inhibitory domain. The actin-activated ATPase activity of full-length DmM5 is significantly stimulated by micromolar level calcium. In contrast, the actin-activated ATPase activity of the truncated DmM5 without GTD is partially inhibited by calcium and this inhibition could not be rescued by exogenous calmodulin (CaM). Among the two cargo-binding proteins of DmM5 in Drosophila compound eye, GFP-bound dRab11 significantly activates the actin-activated ATPase activity of DmM5, whereas LTD has little effect on it. A single residue mutation in the GTD, Q1689A, disrupts the interaction between the GTD and dRab11 and abolishes the dRab11-dependent activation of the actin-activated ATPase activity of DmM5, indicating that dRab11 abolishes the inhibition of DmM5 by the GTD. Based on these results, we propose that DmM5-dependent transport of pigment granule is directly activated by light-induced calcium influx and DmM5-dependent transport of rhodopsin-bearing vesicle is activated by active GTP-bound dRab11, whose formation is stimulated by light-induced calcium influx.

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14-3-3 Proteins Tune Non-Muscle Myosin-II Assembly, Providing a Possible Bridge between Cell Mechanics and Cancer Metastasis

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The 14-3-3 family comprises a group of small acidic regulatory proteins which are evolutionarily conserved and highly conserved across eukaryotes. Overexpression of the 14-3-3s sigma, epsilon, zeta, and eta correlate with a higher metastatic potential and poorer clinical outcomes in different cancers. We have uncovered a role for 14-3-3s in regulating the assembly of non-muscle myosin-II. This demonstrates that 14-3-3s could tune cell mechanics directly and therefore contribute to the progression of metastatic cancers. Here, we examine how myosin-II assembly is regulated by 14-3-3 in Dictyostelium (one 14-3-3, one non-muscle myosin-II) and humans (seven 14-3-3s, three non-muscle myosin-IIIs). In Dictyostelium, 14-3-3 mediates a pathway between microtubules and the rae small GTPase to regulate myosin-II assembly. Here, 14-3-3’s expression levels negatively correlate with BTF accumulation. In vitro assembly assays using purified myosin-II tail fragments and 14-3-3 demonstrate that this interaction is direct, phosphorylation-independent, and high affinity (Kd ~300 nM). We also found that the seven human paralogs of 14-3-3 affect the assembly of human non-muscle myosin-II filaments in different ways, some causing overassembly and others inhibiting assembly. These two 14-3-3 classes directly compete to govern the overall level of myosin-II assembly. Examining assembled myosin-II filaments by electron microscopy confirmed that the average filament size correlates with the overall assembly level. Furthermore, we mapped three critical residues which differ between the two 14-3-3 classes and discovered that alterations of any of these residues convert an assembler to a disassembler. Our findings demonstrate a novel phosphorylation-independent method for regulating myosin-II assembly that is mechanistically conserved from amoeba to humans. These findings imply that altered 14-3-3 expression profiles could directly modulate cell mechanics in metastatic cancers, which would be of great interest for basic and clinical sciences alike.

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Filopod Extension Requires MyTH4-Ferm Myosin Motor Activity

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Filopodia are cellular protrusions composed of bundled parallel actin filaments that initiate from the cortex and have roles in cell adhesion and signaling during directed cell migration. MyTH4-Ferm myosins (including Myosin 7, 10 and 19) are known to localize to the distal tips of filopodia, stereocilia, and microvilli. Myo10 is required for filopod growth in mammalian cells while Myo7 is essential for filopod growth in Dictyostelium. We performed live cell imaging using GFP-tagged Myo7 in null cells. Myo7 rescued filopod formation and localized to filopodia tips and actin-based pseudopods. In pseudopods, enrichment at the leading edge near the cell membrane preceded the growth of filopodia. Tip extension velocity remained constant throughout the growing phase independent of Myo7 accumulation at the tip. A construct lacking MyTH4-Ferm domain but including the putative SAH domain (Myo7 Motor) failed to rescue filopodia and did not localize with cortical actin. Neither of the two FERM domains was essential for rescue of filopod formation. Deletion of the C-terminal FERM led to increased production of substrate-adhered filopodia suggesting this domain might negatively regulate Myo7 activity in vivo. We tested this by mutating conserved residues implicated in auto-inhibition of Myo10 and expressing a mutated Myo7 (KK233/2336AA) that also showed increased production of filopodia relative to wild type Myo7. Tip extension velocity in these mutants was similar to wild type despite the increased number of filopod tips. A mutant lacking the motor domain (Myo7 Tail) was enriched near the cell membrane but failed to rescue filopod formation. Neither FERM domain was essential for localization of the tail. These results suggest that normal formation of filopodia requires membrane-localized Myo7 with precisely regulated motor activity. (This work is supported by an NSF grant to MAT. KJP is supported by NIH grant T32 AR070612.)

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Myosin 19 is Anchored to the Mitochondria, Affecting its Localization and Morphology

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Mitochondria undergo continuous cycles of fusion and fission creating a highly dynamic network, which is essential for its proper functions in apoptosis, ATP generation, and calcium homeostasis. Mitochondria long-range motility relies on the microtubule motors kinesin and dynein. Recently, actin and myosin 19 have been implicated in mitochondrial motility in vertebrates. However, the interaction of endogenous myosin 19 with the mitochondria remains unknown. Here, we show using multiple complementary approaches that endogenous myosin 19 is anchored directly to the outer mitochondrial membrane (OMM) in a monotypic fashion. We have identified a region of 30 residues at the tail domain of myosin 19, which is both essential and sufficient for myosin 19-OMM interaction. Furthermore, we have purified to near homogeneity a 45 long peptide comprised of this region to study its biochemical and biophysical properties. We performed in-vitro binding assay by fluorescence anisotropy of this specific purified peptide to vesicles with different phospholipid compositions. Our results revealed that this peptide binds to vesicles mimicking the OMM with the highest affinity. To relate this tight binding to the mitochondria to myosin 19 ATPase activity, we have purified myosin 19-3IQ construct and measured its actin-dependent steady state ATPase activity. Interestingly, we found that it is completely inhibited by very low calcium concentration, suggesting that myosin 19 activity may be regulated by local calcium concentration. The interaction between a motor protein and an organelle, and the calcium dependence implicates that myosin 19 plays a role in mitochondria network dynamics.

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In vivo and in vitro Studies of Myosin-XII Dynamics

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Myosin-XII is one of only two myosin isoforms found in the genome of the Leishmania parasite. It is involved in the assembly and elongation of the flagellum and in intracellular trafficking. Using mammalian cell transfections, FRAP (fluorescence recovery after photobleaching) and single particle tracking, we elucidated myosin-XII dynamics in vitro and in vivo. Transfections with myosin-XII resulted in an accumulation of myosin at the plasma membrane and inside the tips of filopodia. A minimal construct encoding the N-terminal SH3 domain, motor domain, and parts of the tail up to aa 808 was required for this localization pattern. Notably, the myosin-XII tail contains several lipid binding sites. Live imaging revealed that filopodial myosin accumulations endured, fourth changes of filopodia, pointing toward the possibility of myosin-XII being stably bound to the plasma membrane. Additionally, we observed an increase in the length and density of filopodia in transfected cells, indicating that the myosin might tether the actin cytoskeleton to the plasma membrane thus stabilizing the architecture of membrane protrusions. To investigate the dynamic behavior of this myosin when bound to membranes, we anchored myosin-XII to planar supported lipid bilayers via a biotin-streptavidin link and investigated its diffusion by FRAP and single particle imaging. Furthermore, we studied the myosin’s capability of moving filamentous actin when bound to lipids. In filament gliding assays on lipid vesicles, vesicle size appeared to influence filament motility. Accordingly, we tested myosin-XII for curvature sensitivity employing SLiC (single liposome curvature) assays.