Myosin V takes multiple 36-nm steps along an actin filament by coordinating its two motor domains. The properties of myosin V have been addressed by means of several assays. In particular, optical tweezers techniques and FIONA have contributed widely to enlightening the motion mechanism. However, these two assays cannot be applied when studying the mechanism for coordinated force generation by a single motor domain during motion. For myosin V, optical tweezers assays are restricted to studying the mechanical properties of both motor domains when an optically-trapped bead is attached to the Myosin V tail. On the other hand, although FIONA is capable of observing the motion of a single motor domain during gait motion, it is not capable of revealing the mechanical properties because these studies do not apply external force.

Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V based on the previous report by Block's lab. The DNA linker is used to connect a bead to one of the two motor domains. In this experimental setup, external force by the optical tweezers is applied to a single motor domain directly via the DNA linker during gait motion. By using this measurement system, we succeeded to observe single head behavior while force is applied to it.

2902-Pos

Reconstituting a Native Actin Track for Myosin V Transport

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The budding yeast *S. cerevisiae* is an excellent model system for the study of cargo transport by myosin, given the relative importance of actin cables versus microtubules in this cell. Despite this, no *in vitro* studies have tried to mimic the actin-tropomyosin cables along which the class V myosin Myo2p transports secretory vesicles, vacuoles, mitochondria, and other organelles to the growing bud. We find that Myo2p is non-processive *in vitro*,in agreement with previous results [1-2]. These experiments were, however, performed using chicken skeletal actin, which is only 87% identical to yeast actin. Accordingly, we are investigating if Myo2p behavior changes when the *in vitro* conditions more closely match those found in the yeast cell. Actin cables will be reconstituted *in vitro* from yeast actin, yeast tropomyosin, and the actin bundling protein fimbrin. The effects of yeast versus skeletal actin, of bundled actin versus single filaments, and of the presence of each of the two different tropomysin isoforms will be tested. Myo2p motility, processivity, and actin binding affinity will be assessed with these different tracks. The effect of varying ionic conditions, nucleotide concentration, and viscosity will also be tested, to determine if Myo2p behavior changes as conditions more closely match those of the intracellular milieu.

1. Reck-Peterson et al., *JCB* 153 (2001).
2. Dunn et al., *JCB* 178 (2007).

2903-Pos

Liposomes as Model Cargo for Myosin Va

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Myosin Va (myoVa) is a processive, actin-based motor involved in the transport of membrane bound secretory vesicles and organelles. How multiple motors attached to such cargo generate productive forward motion is unclear. To address this, we have coupled expressed myoVa-HMM with a C-terminal biotin tag to extruded fluorescent, neutravidin-coated liposomes as an in vitro model for intracellular cargo. This model system allows control over liposome size, fluidity, and surface density of attached motors. When observed in TIRF on actin tracks at room temperature, "rigid" 400nm DPPC liposomes with ~160 motors/liposome move at speeds equal to that of a single processive myoVa-HMM (510 ± 227nm/s), whereas, 200nm liposomes with the same surface density of motors move 30% slower (352 ± 121nm/s). In comparison, "softer" 200nm DMPC/cholesterol liposomes that have more fluid phase membranes are slower yet (229 ± 130nm/s). These velocity data suggest a complex relationship between the ensemble of attached motors and the liposome rigidity/fluidity and size. With more fluid membranes, motors may be mobile within the liposome membrane, compromising their contribution to forward motion and thus the slower velocities. In contrast, larger, more rigid liposomes may allow a fixed number of transporters to remain productively engaged. Interestingly, liposomes can be observed "cartwheeling" along actin tracks, suggesting that motors can exchange roles between being an active transporter and a passenger waiting its turn as the liposome effectively rolls down the actin track. By attaching Qdot-labeled myoVa-HMM, the exact spatial relationship between the motor and liposome cargo can be determined to help understand and model the complexities of this simplified in vitro representation of intracellular cargo transport.

2904-Pos

Myosin Va Cargo Transport on Actin Bundles

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Myosin Va (myoVa) walks processively while carrying cargo towards the plus end of actin filaments. In cells, parallel actin filament bundles (e.g. stress fibers and filopodia) present a directional challenge to myoVa cargo transport. Therefore, we formed unipolar (fascin) and mixed polarity (alpha-actinin) actin bundles as tracks for expressed myoVa-HMM with a C-terminal biotin tag. In this assay, a single streptavidin-Qdot served as cargo for one or many (~ 5) myoVa motors. Qdots transported by one or many myoVa molecules traveled in the same direction on unipolar bundles, while moving in either direction on mixed polarity bundles. Qdot speeds were the same regardless of bundle type or number of motors (400nm/s), and similar to that for one or many motors on a single actin filament (Nelson et al., 2009). However, run lengths for single motors were 2-3 times longer on bundles than previously observed on single actin filaments. This suggests that on parallel tracks the leading head has a greater number of actins within its reach, thus decreasing the probability of run termination. Interestingly, on mixed polarity bundles, we observed individual Qdots changing directions in the middle of a run, the frequency of which increases in the multiple motor case. It was not surprising that a Qdot with a single motor can switch directions on a mixed polarity bundle, given myoVa's inherent flexibility that allows it to turn up to 150o at actin filament intersections (Ali et al., 2007). These data also suggest that one or many myoVa molecules bound to a single cargo have the ability to jump tracks to neighboring actin filaments. With Qdot-labeling of the individual heads, high spatial resolution studies will confirm this on mixed polarity bundles, and determine whether the motors also wander on unipolar bundles.

2905-Pos

Flexibility of Stepping Manner of Myosin V and X Processive Movement on 2D Actin Structures

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In a TIRF *in vitro* motility assay, we investigated the processivity and stepping characteristics of myosin V HMM and myosin X HMM with a leucine zipper on single actin filaments and actin bundles. Actin was polymerized and cross-linked on a charged lipid monolayer in Teflon wells in order to create regular 2D or 3D structures. Two cross-linking proteins were used: alpha-actinin which produces non-polarized bundles with 40 nm filament spacing and fimbrin which produces polarized actin bundles with 14 nm filament spacing. We were determined the velocities and the run length for processive movement on those 2-D actin bundles by using modified particle tracking softwear. Myosin V moved processively on all types of in vitro actin structures. Myosin X moved well on polarized fimbrin cross-linked bundles but movement was impaired or non-existent on non-polarized alpha-actinin bundles. Furstermore, we have measured the stepping manner of myosin V and X by using FIONA analysis, which allow us to measured nano-meter precision. Myosin V steps along single actin filaments, while myosin X steps over several actin filaments on the 2-D actin filaments. We hypothesize that forward runs of myosin X on alpha-actinin cross-linked bundles are inhibited because myosin X "sidesteps" to a parallel oppositely polarized filament and the run stalls. The presence of a SAH domain in the lever arm of myosin X could increase the working stroke or flexibility of the lever arm and allow it more easily sidestep the larger alpha-actinin filament spacing.

2906-Pos

Mechanical and Kinetic Properties of a Myosin 5-SAH Chimera

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We have determined the kinetic and motile properties of a myosin 5a HMM construct in which four calmodulin-binding IQ motifs are replaced by the putative single alpha helical domain (SAH) of similar length from Dictyostelium myosin, MyoM. Electron microscopy of this chimera showed that the SAH domain was straight and 17 nm long as predicted, restoring the truncated lever to the length of wild type (Myo5-6IQ). The powerstroke (21.5 nm) measured in