These values were compared to the constitutively active truncated HMM-MyoVa motor over a range of ionic strengths (25-200mM KCl). Surprisingly, at 25mM KCl 16% of actin-associated FL-MyoVa motors are processive, but with significantly slower velocities and shorter run lengths than HMM-MyoVa. The slower velocities result from FL-MyoVa transitioning between periods of "fast" (V_{fast}) and "slow" (V_{slow}, <50nm/s) processivity. To understand the stepping dynamics during these two processes, the two heads of FL-MyoVa were labeled with different colored Qdots. The stepping dynamics during V_{fast} are indistinguishable from HMM-MyoVa, with each head taking 72nm steps ($10s^{-1}$) in a hand-over-hand manner. In contrast, during V_{slow}, each head takes slow ($2s^{-1}$) short (35nm) steps with one head trailing the other, typical of inchworm-like stepping. Moreover, while HMM-MyoVa rarely steps backward under unloaded conditions, during V_{slow} FL-MyoVa take 20% backwards steps, indicating altered gating, potentially due to the head domains experiencing an internal resistive load imparted by tail interactions with one/both heads. FL-MyoVa thus associates with actin in three distinct mechanical states: a fully inhibited stationary state; a fully processive state; and a partially active state with unique stepping dynamics. We propose that this intermediate conformation is in the equilibrium pathway between the inhibited and active states, and provides an additional checkpoint for regulating the motor *in vivo*. This state may allow MyoVa to be poised to switch "on" when cargo binds, or "off" when cargo detaches, thus allowing finely-tuned transport under varying cellular conditions.

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How Varying the Processivity of Myosin V Affects its Motion in Cos-7 Cells

Hailong Lu, Shane R. Nelson, Elena B. Krementsova, David M. Warshaw, Kathleen M. Trybus.

The processive, hand-over-hand mechanism of myosin Va (myoVa) walking on actin has been intensively studied *in vitro*, but less is known about its behavior within cells. We previously showed that myoVa undergoes a random walk in COS-7 cells as it processively steps along actin tracks within the dense and randomly oriented cortical actin network (Nelson et al. BJ 97:509, 2009). Here we test how the processivity of myoV impacts on the observed cellular motion. A mutant construct with 3-fold shorter run lengths than wild-type myoVa (WT), and one with ~1.5-fold longer run lengths, were introduced into cultured COS-7 cells by pinocytosis. The motion of Quantum dot (Qdot)-labeled single motors within the cultured cells was analyzed through high resolution TIRF microscopy and single particle tracking. Mean Squared Displacement (MSD) analysis of the motor:Qdot trajectories appear to be diffusive over short time scale (~1s), and sub-diffusive over longer time scales (~10s). Strikingly, the diffusion coefficients for the short time scales strictly correlate with the processivity of the motor, and range from $0.06\mu m^2/s$ for the least processive motor, to $0.15\mu m^2/s$ for the more processive variant. The non-processive and very slow myoVc, had the lowest diffusion coefficient of any of the constructs tested ($0.019\mu m^2/s$). The observed diffusion coefficients and the sub-diffusive motion for longer time scales was successfully modeled through Monte Carlo simulations assuming that a processive myoVa motor will either cross over, turn or terminate at actin filament intersections within the randomly oriented actin meshwork. Once the motor terminates its run it undergoes restricted diffusion, being potentially confined within domains that are bounded by cytoskeletal or organellar structures. The motor-dependent cellular behavior supports the idea that the apparently wandering trajectories are random walks by active motors.

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Reconstituting a Native Actin Track for Myosin V Transport

Alex R. Hodges, Carol S. Bookwalter, Patricia M. Fagnant, Elena B. Krementsova, Kathleen M. Trybus.

The budding yeast *S. cerevisiae* is an excellent model system to study cargo transport by myosin V. Cargo is transported from the mother cell to the growing bud exclusively by myosin V, and does not involve microtubule-based motors. We find that yeast myosin V (Myo2p) is non-processive *in vitro*,in agreement with previous results [1-3]. This is surprising given that the cellular role of this motor is long-distance cargo transport. However, these experiments were performed using bare skeletal muscle actin filaments, which differ substantially from the native yeast actin track. Our goal is to reconstitute actin cables *in vitro* using yeast actin, yeast tropomyosin, and the actin bundling proteins fascin or fimbrin. Both isoforms of yeast tropomyosin stabilize yeast actin, resulting in much longer filaments. Preliminary data indicate that tropomyosin also enhances Myo2p function. TIRF microscopy was used to observe quantum dots transported by multiple Myo2p motors along the actin track. The presence of tropomyosin dramatically increased the run length and frequency of processive runs relative to bare actin filaments. We are currently testing if a single motor is capable of processive movement in the presence of tropomyosin. The effects of actin bundling on Myo2p function will also be assessed. Our results are consistent with the idea that the composition and structure of the actin track can greatly influence the properties of the motor.

(1) Hodges et al., *Curr Biol* 19 (2009); (2) Dunn et al., *JCB* 178 (2007); (3) Reck-Peterson et al., *JCB* 153 (2001).

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Processive Cargo Movement by Multiple Non-Processive Motors Bound to a Tetrameric Adapter Protein

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Class V myosins can be processive or non-processive, but both support cargo transport. Here we investigate the mechanism by which Myo4p, the single-headed non-processive class V myosin of Saccharomyces cerevisiae, can transport mRNA cargo from the mother to the bud tip. The adapter protein that couples the Myo4p/She3p motor complex to mRNA (She2p) is tetrameric, and thus can in principle recruit multiple motors. Total internal reflection fluorescence (TIRF) microscopy was used to show that one She2p tetramer recruits enough motors to support processive runs. Metal-shadowed images show two motors attached to a She2p tetramer. Deletion of a prominent α-helix that protrudes from the middle of She2p abolishes correct cellular localization of *ASH1* mRNA, suggesting that it is a binding site for She3p. These results highlight that one strategy used by non-processive motors is to work together in small groups, which functionally allows them to support transport that is as robust as a single processive motor.

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Liposome Transport by Myosin Va Motors: Coupling Through Lipid Membranes Modulates Cooperative Motor Interactions and Mechanics

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Myosin Va (myoVa) is a processive, actin-based motor involved in intracellular vesicular transport. Although capable of single myoVa transport in vitro, multiple myoVa motors transport intracellular vesicles, composed of phospholipid outer membranes. To determine how membrane fluidity affects the collective transport capacity of a myoVa ensemble, we synthesized liposomes with either fluid, DOPC or rigid, DPPC phospholipids. Varying number of myoVa motors were attached, and the liposome velocity on actin tracks observed by TIRF microscopy. Fluid DOPC liposomes with physiologically relevant myoVa surface densities (8 motors/200nm liposome) move at 498 ± 228nm/s (n=282), faster ($p<0.001$) than a single, unloaded myoVa (430 ± 120nm/s, n=233) and even faster ($p<0.001$) than rigid DPPC liposomes (328 ± 120nm/s, n=128) of the same size and motor density. We proposed and confirmed through supported lipid bilayer studies that myoVa motors rapidly diffuse within fluid DOPC membranes ($D=0.97 \pm 0.62\mu m^2/s$, n=157) and enrich at the liposome:actin track interface, compared to relatively immobile motors in rigid DPPC membranes. We modeled this phenomenon through Monte Carlo simulations and assumed that lipid membrane properties are critical to inter-motor interactions. Based on these simulations, the slower rigid liposome velocities may result from resistive forces being transmitted between motors, thus modulating the stepping kinetics in a load-dependent manner. However, this strain is dissipated in the fluid DOPC membranes, allowing stochastically faster motors to greatly enhance liposome velocities. Higher motor densities (32 motors/200nm liposome) lead to slower velocities for both liposome species but to a greater extent for fluid liposomes, due to motor crowding and interference at the liposome:actin track interface. Therefore, changes in both motor number and vesicular membrane properties dictate the extent of inter-motor interactions within the ensemble, becoming potential in vivo modulators of intracellular cargo transport.

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Mechanism of Locking Myosin VI Converter in the Unique Pre-Stroke Conformation

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Myosin VI is unique in myosin superfamily because it moves toward the (-)-end of actin filaments. This unique motion is caused by a combination of structural effects, including a surprising conformational change of the converter and a large angle of converter rotation compared to myosin II. This work aims to use computational methods to identify key residues dictating myosin VI's unique converter rotation. We first hypothesized that the residues causing this special rotation are conserved within myosin VI family and unique compared to other myosin families. We identified three myosin Vi-specific residues where all myosin VI proteins have an identical amino acid but no other myosin has the same amino acid in the same aligned column: P444 on the HO linker,