Unconventional Myosins

899-Pos Board B654
The Structure of Vertebrate Myosin-I Reveals New Insights into Mechanochemical Tuning of Myosins
Michael J. Greenberg[1], Henry Shuman[1], Adam Zwolak[1], Charles Sindelar[2], Roberto Dominguez[1], E. Michael Ostap[1].
[1]Physiology, University of Pennsylvania Medical School, Philadelphia, PA, USA, [2]Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA.
Myosins are actin based motors that are mechanically and kinetically tuned to function in a myriad of cellular processes. The myosin-I family member Myosin-IB (Myo1b) has very slow kinetics and a force sensitivity that is greater than any other characterized myosin, enabling it to function as a tension-sensing anchor. Because there have been no high-resolution structures of myosins that act as tension-sensitive anchors, we determined the 2.3-Å resolution structure of the motor domain and first IQ-motif of Myo1b with bound calmodulin in the absence of nucleotide. The structure reveals several striking features not yet seen in other myosins, including a unique positioning of the light chain binding domain (LCBD) and converter region. This unique conformation results in a cavity that sandwiches the N-terminal region (NTR) of the heavy-chain between the motor domain and LCBD. Single molecule and ensemble biochemical analyses show that the NTR plays an important role in stabilizing the post-power stroke conformation of Myo1b and in tuning the rate of the force-sensitive transitions that limit actin detachment. The sequence of the NTR is highly variable within the myosin-I family, so we propose that together with unique LCBD and converter conformations, the NTR plays a role in tuning the mechanochemical properties of the motor.

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Myosin-3B and its Light Chains
Sarah M. Heissler, Neil Billington, James R. Sellers.
Laboratory of Molecular Physiology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA.
Unconventional myosin-3B is a single-headed myosin containing an amino-terminal kinase domain. In vitro, we find full length and truncated versions of the molecule to bind the regulatory light chain (RLC) and calmodulin (CaM). The RLC binds to the first IQ-like motif and CaM to the second IQ motif within the neck region of the molecule. Uniquely, we find the RLC to be reversibly exchangeable with CaM in the presence of calcium. Calcium-free conditions favor RLC binding. Exchange of RLC versus CaM to the first IQ motif significantly increases the steady-state ATPase activity in the presence of calcium and modulates the interaction of the molecule with F-actin. This observation shows that the calcium-dependent light chain exchange triggers the activity of human myosin-3B as a molecular motor and suggests that myosin motor function is directly influenced by the set of light chains bound to the neck region.

901-Pos Board B656
Dynamics of the Lever-Arm Swing in Myosin V
Darshan V. Trivedi[1], Jonathan P. Davis[2], Christopher M. Yengo[1].
[1]Cellular and Molecular Physiology, Pennsylvania State University, Hershey, PA, USA, [2]Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA.
Myosins utilize a conserved structural mechanism to convert the energy from ATP hydrolysis to a large swing in the force generating lever-arm. However, there remains an ongoing controversy about the kinetics of lever-arm swing in relation to the steps in the ATPase cycle. To address this question we have developed a novel FRET system in myosin V (MV) that utilizes several donor-acceptor pairs to examine the dynamics of lever arm motion. MV containing a single IQ motif and an N-terminal (NT) tetracysteine site was labeled with the bisarsenical dye FlAsH (MV.NT.FlAsH). The first IQ motif of MV.NT.FlAsH was exchanged either with IAANS labeled CaM, a donor, or QSY-9 labeled CaM, a non-fluorescent acceptor. Steady-state and transient kinetic experiments reveal a decrease in FRET upon ATP binding (recovery stroke) in both donor-acceptor pairs. We utilized transient kinetic experiments to demonstrate that upon mixing the MV.ADP.Pi complex with actin there was a FRET increase that occurred in two phases, and the fast and slow phases correlated well with the release rates of Pi and ADP, respectively. We also labeled the upper-50kDa tetracysteine site with FlAsH (MV.U50.FlAsH) and exchanged the QSY labeled CaM on to the first IQ motif. We observed structural changes during ATP binding that were very similar to the MV.NT.FlAsH results. During actin-activated product release we observed two-phases, a rapid increase in FRET followed by a slower decrease in FRET, which correlated well with ADP release. We find that the force generating motion of the lever arm occurs in two steps which are closely coupled to the product release steps. Our results also indicate that the conformational changes in the lever arm associated with the power stroke may follow a unique pathway that is not simply the reversal of the recovery stroke.

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Two Molecule of Two Headed Myosin 5C on a DNA Scaffold Steps Processively along Actin Filaments
Laura Gunther[1], Kenya Furuta[2], Jianjun Bao[1], Yuwen Mei[1], Howard White[3], Takeshi Sakamoto[1].
[1]Wayne State Univ, Detroit, MI, USA, [2]Advanced ICT Research Institute, National Institute of information and Communication Technology, Kobe, Japan, [3]Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA, USA.
Myosin 5c is a low duty ratio motor and doesn't move processively along actin filaments as a single molecule. Here, we characterized the kinetic properties of double-headed myosin 5c and examined the possibility that multiple myosin 5c molecules could possess processive movement. Two myosin 5c molecules were conjugated to DNA scaffolds and their processivity was analyzed by single molecule techniques with TIRF and FIONA. ATPase activity assay and ADP dissociation kinetics demonstrated that the interaction between the two heads of myosin 5c increases its F-actin-binding affinity via the ''gated-gate'' mechanism. Single molecule study revealed that two myosin 5c molecules in a complex moved processively along actin filaments. Moreover, the space distance between the myosin 5c molecules is an important factor for processive movements. Our results provided a novel possibility for a non-processive motor to achieve processive movement that could be used to transport cargo in cells.

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A Role for Myosin VI in Retinal Pigment Epithelium Phagocytosis
Rebekah Daniel[1], Bianca Nagata[2], David Altman[1].
[1]Department of Physics, Willamette University, Salem, OR, USA, [2]Department of Biology, Willamette University, Salem, OR, USA.
Retinal pigment epithelium (RPE) cell phagocytosis of shed photoreceptor rod outer segments (ROS) is vital for retinal function. We examined the role of myosin VI in this process by utilizing micron-diameter plastic beads as a proxy for ROS waste. When placed in the extracellular medium, the beads were phagocytosed by a cultured human RPE cell line (ARPE-19). At the same time, these cells were made to over-express myosin VI with perturbed motor function. We observed that the rates of trafficking of beads within the cell were significantly reduced in the presence of the perturbed myosin VI compared to control cells, indicating that myosin VI plays a role, either directly or indirectly, in RPE phagocytosis.

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Single Molecule Characterization of Human Myosin VIIA
Osamu Sato[1], Tsuyoshi Sakai[1], Ryosuke Tanaka[2], Takeomi Mizutani[2], Tomonobu M. Watanabe[3], Reiko Ikebe[1], Mitsuo Ikebe[1].
[1]Dept. of MaPS, UMass Med Sch, Worcester, MA, USA, [2]Faculty of Advanced Life Science, Hokkaido University, Sapporo, Japan, [3]QBiC, Riken, Osaka, Japan.
Human myosin VIIA is responsible for Usher Syndrome (USH) type 1B. Although human myosin VIIA is thought to be involved in transportation of USH1 proteins and melanosomes, it is obscure that human myosin VIIA is a suitable motor as a cargo transporter.
In this study, we analyzed the motor characteristics of tail truncated human myosin VIIA (HM7DTail/LZ) at single molecule level. We found that HM7DTail/LZ moves processively with large ~35 nm forward and small ~23 nm backward steps at physiological ATP concentration. The forward step of HM7DTail/LZ was slightly larger than that of Drosophila myosin VIIA [~30 nm, Yang *et al.* (2006) PNAS, 103, 5746-5751]. The average run-length of HM7DTail/LZ was ~0.7 μm on single actin filaments. This means that HM7DTail/LZ can move ~20 steps on single actin filaments on average. Dwell time distribution gave the average waiting time of ~3.4 s, yielding 0.3 s^{-1} for the mechanical turnover rate. This rate is consistent with the V_{max} value of actin activated ATPase activity (~0.3 s^{-1}) of tailless human myosin VIIA. On the other hands, the velocity of HM7DTail/LZ was extremely slow, 11 nm/sec. This value is ~7 times slower than that of Drosophila myosin VIIA [72 nm/s, Yang *et al.* (2006)]. Recent studies have suggested that mammalian myosin VIIA may function in tethering melanosomes on actin filaments. Thus, the slow velocity may imply that human myosin VIIA is more suitable for cargo-actin tethering motor than Drosophila myosin VIIA, and may link transportation processes such as USH protein transportation. We recently found that human myosin VIIA localizes at the filopodia tip in cells when it dimerizes [Sakai *et al.* (2011) PNAS,108, 7028-7033]. Currently, we