strongly backwards. Lead heads attached at the 13 subunit spacing are 98 % in pre-powerstroke state, tethered there by the trail head. However, heads spaced by 11 subunits are more evenly distributed (60:40) pre- to post-powerstroke. No post-powerstroke lead heads are seen in heads spaced by 15 actin subunits. These results are consistent with an energy difference of 10 kJ/mole between the pre- and post-power stroke conformations at 11 and 13 actin subunit separation. The post-powerstoke lead head is a new attached state of myosin: the motor domain has completed its powerstroke at the expense of severe lever distortion, but with little cargo movement. The rate of ADP dissociation from lead heads measured by stopped-flow fluorescence is >30 fold slower than from trail heads. The slower rate can be explained by a mechanism in which ADP only dissociates from the post-powerstroke state. ADP dissociation from the lead head is therefore inhibited by an unfavorable equilibrium between the pre-and post-powerstroke conformations. Supported by NIH EB00209.

1191-Plat
Processive Runs of Full Length Myosin VA Are Interrupted by Pauses and Dwells
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Full length myosin Va (FL-MyoVa) forms an inhibited, folded conformation at low salt, stabilized by interactions between the globular tails and the heads. High ionic strength disrupts this interaction, resulting in an extended, active processive motor. In vivo, it has been postulated that cargo binding disrupts the folded conformation and activates the motor. It is possible that splice variations in the tail (-B+D+F, melanocyte; +B-D-F, brain) could modify the ability of myosin Va to form the inhibited state. Two FL-MyoVa splice variants and an HMM-MyoVa, with biotin tags for Qdot labeling, were expressed in Sf9 cells. Sedimentation velocity experiments showed similar transitions from the folded-to-extended conformation for the two splice variants as a function of salt. TIRF microscopy was then used to observe processive runs on actin. The velocities of both FL-MyoVa splice variants were similar, and increased 270% (171-460nm/sec) with increasing KCl concentration (25-200mM). In contrast, the velocity of HMM-MyoVa increased by a more modest 50% (381-586nm/sec). The trajectories of the FL-MyoVa and HMM-MyoVa were also strikingly different. Both FL-MyoVa splice variants underwent processive runs that were interrupted by periods during which the motor dwelled at fixed points on the actin filament, presumably in the folded, inhibited state. At lower KCl concentration, FL-MyoVa dwelled approximately half of the total trajectory duration. Increasing ionic strength decreased duration of the dwells. HMM-MyoVa was fully active and maintained continuous processive movement at all KCl concentrations. The slower overall velocities for the FL-MyoVa splice variants, compared to HMM-MyoVa, results from inclusion of the dwell periods. We propose that during a processive run, a single FL-MyoVa can switch between an active and inhibited state without dissociating from actin, and that this phenomenon is independent of splice variations in the tail domain.

1192-Plat
Simultaneous Observation of Tail and Head Movements of Myosin V During Processive Motion Provides Insight into Its Stepping Dynamics
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Processive stepping of myosin V (myoV) on actin has been studied either by tracking the position of the tail, which follows the motion of the molecule as a whole, or by tracking the position of one or both heads. Here we combine these two approaches, and attach a quantum dot (Qdot) to one of the motor domains, and a bead to the tail. Using optical trapping and total internal reflection microscopy, the position of one head and the tail are simultaneously observed as myoV moves processively on an actin filament against increasing load. Our results show that the head (Qdot) moves continually with 72.9 ± 10.3 nm step size, while the tail (bead) moves with a step size of 34.7 ± 8.6 nm. For every two tail steps, the head moves only one step. One of the tail steps takes place concurrently with the head step. Back steps were occasionally observed. Analysis shows that before taking a back step, the head moves 68 ± 11nm while the tail moves 31.9 ± 9.7nm, which suggests that the leading head lands on the 11th actin subunit instead of its normal 13th actin subunit. Interestingly, during a backstep the tail moves -28.6 ± 13.7nm, while the step size distribution for the head shows multiple peaks. This suggests that the head has multiple binding positions along the actin filaments, while the tail has a more defined conformation. Our observation supports a hand-over-hand model for processive movement of myoV, and reveals the cause of the back stepping behavior of myosin V under physiologically relevant loading forces (<2pN).

1193-Plat
Contribution of the Myosin VI Tail Domain to Processive Stepping and Intramolecular Tension Sensing
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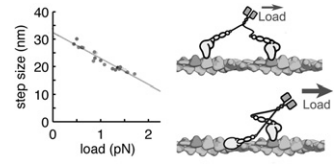
Myosin VI is proposed to act as both a molecular transporter and as a cytoskeletal anchor in vivo. The structural traits and kinetic mechanisms by which myosin VI takes processive, ~36 nm steps along actin are controversial. In particular, the portion of the molecule C-terminal to the canonical lever arm, termed the medial tail (MT), has been hypothesized to act as either a lever arm extension or as a dimerization motif. We created constructs in which the MT is interrupted by glycine-rich molecular swivels in order to test competing models of the MT's contribution to processive stepping. Disruption of the MT results in decreased processive run lengths measured using single-molecule fluorescence microscopy and a decreased step size under applied load as measured in an optical trap (see Figure). We used single-molecule gold nanoparticle tracking and optical trapping to examine the mechanism of coordination between the heads of dimeric myosin VI. We conclude that intramolecular tension prevents ADP release from the lead head. This mechanism likely increases both the motor's processivity and its ability to act as an anchor under physiological conditions.



1194-Plat
Engineering A Controllable Bidirectional Molecular Motor
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Myosin superfamily motors play crucial roles in cellular functions such as motility, cell division and organelle trafficking. Different myosin classes are