Myosin-1C associates with microtubules and stabilizes the mitotic spindle during cell division

Agrani Rump1,*, Tim Scholz2,*, Claudia Thiel1, Falk K. Hartmann1, Petra Uta2, Maike H. Hinrichs2, Manuel H. Taft1 and Georgios Tsiavaliaris1,‡

1Laboratory for Cellular Biophysics, Institute for Biophysical Chemistry, Hannover Medical School, 30625 Hannover, Germany
2Institute for Molecular and Cell Physiology, Hannover Medical School, 30625 Hannover, Germany
*These authors contributed equally to this work
‡Author for correspondence (Tsiavaliaris.Georgios@mh-hannover.de)

Summary

The mitotic spindle in eukaryotic cells is composed of a bipolar array of microtubules (MTs) and associated proteins that are required during mitosis for the correct partitioning of the two sets of chromosomes to the daughter cells. In addition to the well-established functions of MT-associated proteins (MAPs) and MT-based motors in cell division, there is increasing evidence that the F-actin-based myosin motors are important mediators of F-actin–MT interactions during mitosis. Here, we report the functional characterization of the long-tailed class-1 myosin myosin-1C from Dictyostelium discoideum during mitosis. Our data reveal that myosin-1C binds to MTs and has a role in maintenance of spindle stability for accurate chromosome separation. Both myosin-1C motor function and tail-domain-mediated MT–F-actin interactions are required for the cell-cycle-dependent relocalization of the protein from the cell periphery to the spindle. We show that the association of myosin-1C with MTs is mediated through the tail domain. The myosin-1C tail can inhibit kinesin motor activity, increase the stability of MTs, and form crosslinks between MTs and F-actin. These data illustrate that myosin-1C is involved in the regulation of MT function during mitosis in D. discoideum.

Key words: Myosin, Mitosis, Microtubules, Actin

Introduction

With their ability to mediate dynamic interactions between the cytoskeleton and membrane compartments by exerting motor domain driven force along actin filaments, the single-headed, non-filamentous class-1 myosins have key roles in cell motility, organelle transport and cytoskeleton remodeling (Coluccio, 1997; McConnell and Tyska, 2010). Class-1 myosins localize to cortical regions of high actin turnover during cell migration, associate with cell–cell junctions, bind endocytic vesicles, and support the formation and maintenance of actin-rich protrusive structures during endocytosis (Kim and Flavell, 2008). The eukaryotic model system Dictyostelium discoideum has been used extensively to study class-1 myosins in processes of the endocytic pathway and cell movement (Falk et al., 2003; Jung et al., 1996; Neuhaus and Soldati, 1999). Of the seven class-1 myosins produced in D. discoideum, the long-tailed members myosin-1B, myosin-1C and myosin-1D have been implicated in the management of cortical tension during endocytosis (Novak et al., 1995; Morita et al., 1996); they are important for efficient cell motility (Wessels et al., 1991; Jung et al., 1996), and essential for the recycling of plasma membrane components from endosomes back to the cell surface (Neuhaus and Soldati, 2000). Compared with the shorter isoforms with which they share the small basic phospholipid-binding TH1 domain that mediates membrane interactions, long-tailed class-1 myosins are characterized by two additional tail homology domains (TH2 and TH3) (Hokanson and Ostap, 2006). TH2 is a Gly–Pro–Ala-rich domain capable of F-actin binding (Doberstein and Pollard, 1992; Vargas et al., 1997), which, together with TH3, a Src-homology 3 domain, links the motors to the machinery responsible for actin dynamics (Soldati, 2003).

Although class-1 myosins have been widely investigated in vegetative cells during interphase (Falk et al., 2003; Rivero et al., 2008), their functions during mitosis remain still elusive. Here, we characterized D. discoideum myosin-1C, in addition to its endocytotic roles (Peterson et al., 1995; Dieckmann et al., 2010), as a high-affinity MT-binding myosin that associates with the spindle throughout mitosis. We show that the myosin-1C tail domain affects the dynamic instability of MTs by protecting them from depolymerization. Impairment of myosin-1C function causes defects in spindle assembly and positioning, which results in perturbed mitotic progression. Thus, myosin-1C appears to be important for the structural integrity of the entire spindle to facilitate high-fidelity chromosome separation during mitosis.

Results and Discussion

Myosin-1C associates with microtubules of the mitotic spindle

The subcellular distribution of myosin-1C in D. discoideum was analyzed using AX2 cells and myosin-1C-knockout cells, producing recombinant full-length myosin-1C tagged either at the N- or C-terminus with YFP. Western blot analysis of whole cell lysates revealed comparable protein levels of recombinant and endogenous myosin-1C (supplementary material Fig. S1A). Confocal analysis of fixed and living cells showed myosin-1C at the plasma membrane, crown-like cortical structures, and endosomes that were formed during phagocytic and pinocytic uptake (Fig. 1A,B). This localization pattern resembles that of endogenous protein (Peterson et al., 1995), indicating that the presence and position of the YFP tag did not affect the dynamic distribution of the motor. During yeast internalization, myosin-1C gradually accumulated at the
entire phagosomal membrane and disappeared from the proximal phagosomal region after completion of phagocytosis. The enrichment at phagocytic cups suggests a similar role of myosin-1C in exerting force at membrane extensions of endocytic cup pseudopods as reported for myosin-1D (Morita et al., 1996; Dai et al., 1999). During mitosis, however, myosin-1C was completely absent from the cell periphery and localized to the cell center. Live imaging of mitotic cells revealed a well-defined enrichment of the protein at structures indicative of the spindle apparatus. This new and striking myosin-1C localization pattern is depicted in Fig. 1C as a confocal time-lapse sequence from metaphase until the complete detachment of the daughter cells.

To obtain a more detailed picture of the temporal and spatial localization of myosin-1C with the spindle, we analyzed the distribution of the protein in synchronized and fixed cells at different mitotic phases (Fig. 1D). D. discoideum undergoes a closed mitosis in which the spindle is formed inside the nucleus and MTs penetrate the intact nuclear envelope (Moens, 1976). Because the nuclear envelope is maintained throughout the cell cycle, it forms a barrier for nuclear import. This excludes unspecific enrichment of YFP–myosin to the nucleus. During prometaphase, myosin-1C was mainly present at the spindle poles. In metaphase cells, myosin-1C decorated the entire length of the central spindle. From metaphase to telophase, spindle association persisted and extended to astral MTs. During cytokinesis, myosin-1C was mainly present at the spindle poles and astral MTs that reached the cell cortex. The disappearance of myosin-1C from the center of the spindle is concomitant with the decomposition of MTs during spindle breakdown upon cleavage furrow constriction. Closer analysis of mitotic cells showed colocalization of myosin-1C with both α-tubulin and γ-tubulin (Fig. 2A, supplementary material Fig. S1B). In synchronized, G2-phase-arrested cells (Weijer et al., 1984), myosin-1C exclusively localized at the periphery of centrosomes (supplementary material Fig. S1C), but did not colocalize with the centrosomal protein Spc97 (supplementary material Fig. S1D), suggesting that before mitosis, myosin-1C specifically binds to MTs that emanate from the centrosomal corona (Euteneuer et al., 1998). The observed stepwise association of myosin-1C with centrosomal, interzonal, and astral MTs during mitotic progression supports a role of the motor in contributing to MT dynamics of the spindle, a process that needs to be tightly regulated to ensure
proper spindle assembly or positioning, and subsequent spindle breakdown for successful cell division.

**The myosin-1C tail binds MTs and mediates crosslinking of MTs to F-actin**

To test whether myosin-1C has a role in MT dynamics, we studied the protein in more detail in vitro. Because the elements targeting class-1 myosins to distinct cellular locations are implicated to reside within the C-terminal domains (Ruppert et al., 1995), we generated a set of recombinant tail-truncation constructs (Fig. 2C) to test for direct MT binding and dissect the functional properties of the individual TH domains. Quantitative analyses of GST pull-downs and co-sedimentation assays revealed nanomolar affinity binding of the entire tail (TH1-TH2-TH3) to MTs (supplementary material Fig. S2). The specificity of this interaction was further investigated by in vitro TIRF microscopy. A fluorescent myosin-1C tail construct either fused to YFP (Fig. 2B) or (immuno-) labeled with rhodamine or quantum dots (supplementary material Fig. S3B,C), bound in all examined cases along the entire length of MTs (n=232 MTs). In addition to the previously reported F-actin binding property of the myosin-1C tail domain (Jung and Hammer, 1994), which we could confirm by TIRF assays (Fig. 2D, supplementary material Fig. S3E), our experiments revealed further that the tail is capable of binding MTs and F-actin simultaneously (Fig. 2E, supplementary material Fig. S3D); thus crosslinking of both filamentous structures occurs without the need for additional factors. Consistent with this finding, we observed that in mitotic cells, myosin-1C colocalized with astral MTs (Fig. 2A) and F-actin in regions close to the spindle poles and the cell cortex (Fig. 2F). In other cytoplasmic compartments they were clearly separated. This suggests a role of myosin-1C in mediating spindle attachment to cortical actin sites of the leading edge of dividing cells (Hestermann et al., 2002).

To elucidate the functional relevance of the observed MT interactions, we first analyzed the effect of tail binding on MT dynamics by TIRF microscopy. The cold-inducible depolymerization of reconstituted MTs occurred at rate of $k_{\text{depoly}}=0.5$ µm minute$^{-1}$ (Fig. 3A,B). MTs pretreated with saturating concentrations of tail construct TH1-TH2-TH3, however, maintained their initial length (Fig. 3A). By taking advantage of
this stabilizing effect, we performed titration experiments with different tail domain constructs to test for their ability to prevent MT decomposition and determine the apparent equilibrium constants of this process. In the case of the entire tail, depolymerization rates decreased hyperbolically yielding a half maximal effector concentration of $K_i = 2.4 \pm 1$ nM. The TH1-TH2 domain alone stabilized MTs with $K_i = 2.2 \pm 1$ nM, indicating that the TH3 domain is not essential for MT stability (Fig. 3B). The TH1 domain had no effect on MT dynamics, although it bound to MTs in cosedimentation assays (supplementary material Fig. S2A). The TH2 domain prevented MT depolymerization with 100-times lower efficiency ($K_i = 246 \pm 55$ nM) than constructs containing both TH1 and TH2 domains. However, an equimolar mixture of the isolated TH1 and TH2 domains had no influence on MT disassembly (Fig. 3B, filled circle). Taken together, the results reveal that effective stabilization of MTs requires the action of the entire TH1-TH2 polypeptide chain. The data imply that the TH2 domain harbors the MT stabilizing properties, whereas the TH1 domain contributes to increasing MT affinity. Because the TH1-TH2 domains are composed of highly repetitive Pro-Lys-rich motifs, it is possible that charge–charge interactions are responsible for high-affinity binding. This is supported by our observation that the affinity for MTs is ionic strength-dependent ($K_i = 2 \pm 1.4$ nM at 25 mM KCl versus 80±50 nM at 150 mM KCl) (supplementary material Fig. S2B). We further assessed for a possible role of the myosin-1C tail in MT assembly by monitoring the time-dependent turbidity changes with increasing concentrations of tubulin. However, MT nucleation and elongation were not affected (supplementary material Fig. S4).

**Myosin-1C tail affects kinesin motility along MTs**

Because the binding sites of MAPs on the MT lattice can partially overlap with those of kinesin (Seitz et al., 2002), we tested whether myosin-1C binding to MTs interfered with the processive properties of kinesin motors. Indeed, we observed a myosin-1C tail concentration-dependent reduction of the attachment frequency of single kinesin-1 molecules on MTs (Fig. 3C). Although velocity and processive run length were not affected (Fig. 3C, inset), kinesin attachment frequencies decreased hyperbolically, yielding a half-maximal effector concentration of 1.2 nM. This value is comparable with the apparent equilibrium constant ($K_i$) for tail binding to MTs. Kinesin itself did not affect myosin-1C tail binding to MTs (supplementary material Fig. S3F). These results indicate that the motors do not share the same MT interaction sites. Secondary structural analysis of the tail domain by circular dichroism (data not shown) revealed that both TH1 and TH2 are highly unstructured domains with low $\alpha$-helical and $\beta$-strand contents. This predicted intrinsically disordered tail structure might effectively hinder kinesin molecules from efficient MT binding by blocking sterically and/or electrostatically interaction sites on the MT lattice. Myosin-1C could thus have a role in regulating kinesin function in mitotic processes.

**Myosin-1C contributes to spindle stability**

To test whether the tail domain itself targets myosin-1C to the mitotic spindle or whether motor function is an additional prerequisite for the relocalization mechanism, we expressed YFP-tagged constructs lacking either the motor domain or the entire tail in AX2 cells. The motor domain exhibited diffuse cytoplasmic staining (supplementary material Fig. S5B), whereas the tail accumulated in punctuated structures of the cytoplasm, with no obvious spindle association (supplementary material Fig. S5C). A full-length myosin-1C construct lacking the TH3 domain, however, displayed normal spindle association (supplementary material Fig. S5D). The latter observation agrees with the results discussed
earlier that the TH3 domain neither contributes to MT binding nor affects MT stability. In summary, these observations indicate that both a functional motor domain and the TH1-TH2 domains are essential for the targeting mechanism of myosin-1C to MTs of the spindle apparatus.

In agreement with a role for myosin-1C in endocytosis, myosin-1C-deficient cells (myosin-1CKO) exhibited reduced efficiency of fluid and particle uptake (supplementary material Fig. S6C,D), which, in turn, affected their axenic (Fig. 4D) and bacteria-assisted growth (supplementary material Fig. S6B). During mitosis, myosin-1CKO cells displayed no apparent phenotype related to MT organization. Confocal analysis revealed typical MT patterns and normal spindle architecture (supplementary material Fig. S5A).

We therefore followed a dominant-negative approach to investigate a possible mitotic function of myosin-1C. Dominant-negative inhibition has been a successful tool for the characterization of myosins in vivo (Tsiavaliaris et al., 2002; Mulvihill et al., 2006). In particular, this approach can be helpful when morphological changes from gene deletion are not apparent because of the presence of functionally redundant protein isoforms. The coexpression of a full-length myosin-1C construct (YFP–myosin-1C<sup>G395A</sup>) containing a single point mutation in the motor domain, which is known to reduce actin affinity and perturb motor properties (Sasaki et al., 1998) indeed induced similar defects in growth and endocytic behavior, as seen for myosin-1CKO cells (supplementary material Fig. S6). More interestingly, we observed morphological changes
in the MT patterns of these cells during mitosis, indicating that the mutant competes with endogenous myosin-1C function. In comparison with wild-type cells (Fig. 4A, bottom row), cells overexpressing YFP–myosin-1C<sup>G395A</sup> showed irregular spindle patterns with misaligned chromosomes (Fig. 4A, first row). In some cases, cells underwent cytokinesis before chromosomal separation was completed (Fig. 4A, second row), and the formation of interzonal MTs in prometaphase cells was strongly affected (Fig. 4A, third row). As a consequence of impaired spindle function, we observed that YFP–myosin-1C<sup>G395A</sup> cells contained nuclei that were on average twice as large as those in non-transfected cells or in cells producing wild-type myosin (Fig. 4B). In agreement with potential defects in chromosome separation, the nuclear chromatin content of these cells was increased twofold (Fig. 4C). Compared with the wild type, mitosis of dominant-negative cells was prolonged approximately threefold (supplementary material Fig. S7A). In particular, the progression from prometaphase to metaphase, where the assembly of interzonal spindle MTs takes place, was delayed in the mutant cells (supplementary material Movies 1, 2) compared with the wild type (supplementary material Movie 3). When grown in suspension, the fraction of myosin-1C<sup>KO</sup> and myosin-1C<sup>G395A</sup> cells with one nucleus was decreased in favor of two and more nuclei (supplementary material Fig. S7B), indicating an involvement of myosin-1C in the temporal coupling of mitosis to cytokinesis. Multiple nuclei were not observed in surface-attached cells, because traction-mediated cytoplasmic fission and motile activity of the cells support the process of cytokinesis (Neu jahr et al., 1997).

Because Dictyostelium class-1 myosins share overlapping interphase functions (Jung et al., 1996), members of the same subclass might also compensate for depletion or dominant-negative impairment of myosin-1C during mitosis. Two potential candidates are myosin-1B and myosins-1D. Myosin-1D binds and stabilizes MTs as efficiently as myosin-1C does (data not shown). Moreover, the localization pattern of myosin-1D throughout the cell cycle resembles in many aspects that of myosin-1C. Myosin-1D decorates the cortical membrane during interphase (supplementary material Fig. S8E), and is concentrated at the central spindles and nuclear envelope during mitosis (supplementary material Fig. S8B). This association is observed throughout mitosis, indicating additional roles of this motor, for example, in nuclear membrane fission to cytokinesis. Multiple nuclei were not observed in surface-attached cells, because traction-mediated cytoplasmic fission and motile activity of the cells support the process of cytokinesis (Neu jahr et al., 1997).

Materials and Methods

Plasmid construction

The sequence encoding myosin-1C comprising 1182 amino acids (aa) was amplified from <i>D. discoideum</i> genomic DNA by PCR (using the primers myoCfw and myoCrv and cloned in pDXA–3H–eYFP–mcs (Knetoch et al., 2002). Mutation G395A was introduced by PCR-directed mutagenesis using primer myoG395A. Sequences encoding myosin-1C tail domain constructs TH1–TH2–TH3 (aa 770–1182), TH1–TH2 (aa 770–1136), TH1 (aa 770–982) and TH2 (aa 975–1136) were amplified by PCR and cloned in pGEX-6P-2 for expression in <i>E. coli</i> as N-terminal GST fusions and in pDXA–YFP–mcs as N-terminal YFP fusion for the expression in <i>D. discoideum</i> cells, respectively. The myosin-1C tail construct YFP–TH1–TH2–TH3 with N-terminal His<sub>6</sub> tag fused to YFP was obtained from pDXA–YFP–MIC-tail by PCR and ligated into the pProExHta vector using restriction sites EcoRI and BclI. All constructs were confirmed by sequencing.

Cell lines, protein expression and purification

Cultivation of <i>D. discoideum</i> cells was performed as described previously (Dürwangen et al., 2006). Synchronization experiments were performed as described (Nellen and Saur, 1988; Tuxworth et al., 1997). GST fusion constructs were expressed in <i>E. coli</i> Rosetta pLys-S cells (Merck, Darmstadt). Cells were grown at 30°C in YT medium (16 g Tryptone, 10 g yeast extract, 5 g NaCl per liter pH 7.0) and induced with 0.5 mM IPTG at OD<sub>600</sub>=0.6, grown at 21°C for 16 hours, harvested by centrifugation (4°C, 4000 g) and lysed with glass beads in PBS containing 10 mM reduced glutathione, 300 mM NaCl, 1 mM EDTA, 2 mM benzamidine, 5 mM DTT, 4 mM PMSF, 0.5 mg/ml lysozyme, four pellets Complete inhibitor cocktail (Roche, Penzberg, Germany), 1000 U benzonase (Merck, Darmstadt, Germany) and lyzed with Triton X-100 (1% final concentration). After centrifugation at 20,000 g at 4°C (Avanti J-30, Beckmann Coulter) the supernatants were applied to a glutathione-Sepharose column, washed with buffer (50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM EDTA, 2 mM DTT, 2 mM benzamidine). A linear gradient of Buffer A (50 mM Tris-HCl, pH 8.0, 300 mM NaCl) and Buffer B (Buffer A containing 10 mM reduced glutathione) eluted the protein containing fractions, which were dialyzed against Storage Buffer (25 mM Tris-HCl, pH 7.5, 50 mM arginine, 50 mM glutamate, 300 mM NaCl, 2 mM DTT, 3% Sucrose), snap-frozen in liquid nitrogen, and stored at −80°C.

Fluorescence microscopy

Phagocytosis and pinocytosis assays were performed as described previously (Rivero and Manaik, 2006). For live cell imaging, cells were seeded and washed twice with Mes buffer (20 mM Mes, pH 6.8, 0.2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>). For immunofluorescence, cell fixation was performed in 20 mM PIPES (pH 6.8) containing 3% paraformaldehyde. Cells were permeabilized in PBS containing 0.1% Triton X-100 for 5 minutes, washed with PBS, and blocked with Image-iT Signal Enhancer. Cells were incubated with 4 µg/ml anti-α-tubulin antibody in PBS containing 3% BSA for 1 hour and washed with PBS followed by incubation with Alexa-Fluor-633-labeled secondary antibody (1:500 in PBS) for 1 hour. Chromatin was stained with 0.1 µg/ml 4′,6′-diamidino-2-phenylindole (DAPI) for 5 minutes. Coverslips were mounted on glass slides with Slow Fade Gold. Images were recorded with an IX81 inverted microscope (Olympus, Germany). The microscope set-up Scan<sup>®</sup> (Olympus, Munich) was used for the statistical analysis of the chromatin content in wild-type and mutant cell lines. For confocal microscopy Leica TCS-SP2 AOBS and Olympus FV-1000 systems equipped with 63×, 1.4 NA oil objectives were used.
Function of myosin-1C in mitosis

Euteneuer, U., Graf, R., Kube-Granderath, E. and Schiwa, M. (1998). Dictyostelium myosin-1C: molecular characterization and ultrastructural localization. J. Cell Sci. 111, 405-412.

Falk, D. L., Wessels, D., Jenkins, L., Pham, T., Kuhl, S., Titus, M. A. and Soll, D. R. (2003). Shared, unique and redundant functions of three members of the class I myosins (MyoA, MyoB and MyoD) in motility and chemotaxis in Dictyostelium. J. Cell. Sci. 116, 3985-3999.

Fujita-Becker, S., Dürrenglung, Ü., U. Erent, M., Clark, R. J., Geves, M. A. and Manstein, D. J. (2005). Changes in Mg2+ ion concentration and heavy chain phosphorylation regulate the motor activity of a class I myosin. J. Biol. Chem. 280, 6064-6071.

Helehurtz, J., Brouhard, G., Kalaidzidou, V., Diez, V. and Howard, J. (2006). The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. Nature 441, 115-119.

Hestermann, A., Rebberg, M. and Graf, R. (2002). Centrosomal microtubule plus end tracking proteins and their role in Dictyostelium cell dynamics. J. Muscle Res. Cell Motil. 23, 621-630.

Hokanson, D. E. and Ostap, E. M. (2006). Myo1c binds tightly and specifically to phosphatidylinositol-4,5-biphosphate and inositol 1,4,5-trisphosphate. Proc. Natl. Acad. Sci. USA 103, 3118-3123.

Hwang, K. J., Mahmodian, F., Ferretti, J. A., Korn, E. D. and Gruschus, J. M. (2007). Intramolecular interaction in the tail of Acanthamoeba myosin IC between the SH3 domain and a putative pleckstrin homology domain. Proc. Natl. Acad. Sci. USA 104, 784-789.

Hyman, A. A., Salser, S., Drechsl, D. N., Unwin, N. and Mitchison, T. J. (1992). Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPPCP. Mol. Biol. Cell 3, 1155-1167.

Johnson, K. A. and Borisy, G. G. (1975). The equilibrium assembly of microtubules in vitro. Soc. Gen. Physiol. Ser. 10, 65-78.

Jung, G. and Hammer, J. A., 3rd (1994). The actin binding site in the tail domain of Dictyostelium myosin IC (myoc) resides within the glycinine- and proline-rich sequence (tail homology region 2). FEBS Lett. 342, 197-202.

Jung, G. A., Wu, X. and Hammer, J. A., 3rd (1996). Dictyostelium mutants lacking multiple classic myosin I isoforms reveal combinations of shared and distinct functions. J. Cell Biol. 133, 305-323.

Keller, C. A., Sellers, J. R., Gard, D. L., Bui, D., Adelstein, R. S. and Baines, I. C. (1996). Xenopus oocyte myosin heavy chain isoforms have different subcellular localizations and enzymatic activities. J. Cell Biol. 134, 675-687.

Kim, S. V. and Flavel, R. A. (2008). Myosin I: from yeast to human. Cell. Mol. Life Sci. 65, 2128-2137.

Knetes, M. L., Tsaiavaliaris, G., Zimmermann, S., Ruhl, U. and Manstein, D. J. (2002). Expression vectors for studying cytoskeletal proteins in Dictyostelium. J. Muscle Res. Cell Motil. 23, 605-611.

Kurzawa, S. E. and Graves, M. A. (1996). A novel stopped-flow method for measuring the affinity of actin for myosin head fragments using microgram quantities of protein. J. Muscle Res. Cell Motil. 17, 669-676.

McConnell, R. E. and Tyska, M. J. (2010). Leveraging the membrane-cytoskeleton interface with myosin-1. Trends Cell Biol. 20, 418-426.

Moenes, B. P. (1976). S. Trendeholm and myosin. Proc. Natl. Acad. Sci. USA 73, 2027-2032.

Nelson, W. and Saur, U. (1988). Cell-cycle dependent transcription partner in Dictyostelium discoideum. Biochem. Biophys. Res. Commun. 154, 54-59.

Neuhau, E. M. and Soldati, T. (1999). Molecular mechanisms of membrane trafficking. What do we learn from Dictyostelium discoides? Protist 150, 235-243.

Neuhau, E. M. and Soldati, T. (2000). A myosin I is involved in membrane recycling from early endosomes. J. Cell Biol. 150, 1013-1026.

Neuzhur, R., Heizer, C., and Gerisch, G. (1997). Myosin II-independent processes in mitotic cells of Dictyostelium discoideum: redistribution of the nuclei, re-arrangement of the actin system and formation of the cleavage furrow. J. Cell Sci. 110, 123-137.

Novak, K. D., Peterson, M. D., Reedy, M. C. and Titus, M. A. (1995). Dictyostelium myosin I double mutants exhibit conditional defects in pinocytosis. J. Cell Biol. 131, 1205-1212.

Novak, K. D., Peterson, M. D., Reedy, M. C., Ruman, J. I. and Titus, M. A. (1996). Molecular genetic analysis of myoC, a Dictyostelium myosin I. J. Cell Biol. 138, C347-C359.

Ogawa, K., Itoh, T., Fujisawa, M., Shimizu, K., Koketsu, S., Nakajima, K., Endou, M. and Machesky, L. M. (2003). The function of myosin-1C in mitosis. J. Cell Sci. 116, 123-137.

Peterson, M. D., Novak, K. D., Reedy, M. C., Ruman, J. I. and Titus, M. A. (1996). A novel stopped-flow method for measuring the kinetic parameters of actin-myosin interaction. J. Biol. Chem. 271, 2128-2137.

Peterson, M. D., Novak, K. D., Ruman, J. I. and Titus, M. A. (1997). Myosin I contributes to the generation of resting cortical tension. J. Cell Biol. 137, 1241-1249.

Rodriguez, O. C., Schaefer, A. W., Mandato, C. A., Forscher, P., Bement, W. M. and Waterman-Storer, C. M. (1992). Myosin Va maneuvers through actin intersections during phagocytosis. Proc. Natl. Acad. Sci. USA 89, 7777-7781.

Sakai, N., Shimada, T. and Sutoh, K. (1998). Mutation analysis of the switch II loop in Dictyostelium myosin II J. Biol. Chem. 273, 20334-20340.
Seitz, A., Kojima, H., Oiwa, K., Mandelkow, E. M., Song, Y. H. and Mandelkow, E. (2002). Single-molecule investigation of the interference between kinesin, tau and MAP2c. *EMBO J.* 21, 4896-4905.

Soldati, T. (2003). Unconventional myosins, actin dynamics and endocytosis: a menage a trois? *Traffic* 4, 358-366.

Stöffler, H. E. and Bähler, M. (1998). The ATPase activity of Myr3, a rat myosin I, is allosterically inhibited by its own tail domain and by Ca2+ binding to its light chain calmodulin. *J. Biol. Chem.* 273, 14605-14611.

Tsiavaliaris, G., Fujita-Becker, S., Batra, R., Levitsky, D. I., Kull, F. J., Geeves, M.A., and Manstein, D.J. (2002) Mutations in the relay loop region result in dominant-negative inhibition of myosin II function in *Dictyostelium*. *EMBO Rep.* 3, 1099-1105.

Tuxworth, R. I., Cheetham, J. L., Machesky, L. M., Spiegelmann, G. B., Weeks, G. and Insall, R. H. (1997). *Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth. *J. Cell Biol.* 138, 605-614.

Vargas, M. A., Voigt, H., Sansonetti, P. and Guillen, N. (1997). The tail domain of Entamoeba histolytica myosin IB bind F-actin. *Arch. Med. Res.* 28, 137-138.

Weber, K. L., Sokac, A. M., Berg, J. S., Cheney, R. E. and Bement, W. M. (2004). A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* 431, 325-329.

Weijer, C. J., Duschl, G. and David, C. N. (1984). A revision of the *Dictyostelium discoideum* cell cycle. *J. Cell Sci.* 70, 111-131.

Wessels, D., Murray, J., Jung, G., Hammer, J. A., 3rd and Soll, D. R. (1991). Myosin IB null mutants of *Dictyostelium* exhibit abnormalities in motility. *Cell Motil. Cytoskeleton* 20, 301-315.

Woolner, S., O’Brien, L. L., Wiese, C. and Bement, W. M. (2008). Myosin-10 and actin filaments are essential for mitotic spindle function. *J. Cell Biol.* 182, 77-88.

Wu, X., Bowers, B., Rao, K., Wei, Q. and Hammer, J. A., 3rd (1998). Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function in vivo. *J. Cell Biol.* 143, 1899-1918.