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N-terminal extension that shows no similarity to any known protein domain. Here, we show that the human myosin-18B motor domain binds to F-actin with an affinity of 4 μM. The isolated motor domain binds ATP but has no intrinsic ATPase activity. The large N-terminal extension is shown to directly bind to F-actin with an affinity of 7 μM. This interaction is nucleotide-independent but shows strong ionic strength dependence, which is indicative for a charge-mediated actin binding mechanism. We further analyzed the molecular function of the N-terminal extension by means of actin polymerization assays and found that the myosin-18B N-terminus inhibits F-actin assembly *in vitro*. Myosin-18B has previously been shown to be located in the cytoplasm of undifferentiated myoblasts. At later stages of differentiation it accumulates in myonuclei. Furthermore, it has been shown that cardiomyocytes display a partial sarcomeric pattern of myosin-18B alternating that of α-actinin-2. Based on our data, we propose a role for myosin-18B in the regulation of muscle sarcomere architecture during differentiation and the regulation of the nuclear actin pool.

910-Pos Board B665

Calmodulin and Lipid Binding Regulate Dimerisation and Motility of Myosin-XXI in Leishmania

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In Leishmania parasites myosin-XXI seems to be the only myosin expressed. Although it has been suggested that it performs a variety of motile functions, the motor's oligomerisation states, cargo-binding and motility are unknown. We found that binding of a single calmodulin causes the motor to adopt a monomeric state and to move actin filaments at ~18 nm.s-1. In the absence of calmodulin, non-motile dimers were formed that cross-linked actin filaments. The dimerisation domains include the calmodulin-binding neck region, which is essential for the generation of force and movement in myosins. We also found that monomeric myosin-XXI bound to mixed liposomes, while the dimers did not. The lipid binding sections overlapped with the dimerisation domains. They also included a phox-homology (PX) domain in the converter region. We propose a novel mechanism of myosin regulation, where dimerisation and motility are regulated by binding of calmodulin and lipids. While myosin-XXI dimers could act as ATP-dependent, non-motile actin crosslinkers, the calmodulin-binding monomers might transport lipid cargo. Sponsored by DFG-SFB 863 and Baur-Stiftung.

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Class III Myosin Motor Activity Correlates with Localization in Actin Protrusions

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Class III myosins (myo3A and myo3B) contain a N-terminal kinase domain, central motor domain, and an isoform specific C-terminal tail. Myo3A contains a longer tail and has an actin-binding motif at the C-terminus that is not present in the shorter tail of myo3B. Modifications to the myo3A gene result in late onset human deafness (DFNB30), suggesting myo3B may be able to compensate for myo3A only early in life. Thus, it is critical to understand differences in the motor properties of myo3A and myo3B. We investigated the in vitro motility of myo3A and myo3B, each with the kinase domain removed and containing two IQ domains and a C-terminal GFP fusion (myo3AΔK.GFP and myo3BΔK.GFP). The sliding velocity measured in the in vitro motility assay was ~20% faster in myo3AΔK.GFP (76 nm/sec) compared to myo3BΔK.GFP (62 nm/sec). The sliding velocity correlated well with the maximum actin-activated ATPase activity (k_{cat} = 1.0 sec^{-1} and 0.7 sec^{-1}, respectively). Interestingly, myo3B.ΔK.GFP contained a 25-fold weaker actin affinity compared to myo3A.ΔK.GFP as assessed by the actin-dependence of the ATPase activity (K_{ATPase} = 3 and 76 μM, respectively). We transfected N-terminally GFP tagged myo3A.ΔK containing the full length tail domain into COS7 cells and examined the efficiency of localizing to the filopodia tips by examining the tip to cell body ratio. Since the tail actin binding motif is required for localization to filopodia tips, we generated a chimera that contained the myo3B motor and the actin binding motif of the myo3A tail. The tip localization efficiency was approximately 17% ($p<0.05$) reduced in myo3B compared to myo3A. Thus myo3B is a slightly slower motor with a weaker actin affinity compared to myo3A, which may explain why it is unable to compensate for the loss of myo3A in DFNB30.

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Structural Basis of Myosin 1C Ca^{2+} Regulation

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Myosins are actin-based motor proteins that hydrolyze ATP to generate force and movement. Class I myosins are single-headed and can be subdivided into short and long-tailed isoforms. Myosin 1c is a short-tailed isoform involved in the insulin-dependent transport of GLUT4-storage vesicles and the adaptation response of the stereocilia in the inner ear. Both these processes are Ca^{2+}-regulated. Here, we show how myosin 1c motor activity is regulated by calcium ion binding to calmodulin at the first IQ motif. We present the crystal structure of the human myosin 1c motor domain in complex with calmodulin bound to the first IQ motif in the pre-power stroke state at 2.7 Å. The structure reveals a lever arm orientation that positions the Ca^{2+}-binding lobes of calmodulin closely to the HO-linker of the motor domain. Deletion of a unique 6 amino acids insertion in the HO-linker abolished Ca^{2+}-regulation of myosin 1c, as evidenced by a detailed comparative kinetic analysis of wild-type and deletion mutants. Our findings reveal an allosteric communication pathway, where the extended HO-linker senses Ca^{2+}-induced changes in calmodulin. These changes are transduced via the HO-helix and the central β-sheet to residues that mediate coupling between the actin and nucleotide binding sites.

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Remote Control of Diverse Cytoskeletal Motors using Light-Activated Gearshifting

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Cytoskeletal motors perform critical force generation and transport functions in eukaryotic cells. Protein engineering has been used to modify cytoskeletal motors for dynamic control of activity and directionality, providing direct tests of structure-function relationships and potential tools for controlling cellular processes or for harnessing molecular transport in artificial systems. We have previously[1] created myosin motors that can be signaled to switch directions in response to changes in [Ca^{2+}]. Light is a more versatile control signal[2] because it can be precisely modulated in space and time, and is generally orthogonal to cellular signaling. Here we report the design and characterization of a panel of cytoskeletal motors that reversibly change gears - speed up, slow down, or switch directions - when exposed to blue light. Our structural designs incorporate a photoactive protein domain to enable light-dependent conformational changes in an engineered lever arm. We have used *in vitro* motility assays to confirm robust spatiotemporal control over motor function and to characterize the kinetics of optical gearshifting. Our modular approach has yielded controllable motors for both actin-based and microtubule-based transport. Genetically encoded light-responsive motors will expand the optogenetics toolkit, complementing precise perturbations of ion channels and intracellular signaling with spatiotemporal control of cytoskeletal transport and contractility.

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Myosin Lever Arm Directs the Collective Movement Patterns of Motor Proteins

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The emergence of movement patterns, as in flocking of birds and schooling of fish, is a widespread phenomenon in nature. This collective movement can also be seen at the nanoscale in how the molecular motor myosin teams up to drive muscle contraction, membrane traffic, and cell division in biological cells. To systematically dissect the role of intra- and inter-motor interactions on collective function, we engineered a model system consisting of a branched polarized actin network and a biomimetic DNA origami scaffold patterned with defined number of myosin V and VI. Here, we report on the emergent movement patterns of scaffolds patterned with myosin V and VI. Quantitative analysis of the scaffold movement patterns shows that most myosin VI trajectories are linear while a substantial fraction of myosin V trajectories are highly skewed. We find the flexibile lever arm of myosin VI gives rise to the observed linear movement patterns, while the skewed trajectories of scaffolds with myosin V motors is driven by their rigid lever arm. By pairing simulations and experiments with chimeras, we find that the interplay between the torsional strain on the motor lever arm and inter-motor tension dictates collective motion in groups of