filaments within the bundle with a high probability (24%). Although a single myosin Va is sufficient to transport cargo in vitro, intracellular cargo transport is driven by multiple motors. To understand the collective behavior of multiple motors, we have linked two myosin Va motors, with only one head of each motor labeled with either a red or green Qdot, via a third Qdot which acted as a cargo. If each motor walks on a different actin filament within the bundle, then the two motors may experience an off-axis load. The velocity and the run length of the 2-motor complex was reduced significantly from that of a single motor, suggesting that the motors interfere with each other's motion. Interestingly, the leading motor takes ~10% back steps, indicating that it experiences a resistive load from its partner. Both the run length of the complex and the step lifetimes of the motors were correlated to the inter-motor distance, with the run length decreasing and the step lifetimes increasing with greater motor separation. Our data suggest that the two motors step independently when close together. However, when far apart, tension increases in their cargo-linkage, which results in inter-motor mechanical coupling. This study will provide insight into the mechanism of how multiple motors mechanically interact to transport cargo in vivo.

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Stepping Mechanisms of Myosin Va on Various Actin Cytoskeleton Structures

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Myosin Va plays a central role in intracellular polarized transport through its actin-based processivity. Cargo transport by myosin Va has been intensively investigated. However, it is not known yet whether a myosin Va molecule moves over all the types of actin filaments in cells. To examine the effects of different types of actin filaments on myosin Va's processivity, we have used actin cytoskeleton structure in cells that were de-membraned by triton treatment. We then observed the movement behavior of single myosin Va molecules on these different areas of actin filaments which include stress fibers, filopodia, leading edges, and lamellipodia. The run-length, direction of step and step-size of myosin Va, at each region of cytoskeletal actin were measured. We found that myosin Va molecules moved well on stress fibers (run-length: 1.8 ± 0.35 μm), leading edge (run-length: 2.1 ± 0.43 μm), and filopodia (run-length: 1.6 ± 0.21 μm), but not at lamellipodia area (run-length: 0.54 ± 0.35 μm). The step-size of myosin Va molecule was 35.2 ± 8.2 nm at the stress fiber area, while it exhibited two peaks (Major peak: 34.6 ± 9.1nm, and Minor peak: 18.5 ± 5.5 nm) on filopodia. On the actin filaments at the leading edge, the distribution of myosin Va step-size was larger than others (35.5 ± 16.2 nm), suggesting that the myosin Va may take side-steps on actin filaments at the leading edges. We further investigate the movement and mechanism of myosin Va on a restricted subset of actin filaments.

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More than Just a Cargo Adapter: Melanophilin Prolongs Slow Processive Runs of Myosin Va

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Melanophilin (Mlph) is a cargo adapter protein that links melanosomes via Rab27a to myosin Va (myoVa) for transport along actin. In the absence of cargo, full length myoVa is in equilibrium between a folded, inactive and an extended, active conformation. This equilibrium causes a deviation from a hand-over-hand stepping pattern due to altered gating between the heads (Armstrong et al. 2012). Cargo binding has been suggested to activate myoVa for transport. Here we used single molecule TIRF assays at near physiological ionic strength (150mM KCl) to determine the effect of Mlph on myoVa processivity. In the absence of Mlph, Qdot labeled full-length myoVa moved at a median velocity of 443nm/s, and showed the altered stepping pattern previously seen at lower ionic strength. Addition of Mlph recruited 14-times more motors to move processively, consistent with a simple model of cargo activation. The myoVa-Mlph complex also showed increased run lengths, with many motors traveling to the ends of the actin filament. In the presence of Mlph, myoVa moved much more slowly (median velocity=75nm/s). The speed distribution was asymmetrical and similar to speeds of melanosome movement observed in vivo. In the presence of Mlph, myoVa showed normal gating between the heads, and hand-over-hand steps on actin typical of a fully-active motor. Based on mutagenesis of Mlph the enhanced processivity depended on a positively charged cluster of amino acids in the actin binding site of Mlph. This suggests that Mlph acts as an electrostatic tether to limit myoVa dissociation from actin, a property likely to favor the transfer of melanosomes to adjacent keratinocytes in vivo. More generally, our results suggest that adapter proteins which link motors to cargo can affect motor properties in ways favorable for their biological role.

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Engineering of the Myosin Viia Nucleotide-Binding Pocket to Create Selective Sensitivity to N^6-Modified ADP Analogs

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Mutations in myosin VIIa (MYO7A), an unconventional myosin, lead to sensory disorders that include congenital deafness or progressive hearing loss. MYO7A that is expressed in the cochlear neuroepithelium has been shown to play a role in maintenance of tension and the organization of stereocilia. Recent studies showed that MYO7A is located at the upper end of the tip link (Grati et al. 2011), which led us to ask whether MYO7A plays a role in adaptation, a role previously thought to be performed by myosin 1c (MYO1C). Identification of the Y61G mutation in MYO1C, which is selectively inhibited by the N^6-modified ADP analog N^6-(2-methylbutyl)ADP, motivated the development of MYO1C-Y61G transgenic mice, which were used to evaluate MYO1C's role in transduction and adaptation. We engineered and characterized an analogous MYO7A mutant, Y114G-S1-SAH, to test ADP analogs for selective inhibition of mutant MYO7A. MYO7A-Y114G-S1-SAH was created through site-directed mutagenesis, and MYO7A-S1-SAH (WT) and MYO7A-Y114G-S1-SAH were expressed in SF9 insect cells with a baculoviral expression system. An NADH-coupled steady-state assay showed that the basal ATPase of MYO7A-Y114G-S1-SAH is the same as WT ($0.16s^{-1}$); at $2.25s^{-1}$, the actin-activated ATPase activity of the mutant is about 4-fold higher than WT. The rate of ADP release from the Y114G-actomyosinADP complex is 10-fold faster ($k_{-AD}=10s^{-1}$) compared to WT. The rate of ATP hydrolysis (k_H+k_{-H}) measured by tryptophan fluorescence is $>12s^{-1}$, similar to WT. MYO7A-Y114G-S1-SAH had an affinity of 0.36 μM for the N^6-(2-methylbutyl)ADP, 71-fold higher than WT (25.6 μM). These data show that activity of Y114G-MYO7A is similar to WT, except that it can be selectively inhibited by N^6-modified ADP analogs. A mouse line is being generated with the Y114G mutation knocked into the *Myo7a* locus for electrophysiological studies of transduction and adaptation.

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Myosin-XXI, a Motor with many Missions

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Myosin-XXI is one of only two myosins found in the Leishmania parasite genome. While no expression of myosin-IB has been found in the organism to date, myosin-XXI has been detected in both the amastigote and promastigote stages of the Leishmania life cycle. The presence of only a single myosin isoform suggests that this myosin carries out a variety of functions within the protozoa, including membrane anchorage, longer range directed movements of cargo and possibly roles in cell signalling. Our aim is to investigate how a single myosin can carry out several different tasks within the cell and to identify molecular mechanisms controlling this. To determine the directionality of motor movement we performed gliding filament assays using myosin-XXI constructs expressed using a baculovirus/SF21 system and dual labelled F-actin with actin-filaments capped by gelsolin and labelled with phalloidin-TRITC at their barbed ends and phalloidin-FITC at their pointed ends. These experiments showed that myosin-XXI is a plus-end directed motor. Our in vitro studies also showed that myosin-XXI binds to a variety of lipids including PIP2 and PIP3 as well as a number of other phospholipids. Furthermore, the motor can adopt both a monomeric and a dimeric conformation in vitro. using a variety of tail constructs we found that only the monomeric conformation has the ability to bind lipids. We identified several distinct lipid-binding sites in the tail domain with different lipid binding specificities. Preliminary data suggest that motor dimerisation and lipid binding are regulated by binding of calcium-calmodulin which might play a key role in the cellular distribution of the motor and its ability to perform a variety of motile roles within the parasite. Sponsored by DFG-SFB 863 and Baur-Stiftung.

Actin and Actin-binding Proteins

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Structural Polymorphism of F-Actin is Coupled with its Mechanical Properties

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