10-24-2007

The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation

Hiroshi Tokuo  
*University of Massachusetts Medical School*

Katsuhide Mabuchi

Mitsuo Ikebe  
*University of Massachusetts Medical School*

Follow this and additional works at: [http://escholarship.umassmed.edu/oapubs](http://escholarship.umassmed.edu/oapubs)

Part of the [Life Sciences Commons](http://escholarship.umassmed.edu/oapubs), and the [Medicine and Health Sciences Commons](http://escholarship.umassmed.edu/oapubs)

Repository Citation

Tokuo, Hiroshi; Mabuchi, Katsuhide; and Ikebe, Mitsuo, "The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation" (2007). Open Access Articles. 1277.  
[http://escholarship.umassmed.edu/oapubs/1277](http://escholarship.umassmed.edu/oapubs/1277)

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Open Access Articles by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation

Hiroshi Tokuo,1 Katsuhide Mabuchi,2 and Mitsuo Ikebe1

1Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01655
2Muscle Research Group, Boston Biomedical Research Institute, Watertown, MA 02472

Filopodia are actin-rich fingerlike protrusions found at the leading edge of migrating cells and are believed to play a role in directional sensing. Previous studies have shown that myosin-X (myoX) promotes filopodia formation and that this is mediated through its ability to deliver specific cargoes to the cell periphery (Tokuo, H., and M. Ikebe. 2004. Biochem Biophys. Commun. 319:214–220; Zhang, H., J.S. Berg, Z. Li, Y. Wang, P. Lang, A.D. Sousa, A. Bhaskar, R.E. Cheney, and S. Stromblad. 2004. Nat. Cell Biol. 6:523–531; Bohil, A.B., B.W. Robertson, and R.E. Cheney. 2006. Proc. Natl. Acad. Sci. USA. 103:12411–12416; Zhu, X.J., C.Z. Wang, P.G. Dai, Y. Xie, N.N. Song, Y. Liu, Q.S. Du, L. Mei, Y.Q. Ding, and W.C. Xiong. 2007. Nat. Cell Biol. 9:184–192). In this study, we show that the motor function of myoX and not the cargo function is critical for initiating filopodia formation. Using a dimer-inducing technique, we find that myoX lacking its cargo-binding tail moves laterally at the leading edge of lamellipodia and induces filopodia in living cells. We conclude that the motor function of the two-headed form of myoX is critical for actin reorganization at the leading edge, leading to filopodia formation.

Correspondence to Mitsuo Ikebe: Mitsuo.Ikebe@umassmed.edu

Abbreviations used in this paper: FKBP, FK506-binding protein; FN, fibronectin; myoV, myosin-Va; myoVII, myosin-VIIa; myoX, myosin-X; SAH, stable α helix; shRNA, small hairpin RNA.

The online version of this article contains supplemental material.
**Results and discussion**

**Dimerization with proper neck length is critical for the filopodial tip localization of myoX**

To eliminate the effect of the tail-binding molecules on filopodia formation, we constructed a GFP-tagged tailless myoX (Fig. 1). We produced constructs having the stable two-headed structure because that structure is necessary for continuous movement of the processive myosins (Veigel et al., 2002; Yildiz et al., 2003; Park et al., 2006). We added the coiled-coil domain of myosin-Va (myoV) at the C-terminal end of the SAH domain of myoX (Fig. 1 A, b). GFP signals of this construct showed a distinct localization at the tip of filopodia in COS7 cells (Fig. 1 A, b). Interestingly, the construct lacking the SAH domain (Fig. 1 A, a) failed to show tip localization, although it formed the two-headed structure. On the other hand, GFP-M10MoIQ3SAH and GFP-M10MoIQ3, having no stable coiled-coil domain, failed to localize at the tip (unpublished data). These results suggest that the formation of the two-headed structure and the presence of the SAH domain are required for the movement of myoX toward the tip of filopodia.

**The regulated dimerization system of myoX**

To further elucidate the relationship between the dimer formation of myoX and the initiation of filopodia in the living cells, we used the regulated homodimerization system (described in Materials and methods). In the NIH3T3 cells expressing GFP-M10MoIQ3SAH-FK506-binding protein (FKBP), GFP signals were distributed throughout the cell body (Fig. 1 B, a). After the addition of the homodimerizer AP20187, GFP signals became distinctly concentrated at the tip of filopodia (Fig. 1 B, b). In contrast, GFP-M10MoIQ3SAH, having no FKBP domain, did not show the AP20187-induced tip localization (unpublished data). When we used the heterodimerizer AP21967 as a control, GFP-M10MoIQ3SAH-FKBP did not localize at the tip (unpublished data). These results further support the notion that dimer formation is critical for the movement of myoX toward the tip of filopodia.

**Induction of microspikes/filopodia by dimerization of tailless myoX**

To further investigate the potential role of the monomer to dimer transition of myoX in filopodia initiation, we performed quantification of microspikes/filopodia in COS7 cells (Fig. 1 C). The production of microspikes/filopodia of GFP-M10MoIQ3SAH-M5CC was four- to fivefold greater than that of GFP-M10MoIQ3-M5CC (Fig. 1 C). Furthermore, addition of the dimerizer considerably induced the microspike formation in GFP-M10MoIQ3SAH-FKBP–expressed cells, and the number of microspikes was four- to fivefold greater than without dimerizer (Fig. 1 C). These results suggest that the initiation of microspikes/filopodia takes place with the dimer formation of myoX.

**Motor function and proper length of the lever arm of myoX**

The aforementioned results indicate that three domains (motor, IQ, and SAH) of myoX are essential for the induction of filopodia. It was hypothesized that the motor activity of myoX plays a critical role in the induction of filopodia. To address this idea, we produced the two constructs having motor-dead mutation in switch I (R220A) or switch II (G437A) of GFP-M10MoIQ3SAH-FKBP. These two mutations were found to inhibit both the localization of myoX at the tip of filopodia and the induction of filopodia even after the addition of dimerizer (Fig. 2 A). These results support a critical role for the motor activity of myoX in filopodia formation.

It has been proposed that the SAH domain may function as part of the myoX lever (Knight et al., 2005). To investigate a correlation between the neck length and filopodia formation, we constructed the myoX vectors that have different neck lengths by introducing a different number of IQs from myoV (Fig. 2 B). The introduction of one IQ domain from myoV to the SAH-deleted construct (Fig. 2 B, a) considerably (four- to fivefold) increased the initiation of microspikes, and the expressed molecules were concentrated at the tip of filopodia (unpublished data). There was no difference in the number of microspikes/filopodia between the constructs having one or two additional IQs (Fig. 2 C). The addition of even more IQs (M5456, M53456, and M523456) did not further increase the number of microspikes (unpublished data).

It was reported that myosin-VIIa (myoVII) also has a SAH domain in the predicted coiled-coil region (Knight et al., 2005). To investigate whether the function of the SAH domain of myoX (M10SAH) is caused by the specificity of the M10SAH structure, we swapped M10SAH for M7SAH of the original construct (Fig. 2 B, c). The swapped construct induced the microspike/filopodia after the addition of dimerizer, similar to the original construct (Fig. 2 C). It was calculated that the length of the SAH domain used in this study was ~5.4 nm, and one IQ domain was 3.5 nm (Knight et al., 2005). Based on this calculation, the lengths of three IQs and a SAH (Fig. 1 A, b), four IQs without the SAH (Fig. 2 B, a), and five IQs without the SAH (Fig. 2 B, b) were 15.9 nm, 14.0 nm, and 17.5 nm, respectively. These results suggest that deletion of the SAH hampered the proper movement of myoX because of the lack of sufficient neck length. The results also indicate that it is important for myoX movement to have the certain minimum neck length. Therefore, it is likely that the SAH domain provides enough span and flexibility for myoX heads to search for the proper binding sites on actin filaments.

**Dimerizer-induced formation of the two-headed structure of myoX**

To examine whether AP20187 actually induces dimer formation in the cells, we expressed myc-M10MoIQ3SAH-FKBP along with GFP-M10MoIQ3SAH-FKBP-HA in COS7 cells (Fig. 3 A). The cells cotransfected with the aforementioned two constructs were incubated with or without AP20187. The myoX construct immunoprecipitated with anti-HA antibodies was recognized by both anti-myc and anti-HA antibodies only when the cells were incubated with AP20187 (Fig. 3 B, lane 4). The result indicates that AP20187 induces the dimer formation of M10MoIQ3SAH-FKBP in cells. It should be noted that the dimer formation of the same construct was not detected with AP21967 (unpublished data). To directly visualize the structure of the molecules of the myoX constructs, the isolated myoX...
Figure 1. Dimerization with proper neck length of myoX is critical for filopodia formation. (A) Localization of the tailless myoX constructs franked with the coiled-coil domain of myoV. COS7 cells were transfected with GFP-M10MoIQ3-M5CC (a) or GFP-M10MoIQ3SAH-M5CC (b) and stained with rhodamine-phalloidin (red). Arrow indicates GFP-M10MoIQ3SAH-M5CC at the tip of filopodium. (B) The myoX constructs with the homodimerization domain induced filopodia formation upon addition of the dimerizer. NIH3T3 cells were transfected with GFP-M10MoIQ3SAH-FKBP (green) and replated on FN for 3 h with 100 nM AP20187. Red indicates rhodamine-phalloidin staining. Arrow indicates GFP-M10MoIQ3SAH-FKBP at the tip of filopodium. (C) Quantification of microspikes/filopodia. Microspikes/filopodia per 20 μm of the cell leading edge were counted in COS7 cells untransfected or transfected with the indicated constructs. The frequency of microspikes/filopodia was increased significantly by the addition of an SAH domain to M10MoIQ3 or forced dimerization with the SAH domain \( P < 0.001 \). Error bars represent the mean ± SD. Bars, 10 μm.
molecules were subjected to electron microscopic observation. Approximately 70% of the molecules of M10MoIQ3SAH-FKBP were two headed in the presence of AP20187, whereas all of the molecules were monomeric in the absence of AP20187 (Fig. 3 C). These results clearly show that AP20187 induces the two-headed structure of M10MoIQ3SAH-FKBP.

The tailless myoX induces filopodia upon dimerization in living cells

Using the induced dimerization technique described in Fig. 1 B, we examined whether the formation of the two-headed structure of myoX directly related to the initiation of filopodia in the living cells. The representative images of spreading cells...
plated on the fibronectin (FN)-coated coverslip are shown in Fig. 4 A and Video 1 (available at http://www.jcb.org/cgi/content/full/jcb.200703178/DC1). Before the addition of the dimerizer, GFP-M10MoIQ3SAH-FKBP showed diffuse localization throughout the cytosol, and fewer than three of the fluorescent puncta (the tip of filopodia) per cell appeared from the cell periphery.
(Fig. 4 A, arrowhead). After the addition of the dimerizer, >20 additional fluorescent puncta appeared within 7 min (Fig. 4 A, bottom). Note that the induced new filopodia were produced only from the active ruffling area and quickly retracted to the edge (Video 1).

Fig. 4 B and Video 2 (available at http://www.jcb.org/cgi/content/full/jcb.200703178/DC1) show representative images of the migrating cells on the FN-coated coverslip. The cell shown in Fig. 4 B and Video 2 was observed migrating from the bottom to the top of the frame (Fig. 4 B, arrow). Before the addition of AP20187 (Fig. 4 B, top), substantial accumulation of GFP-M10MoIQ3SAH-FKBP at the leading edge was observed, and the predominant structure of the leading edge was lamellipodia. Approximately 3 min after the addition of AP20187, several short filopodia appeared from the leading edge and elongated in the direction of the migration (Fig. 4 B, bottom). However, the short filopodia retracted quickly to the edge of lamellipodia. The protrusion and retraction of the filopodia continued while the cell was moving forward. It should be noted that newly produced filopodia only protruded from the leading edge but never appeared from the lateral or the rear side of the migrating cells (Video 2). In contrast, AP21967 had no inducible effect on the production of filopodia (unpublished data).

It was also discovered that after the addition of AP20187, GFP-M10MoIQ3SAH-FKBP moved laterally along the leading edge and fused with another tip of filopodia (Fig. 4 C and Video 3, available at http://www.jcb.org/cgi/content/full/jcb.200703178/DC1). It should be emphasized that the lateral movement was not observed before addition of the dimerizer. Lateral movement of the full-length myoX has been reported using another cell line (Sousa et al., 2006). The present results suggest that the tail domain is not necessary for the lateral movement of myoX along the leading edge of lamellipodia and that formation of the two-headed structure is critical not only for intrafilopodial movement but also for lateral movement at the leading edge.

Knockdown of the expression of myoX

To further clarify the function of myoX in filopodia formation, experiments were conducted to eliminate the expression of endogenous myoX, and the effect of the deletion of myoX in actin dynamics was examined. The myoX-specific siRNA markedly reduced the expression of myoX as revealed by both Western blotting (Fig. S1 A) and immunocytochemistry (Fig. S1 B, available at http://www.jcb.org/cgi/content/full/jcb.200703178/DC1). In control double-stranded RNA–treated cells, the endogenous myoX localized at the tips of detectable actin filaments (bundles) that are aligned radially at the leading edge in lamellipodium. The elimination of myoX abolished not only the myoX localization at the tip of lamellipodia but also the radial arrangement of actin bundles at the cell periphery (Fig. S1 B). We also used pSIREN-DNR-DsRed plasmid that coexpresses small hairpin RNA (shRNA) and DsRed simultaneously to monitor the transfected cells. Two target sequences were used as a control: mouse specific (siM10m) and human specific (siM10h; Fig. S1 C). 3 d after the transfection, immunostaining showed that the expression of siM10m but not siM10h shRNAs markedly decreased levels of myoX in mouse NIH3T3 cells (Fig. S1 D). It should be noted that siM10m showed an effect on the actin structure similar to myoX-specific siRNA (Fig. 5 A).

To evaluate the specificity of the myoX knockdown phenotype, we performed rescue experiments. GFP-tagged bovine myoX (GFP-M10MoIQ3SAH-FKBP) that is refractory to siM10m shRNA restored microspike formation in cells expressing siM10m shRNA after the addition of AP20187 (Fig. 5, A and B; rescue). On the other hand, GFP-M10F also restored the formation of protrusion from lamellipodium. However, actin bundles were elongated to the long filopodia (unpublished data), which is consistent with a previous study showing that the full-length myoX induces long, stable filopodia (Berg and Cheney, 2002). These results indicate that the siRNA effect is specific to the loss of myoX but not as a result of off-target silencing. These results support the idea that myoX is important for promoting filopodia initiation in lamellipodia.

Based on these results, we propose the following model (Fig. 5 C). (1) MyoX is present as a dimer and monomer in the cells. The monomeric (single headed) myoX does not localize at the edge of lamellipodia. (2) Once the dimer is produced, myoX moves to the tip of the actin filaments, presumably as a result of its ability to walk on the actin filaments toward the barbed end. (3) The tips move laterally along the leading edge with actin filaments, and the mechanical activity of myoX plays a role in this process. (4) The lateral movement of myoX converges on the barbed end of the actin filaments, thus producing the base of filopodia where the actin polymerization system might gather to induce parallel actin bundles.

According to the convergent elongation model of filopodia formation, the initiation step of filopodia consists of actin filament convergence and the barbed-end interaction in lamellipodia (Svitkina et al., 2003). We think that the lateral movement of dimerized myoX powers these movements and the structural changes of actin cytoskeleton in the lamellipodia. The function of the SAH domain can be related to the step size of myoX or the flexibility of the neck domain to search for an appropriate binding site on an actin protofilament. It has been reported that the shortening of the neck length of myoV markedly diminishes the run length (Purcell et al., 2002). Because the neck length (the number of IQ motifs) of myoX is one half of myoV, it is thought that the SAH domain functions to help myoX to find the proper actin monomer in the filament, thus facilitating the continuous movement.

Although the tailless myoX can initiate filopodia formation, the filopodia produced are short and unstable. These results suggest that the tail portion of myoX is important for the elongation and stabilization of filopodia and that these processes are likely to be controlled by the cargo molecules binding to the tail of myoX. Recently, it was reported that the unconventional myosin myosin-VI is dimerized after binding to Disabled-2 or the lipids phosphatidylinositol 4,5-disphosphate (Spudich et al., 2006). The pleckstrin homology domain of myoX also binds to the lipid phosphatidylinositol 3,4,5-trisphosphate in vitro (Tacon et al., 2004). Thus, it is possible that the dimer formation of myoX is induced by lipid binding. The tail domains of myoX...
have binding partners such as microtubules (Weber et al., 2004), integrins (Zhang et al., 2004), and VASP (vasodilator-stimulated phosphoprotein; Tokuo and Ikebe, 2004). It is plausible that these binding proteins also control the dimer formation of myoX. Understanding the spatio-temporal regulation of the monomer-dimer transition of myoX in cells is a critical problem requiring further study.

**Materials and methods**

**Plasmid construction**

The construction of expression vector GFP-M10F was described previously (Tokuo and Ikebe, 2004). The cDNA encoding the first 811 amino acids, including the motor domain and the three IQ motifs, was amplified by PCR and subcloned in frame to pEGFP-C1 (pEGLFP-M10MoIQ3). Inclusion of the SAH domain (the first 861 amino acids) was also constructed using the same method (pEGFP-M10MoIQ3SAH). The sequence of the coiled-coil...
Figure 5. MyoX is essential for filopodia induction from lamellipodia. [A] Rescue of the knockdown phenotype by myoX. Fewer filopodia are seen in cells transfected with the knockdown construct (siM10m) compared with the control (siM10h). A cell expressing both siM10m shRNA and GFP-M10MoIQ3-SAH-FKBP (rescue) displays numerous microspikes after the addition of 100 nM AP20187. Asterisks show microspikes/filopodia. (B) Quantification of microspikes/filopodia. Microspikes/filopodia in NIH3T3 cells transfected with the indicated constructs were counted as described in Fig. 1. The frequency of microspikes/filopodia was decreased significantly by transfection with knockdown construct (P < 0.001). Error bars represent the mean ± SD. (C) Model of filopodia initiation upon the formation of the two-headed myoX. (1) MyoX is present as both a dimer and a monomer in the cells. The monomeric (single headed) myoX is present throughout the cytosol. (2) Once myoX forms the two-headed structure, myoX moves to the tips of the actin filaments, presumably...
region of mouse myoV (M5CC; 907–1,090 amino acids) was amplified by PCR and fused to the aforementioned expression vectors pEGFP-M10MoIQ3-M5CC and pEGFP-M10MoIQ3SAH-M5CC. Using the same methods, IgQ to the coiled-coil region (885–1,090 amino acids) or IgQ to the coiled-coil region (636–1,090 amino acids) was fused to pEGFP-M10MoIQ3 to create pEGFP-M10MoIQ3-M5(6)-M5CC and pEGFP-M10MoIQ3-M5(6)-M5CC, respectively.

Regulated dimerization system

Based on known FKBP and its small molecular ligands (Mallet et al., 2002), an FKBP was fused to the C-terminal end of the SAH domain of myoX (GFP-M10MoIQ3SAH-FKBP). The membrane-permeable drug created by chemical cross-linking of the two monomeric ligands with short linker (AP20187) can specifically bind to FKBP. If two FKBP's are present, AP20187 binds to both FKBP's, thus creating a dimer of the targeting molecule. As a control, we used AP21967, a chemically modified derivative of rapamycin that can interact with the dimerization of FKBP and FRB-containing fusion proteins. To create pEGFP-M10MoIQ3-FKBP and pEGFP-M10MoIQ3SAH-FKBP, a fragment encoding FKBP was isolated from pC4-Fv1E (provided by ARIAD Pharmaceuticals) by restriction digestion and subcloned into pEGFP-M10MoIQ3 and pEGFP-M10MoIQ3SAH. pEGFP-M10MoIQ3SAH-FKBP was created by swapping M10SAH for the rat myoVII SAH domain (M7SAH; 869–926 amino acids). To determine the dimer formation by AP21807, COS7 cells were cotransfected with myc-M10MoIQ3SAH-FKBP and GFP-M10MoIQ3SAH-FKBPHA. 16 h after transfection, 100 nM AP21807 was added to the culture medium and incubated for 30 min. The cell lysates were subjected to immunoprecipitation with anti-HA antibody. The immunoprecipitated samples were subjected to SDS-PAGE followed by Western blotting with anti-HA and anti-myc antibodies.

Motor-dead construct

The sequence of the switch I loop (NNINSSRFGRG; residues 215–220) of myoX with the exception of the second N is conserved in all nearby myosins sequenced so far (Shimada et al., 1997). The R to A mutation in the switch I loop results in loss of the ATP binding ability of skeletal myosin-II (Li et al., 1998). Interaction is critical for ATP hydrolysis by myosin (Li et al., 1998). Although the G to A mutation in switch II does not abolish ATP binding to the active site, it causes the loss of ATP hydrolysis (Kambara et al., 1999) and actin sliding activity (Sasaki et al., 1998). According to the aforementioned results, we made two types of motor-dead constructs having a mutation in switch I (R220A) or in switch II (G437A) by site-directed mutagenesis (Kunkel, 1985, 1987) of EGFP-M10MoIQ3SAH-FKBP (Fig. 2 A).

Cell culture and transfection

Affrican green monkey kidney COS7 cells and NIH3T3 fibroblasts (American Type Culture Collection) were cultured in DME supplemented with 10% FCS. Transient transfections were performed with FuGene-6 (Roche Biochemicals) or LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s instructions. At 16 h after transfection, cells were trypsinized and replated on FN for 3 h, fixed, and stained with rhodamine-phalloidin. Cells were lysed in lysis buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM MgCl2, 0.2 mM EGTA, 1 mM ATP, 0.5% NP-40, 1 mM PMSF, 10 μg/ml leupeptin, 2 μg/ml pepstatin A, and 1 μg/ml trypsin inhibitor). The samples were centrifuged at 10,000 g for 20 min at 4°C, and the supernatants were incubated with antibodies conjugated to anti-FKBP and FRB. The precipitates were washed five times in ice-cold lysis buffer. The precipitates were dissolved in 5% SDS and 0.5 M NaHCO3 buffer. These samples were then subjected to Western blotting as described previously (Tokou and Ikebe, 2004). Recombinant protein and electron microscopy

M10MoIQ3SAH-FKBP-GFP was subcloned into pFastBac-HT baculovirus transfer vector (Invitrogen) containing a hexa-His-tag. The recombinant M10MoIQ3SAH-FKBP-GFP protein was purified with calmodulin as reported previously (Ihara and Ikebe, 2005) with an additional incubation with or without 100 nM AP21807 (ARIAD Pharmaceuticals) for 30 min at RT before the cell lysis step. Rotary metal-shadowing electron microscopy of M10MoIQ3SAH-FKBP-GFP was performed as described previously (Li et al., 2006). In brief, myoX proteins diluted to ~4 nM were absorbed onto a freshly cleaved mica surface for 30 s. Unbound proteins were rinsed away, and the specimen was stabilized by brief exposure to uranyl acetate as described previously (Mabuchi, 1990). The specimen was visualized by the rotary shadowing technique according to a previously described method (Mabuchi, 1991) with an electron microscope (model 300 Super; Philips) at 60 kV.

siRNA experiments

The mouse myoX Stealth siRNA (target sequence 5'-GGAUAGUUGGCGGUCAUUGAUUGUCUGUA-3' corresponding to nt 4,011–4,035 relative to the start codon) was generated by Invitrogen. Control siRNA was purchased from Ambion or Dharmacon. pSIREN-DNR-DsRed-siM10h and -siM10m were constructed according to the manufacturer’s instructions (BD Biosciences). The selected target sequences for siM10h was nt 135–153 of human myoX (GenBank/EMBL/DDB) accession no. AF_234532) for siM10m was nt 135–153 of mouse myoX (GenBank/EMBL/DDB) accession no. NM_019472). siM10h and siM10m had three base mismatches; thus, siM10h served as a negative control of siM10m. The rescue construct (GFP-M10MoIQ3SAH-FKBP) made from bovine myoX had four base mismatches to siM10m and were refractory to siM10m siRNA. The siRNA and plasmid transfection were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The cells were analyzed 3 d after transfection.

Online supplemental material

Fig. S1 shows the depletion of myoX in NIH3T3 cells. Video 1 shows the movement of tailless myoX (GFP-M10MoIQ3SAH-FKBP) in a spreading COS7 cell. Video 2 shows the movement of tailless myoX in a migrating COS7 cell. Video 3 shows lateral movement of the tailless myoX along the leading edge of a migrating cell upon addition of the dimerizer. Online as a result of its ability to walk on the actin filaments toward the barbed end. (3) The tips of the actin filaments move laterally along the leading edge, with the motor function of myoX playing a role in this process. (4) The lateral drift induces a convergence of the barbed ends of the actin filaments, thus inducing the formation of microspikes. Bars, 5 μm.
supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200703178/DC1.

We would like to thank Ariad Pharmaceuticals for providing the plasmid pC4-Fv1E encoding Phe36Val mutant FKBP, AP20187, and AP21967. We also thank Dr. R.A. Frey, University of Massachusetts Medical School, Worcester, MA, for reading the manuscript!

This work was supported by National Institutes of Health grants AR048526, AR048898, and DC006103.

Submitted: 27 March 2007
Accepted: 22 September 2007

References


