

from dmantADP bound to myosin V IIQ (MV IIQ) or Dicty myosin II S1 (Dicty S1) in the presence of 1 mM MgCl₂. We found that dmantADP bound to acto-MV IIQ contained two lifetime components (8 and 3 ns). Time-resolved anisotropy studies of the two lifetime components in myosin V reveal that the long lifetime component was more immobilized (correlation time = 12 ns) while the short lifetime component was highly dynamic (correlation time = 0.4 ns). Interestingly, Dicty S1 contained a single lifetime component (8 ns). The two conformations of the myosin V active site may allow Mg²⁺ to more efficiently bind and reduce key steps in the ATPase cycle such as ADP release. Overall, our results suggest that differences in the structural dynamics of the active site of myosins may play a role in their dependence on free Mg²⁺, which could explain why Mg²⁺ differentially alters the motile and force generating properties of myosins.

3302-Pos Board B457

Dynamics of the N-Terminal Domain of Myosin V Monitored by FRET

Darshan V. Trivedi¹, William C. Unrath¹, Howard D. White², Christopher M. Yengo¹.

¹Pennsylvania State University, Hershey, PA, USA, ²Eastern Virginia Medical School, Norfolk, VA, USA.

The N-terminus is a highly variable region among the myosin superfamily and has been associated with alterations in rotation of the lever arm and step size. We examined the structural dynamics of the N-terminal region of myosin V using steady-state and stopped-flow FRET. We introduced a tetra-cysteine site at the extreme N-terminus of a Myosin V IIQ construct and labeled it with a bisarsenical fluorescein derivative (FIAsh) (MV NT-FLAsH). Energy transfer between FIAsh and mant or deac labeled nucleotides (dmantATP, dmantADP and deacATP) was monitored. Steady-state FRET experiments with the mant-FIAsh pair demonstrated a high FRET state in the presence of dmantATP and a low FRET state in the presence of dmantADP. Sequential-mix, in which MV NT-FLAsH is first mixed with dmantATP, aged to allow formation of the M.ADP.Pi state, and then mixed with saturating actin allowed us to explore the actin-activated product release steps. Biphasic transients with rates corresponding to the fast and slow rate of dmantADP release were observed. The sequential mix experiments with deacATP yielded three phases; a fast, actin dependent, phosphate release phase followed by the two phases of ADP release. Our results suggest the FRET signal monitors a structural change in the N-terminus associated with the sequential release of products. A high FRET state upon binding of ATP is associated with formation of the pre-powerstroke state of the lever arm and a low or no FRET state in the presence of ADP suggests formation of the post-powerstroke state. Our results allow us to hypothesize that movement of the N-terminal domain follows the movement of the converter/lever arm. Further experiments will explore the dynamics of the N-terminal domain and examine how its motion correlates with the conformation of the nucleotide binding pocket and lever arm.

3303-Pos Board B458

Insights into the Mechanism of Myosin V Processivity from Point Mutations in the Converter Domain

Sergey V. Mikhailenko, Takashi Ohki, Jun-ichi Yoshimoto, Shin'ichi Ishiwata.

Waseda University, Tokyo, Japan.

Dimeric myosin V is an intracellular transporter, which moves cargoes towards the barbed end of actin filaments. Its ability to perform as individual molecules implies that the ATPase cycles in two head domains are coordinated, ensuring the efficient unidirectional motility. Directional loads were shown to modulate the kinetics of nucleotide binding to myosin V, suggesting that the head-head communication may be achieved via intramolecular load, generated when both heads are bound to actin. Here we directly tested the effect of the intramolecular load on the processive stepping of myosin V, using point mutations in the converter domain. The converter is a compact structure, which transmits tiny conformational changes at the nucleotide-binding site to the lever arm. To disturb the transmission mechanism, we replaced with alanines, one at a time, two phenylalanine residues that form a hydrophobic cluster with the C-terminus of the relay helix. These mutations are inferred to reduce intramolecular load but affect neither the nucleotide binding nor actin affinity. We used the combination of bulk kinetic and single-molecule measurements to study in detail the effect of the mutations in the converter on the myosin V performance. The F697A mutation, which completely eliminates intramolecular load, abolishes the motility of myosin V dimers. At the same time, the F749A mutation, which only partly reduces intramolecular load, significantly increases the proportion of backward steps. The obtained results provide strong experimental evidence that the efficient unidirectional processive stepping of myosin V is ensured by the head-head communication based on the intramolecular load.

3304-Pos Board B459

Searching, Stepping, and Stomping: What Polymer Theory can teach us about the Molecular Motor Myosin V

Michael Hinczewski¹, Riina Tehver², Devarajan Thirumalai¹.

¹University of Maryland, College Park, College Park, MD, USA, ²Denison University, Granville, OH, USA.

Though myosin V is among the most extensively studied of motor proteins, improvements in experimental techniques continue to provide new insights into the details of its dynamics. High-speed atomic force microscopy has visualized not only the expected hand-over-hand stepping of the two-headed motor along actin filaments, but additional, less well understood behaviors like "foot stomping", where one head detaches and rebinds to the same site. A comprehensive picture of myosin motility needs to account for all the kinetic pathways, including backstepping and foot stomping, how they vary under load, and their relationship to the structural and chemical parameters of the motor. Starting from a simple polymer model, we develop an analytical theory to understand the minimal physical properties that govern motor dynamics. In particular, we solve the first-passage problem of the head reaching the target binding site, investigating the competing effects of load pulling back at the motor, strain in the leading head that biases the diffusion in the direction of the target, and the possibility of preferential binding to the forward site due to the recovery stroke. The theory reproduces a variety of experimental data, including the power stroke and slow diffusive search regimes in the mean trajectory of the detached head, and the force dependence of the forward-to-backward step ratio, run length, and velocity. The analytical approach yields a formula for the stall force, identifying the relative contributions of the chemical cycle rates versus mechanical features like the bending rigidities of the lever arms. Moreover we can fully explore the parameter phase space, to determine the robustness of the dynamical behavior to perturbations, and the natural constraints that dictate the structure of the motor.

3305-Pos Board B460

Building Complexity In Vitro: Single Molecule Reconstitution of ASH1 mRNA Transport by a Class V Myosin from Budding Yeast

Thomas E. Sladewski¹, Carol S. Bookwalter¹, Myoung-Soon Hong², Kathleen M. Trybus¹.

¹University of Vermont, Burlington, VT, USA, ²NIH, Bethesda, MD, USA.

Sub-cellular localization of mRNA is a widely used mechanism to ensure correct spatial and temporal expression of proteins within the cell. A paradigm for localizing mRNAs is the ASH1 transcript in budding yeast which is transported by Myo4p, a class V myosin motor. We previously showed that Myo4p is a single-headed motor that tightly binds the adapter protein She3p. She3p also interacts with the mRNA-binding protein She2p, which recruits two Myo4p/She3p motors. This double-headed motor complex is processive at low salt, but becomes destabilized at physiological ionic strength, suggesting that other factors are required for Myo4p motility. To understand how Myo4p functions in the cell, we increased the complexity of our system by adding labeled mRNA cargo to the motor complex, forming a fully reconstituted messenger ribonucleoprotein complex (mRNP). This mRNP shows robust processivity at physiological ionic strength, thus providing a checkpoint to ensure that only Myo4p motors that are integrated into an mRNP are motile. The ASH1 transcript contains four sequence elements called "zipcodes", which bind She2p. To understand why ASH1 has multiple zipcodes, we reconstituted ASH1 mRNPs containing varying numbers of zipcodes. We find that transcripts with multiple zipcodes more effectively recruit a paired motor complex for transport. Metal-shadowed images of mRNPs show as many as 8 motors bound to native ASH1 transcripts, suggesting that these particles are likely optimized to move on the actin cables found in the cell. We find that mRNP motility on actin bundles shows dramatic enhancements in both run frequency and run length compared to single actin filaments. Thus, only by building complexity in vitro can one begin to fully understand how motors function in their cellular context.

3306-Pos Board B461

Stepping Dynamics of Two Coupled Myosin Va Motors on Actin Bundles

M. Yusuf Ali¹, Andrej Vilfan², Arthur Michalek¹, Kathleen M. Trybus¹, David M. Warshaw¹.

¹University of Vermont, Burlington, VT, USA, ²J. Stefan Institute, Ljubljana, Slovenia.

Myosin Va is a processive, actin-based molecular motor that is critical for organelle transport. While transporting intracellular cargo, myosin Va faces significant physical barriers and directional challenges created by the complex actin cytoskeleton, a network of intersecting actin filaments and actin bundles. We have created an in vitro model system of fascin cross-linked unipolar actin bundles. While walking on an actin bundle, a single myosin Va motor switches