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” ($V_{\text{fast}}$) and “slow” ($V_{\text{slow}}$, <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 $V_{\text{fast}}$ are indistinguishable from HMM-MyoVa, with each head taking 72nm steps (10s$^{-1}$) in an end-to-end hand-over-hand manner. In contrast, during $V_{\text{slow}}$, 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 $V_{\text{slow}}$ FL-MyoVa take 20% backwards steps, indicating altered gating, potentially due to the head domains experiencing 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.

The budding yeast *S. cerevisiae* is an excellent model system to study cargo transport by myosin V. Myosin V is non-processive 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 appears 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 0.066m$^2$/s for the least processive motor, to 0.15m$^2$/s for the more processive variant. The non-processive and very slow myoVc, had the lowest diffusion coefficient of any of the constructs tested (0.019m$^2$/s). The observed diffusion coefficients and the sub-diffusive behavior 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 organelar structures. The motor-dependent cellular behavior supports the idea that the apparently wandering trajectories are random walks by active motors.
M701 on the SH1 helix, and F763 on a helix of the converter. Focusing on the converter, we examined the mutational effect on F763 using a computational approach. We replaced this amino acid with the corresponding amino acid of scallop myosin II, and created a model structure of F763L. We examined the conformational equilibrium of the mutant pre-stroke structure using molecular dynamics simulation. We found that F763L loses an important hydrophobic interaction which holds the converter and the motor domain tightly. Root mean square fluctuation analysis shows the increase in entropic freedom of the converter of the mutant structure. Principle component analysis also shows a much larger first component along the converter compared to the wild-type one. Our results suggest that F763 play a key role in locking the converter of myosin VI in its pre-stroke state, potentially facilitating a large converter rotation of myosin VI or a drastic structural rearrangement of myosin VI’s converter.

644-Pos  Board B444  The Tail Domain of Myosin-VI Ensures the Directed Processive Movement
Keigo Ikezaki, Tomotaka Komori, Mitsuhiro Sugawa, So Nishikawa, Atsuko Iwane, Toshio Yanagida.

Myosin-VI is a motor protein that plays an important role in a large variety of cellular events such as vesicle transport and the anchoring of actin bundles to the plasma membrane. Myosin-VI is thought to move processively as a dimer along an actin filament in a hand-over-hand fashion with step sizes similar to myosin-V.

Recently we showed by using the FIONA method that myosin-VI contains two forward step types, a large forward step (72 nm) and a small forward one (44 nm), and one backward step type (−44 nm). To establish a new stepping method we generated constructs that included these single-molecule high-resolution colocalization (SHREC), an advanced multi-color FIONA technique that involves labeling the two heads with different colored Qdots. This led to a model where for large steps, myosin-VI heads take a state where they are distant when both are bound; while for small and backward steps, the heads take a state where they are adjacent. This model, however, fails to consider the relationship between the head and tail domain during step generation, which has yet to be explored.

To clarify this relationship, we here performed SHREC measurements of myosin-VI labeled at its head and tail domains with different colored Qdots at various nucleotide concentrations. These studies indicate that the lever arm swings forward following small step in <100 ms (within our frame rate) and that it does not swing backward during a backward step. These results suggest that during the adjacent heads binding state, the lever arm is directed forward. This prohibits successive backward steps, which in turns aids in ensuring directed myosin-VI processive movement.

645-Pos  Board B445  Engineering Controllable Myosin Motors for Bidirectional Transport
Lu Chen, Muneaki Nakamura, Tony Schindler, Zev Bryant.

Directed transport mediated by myosin and other molecular motors is a vital process within cells. Particular myosin classes are specialized for unidirectional transport toward either the (+) or (-) end of actin filaments. Previous work has shown that the directionality of recombinant myosins may be altered via the genetic insertion [1] or removal of structural motifs that redirect the lever arm. We have challenged our understanding of myosin structure and function by constructing novel myosin motors that can reversibly switch their direction of motion in response to an external signal. In one successful design using [Ca2+] as the control signal, we have built myosin VI variants incorporating a "retractable" lever arm composed of an alpha-actinin fragment fused to two or more calmodulin-binding IQ repeats. In vitro motility assays show that the engineered motors reverse directionality in response to physiological levels of [Ca2+]. Dimeric versions of our switchable myosins are able to move processively along actin filaments in single molecule tracking assays. We are continuing a detailed biophysical characterization of these engineered motors, and are exploring complementary designs using alternative control signals. Controllable engineered molecular motors should be useful for both studying and reprogramming transport processes in cells, and may also have applications outside of cellular contexts.


646-Pos  Board B446  Structural Features of Myosin X that Favor Straddling more than One Actin Filament
Ronald S. Rock, Benjamin Ricca, Stanislav Nagy.

In the cell, myosin X (M10) is found almost exclusively at the tips of filopodia. The filopodial core contains a parallel and unidirectional arrangement of actin filaments that are bundled by fascin. We have discovered that M10 selects the fascin-actin bundle in the core of the filopodium for motility, and walks poorly on individual, unbundled actin filaments. Here, we have dissected the features that enhance the processivity of M10 on bundles. The structure of M10 may make it easier to walk by straddling two filaments, rather than placing both motor domains on a single filament in a strained configuration. We have measured the stepsize of M10 using both fluorescence imaging and optical traps, and have found ~18 nm steps in both cases. This stepsize is difficult to achieve on a single actin filament, since the myosin would be forced to spin around the filament as it walked. However, the bundle presents additional options to the motor so that regular 18 nm steps are now possible. Moreover, we have completed a series of domain swaps between M10 and the nonselective myosin V motor, since we reasoned that these domain swaps would help to identify the structures required for bundle selection. Surprisingly, bundle selection is controlled by tail domains (including the SAH domain), a part of the myosin that is presumably far away from the actin track. This work reveals that the cellular cytoskeleton is not simply a collection of identical tracks pointing in every direction. The entire cellular system uses a set of simple and elegant interactions to sort cellular traffic to distinct locations; we expect that this theme will reappear many times as more myosins (and other motor families) are examined.

647-Pos  Board B447  Ca2+ Independent and Tail Dependent Regulation of the Motor Activity of Myosin X
Nobushisa Umeki, Hyun Suk Jung, Tsuyoshi Sakai, Osamu Sato, Reiko Ikebe, Mitsuo Ikebe.

Myosin X is involved in the actin cytoskeletal reorganization and protrusion of filopodia. Here we studied the molecular mechanism of regulation of myosin X. The actin-activated ATPase activity of M10Full was Ca2+ independent and significantly lower than that of M10HMM. The tail domain significantly inhibited the actin-activated ATPase activity of M10HMM regardless of Ca2+. The inhibition showed significant dependence on salt concentration, suggesting that the inhibition is dependent on ionic interaction between the tail domain and the head/neck domain of myosin X. The in vitro actin gliding velocity was markedly inhibited (4 fold) by the tail. These results suggest that the tail domain functions as an intra-molecular inhibitor of the myosin X motor function. The deletion of FERM domain abolished the inhibitory activity of the tail. On the other hand, deletion of the N-terminal PEST domain did not affect the inhibitory activity. Further truncation of the PH domain abolished the inhibitory activity of the tail. These results suggest that both the PH and FERM domains of the tail are required for the inhibition. On the other hand, the elimination of both IQ domains and the SAH/coiled-coil domain showed no effect on the tail induced inhibition. Furthermore, M10IQ5 co-immunoprecipitated with M10PH-FERM. The result indicated that the tail domain (PH- FERM) directly interacts with the motor domain to inhibit the motor activity. Electron microscopy revealed that the full-length myosin X molecules were monomeric, showing the wider molecules in low salt with ATP, while narrow molecules, similar head shapes to the M10HMM, in high salt. Our observation suggested that the tail domain folds backward to the head, such that it appears to interact with the motor domain, and thus inhibits the motor activity of myosin X (Supported by NIH).

Microtubular Motors

648-Pos  Board B448  Function Control of Kinesin using Functionality Loop L5 of Kinesin Modified Photochromic Molecule
Kumiko Ishikawa, Keiko Tanaka, Shinshu Maruta.

L5 is one of the unique loops locates in the vicinity of ATP binding site of kinesin. We have previously demonstrated that the point mutation at the L5 dramatically alters ATPase activity and interaction with microtubules. Therefore, the loop may be functional key region. The novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have determined crystal structure of the novel rice kinesin.