Localization of Myosin 1b to Actin Protrusions Requires Phosphoinositide Binding

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Myosin 1b (Myo1b), a class I myosin, is a widely expressed, single-headed, actin-associated molecular motor. Transient kinetic and single-molecule studies indicate that it is kinetically slow and responds to tension. Localization and subcellular fractionation studies indicate that Myo1b associates with the plasma membrane and certain subcellular organelles such as endosomes and lysosomes. Whether Myo1b directly associates with membranes is unknown. We demonstrate here that full-length rat Myo1b binds specifically and with high affinity to phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3), two phosphoinositides that play important roles in cell signaling. Binding is not Ca2+-dependent and does not involve the calmodulin-binding IQ region in the neck domain of Myo1b. Furthermore, the binding site is contained entirely within the C-terminal tail region, which contains a putative pleckstrin homology domain. Single mutations in the putative pleckstrin homology domain abolish binding of the tail domain of Myo1b to PIP2 and PIP3 in vitro. These same mutations alter the distribution of Myc-tagged Myo1b at membrane protrusions in HeLa cells where PIP3 localizes. In addition, we found that motor activity is required for Myo1b localization in filopodia. These results suggest that binding of Myo1b to phosphoinositides plays an important role in vivo by regulating localization to actin-enriched membrane projections.

Class I myosins are single-headed members of the myosin superfamily that bind actin filaments and produce mechanical force by hydrolyzing ATP. Class I myosins consist of an N-terminal head or motor domain containing the ATP- and actin-binding sites, a neck region containing repeats of a light chain-binding region known as an IQ domain, and a C-terminal tail domain. Class I myosins are widely expressed in protozoans and metazoans. In mammals, there are eight class I myosins, Myo1a–h (1), which play roles in diverse cellular events such as membrane trafficking, formation of membrane protrusions, cell migration, and transcription in the nucleus (2).

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1 The online version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.

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2 The abbreviations used are: Myo1, myosin 1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PH, pleckstrin homology; PLCδ1, phospholipase Cδ1.

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The specificity of Myo1b binding to membranes and whether it resembles that of the other mammalian class I myosins that have been studied to date remain unclear. Thus, in this study, we investigated the interaction of Myo1b with lipids and its specificity. In addition, we examined the roles of Myo1b-lipid binding in determination of Myo1b cellular distribution.

EXPERIMENTAL PROCEDURES

Construction of Recombinant cDNAs—For expression in SF9 insect cells, full-length Myo1b, the Myo1b IQ and tail domains (Myo1b IQ-tail; Asp706–Pro1107), or the tail domain only (tail; Val824–Pro1107) was amplified by PCR to contain a C-terminal FLAG tag using rat Myo1b cDNA as a template (a kind gift of Dr. Martin Bähler, University of Münster). As a control, the IQ and tail domains (Myo1c IQ-tail; Ala690–Arg1028) of mouse Myo1c were amplified by PCR to contain a C-terminal FLAG tag using enhanced GFP-mouse Myo1c (a kind gift of Dr. Thomas Friedman, NIDCD, National Institutes of Health). The PCR products were then cloned into the pFastBac Dual vector (Invitrogen) containing a calmodulin expression cassette (24).

For expression in mammalian cells, Myo1b cDNAs in pFastBac Dual were amplified by PCR and ligated into pMyc (25), followed by verification of DNA by automatic sequencing. Point mutations were introduced by site-directed mutagenesis and verified by DNA sequencing. An espin/pGEM3 construct (untagged) was kindly provided by Dr. James R. Bartles (Northwestern University, Evanston, IL).

Protein Expression and Purification—The constructs were expressed in insect cells according to the manual provided for the Bac-to-Bac baculovirus expression system (Invitrogen). The infected insect cells were pelleted and homogenized in 50 mM Tris-HCl (pH 7.5), 300 mM KCl, 2 mM MgCl2, 1 mM EGTA, 2 mM ATP, and protease inhibitors. After centrifugation at 200,000 × g for 30 min, anti-FLAG M2-agarose (Sigma) was added to the supernatant and incubated for 1 h at 4 °C. The expressed protein was eluted with 100 μg/ml FLAG peptide in 30 mM HEPES (pH 7.5), 100 mM KCl, 2 mM MgCl2, and 1 mM EGTA. Purified protein containing 10% sucrose and 1 mM DTT was stored at −80 °C.

Lipid-Bead-Protein Pulldown Assay—The PIP Beads™ sample pack (Echelon Biosciences Inc., Salt Lake City, UT) consisted of 50% slurries of eight different phosphoinositide-coated beads and control beads containing no phosphoinositide. In each case, 20 μl of the 50% slurry was washed by adding to 1 ml of binding buffer (30 mM HEPES (pH 7.5), 100 mM KCl, 2 mM MgCl2, and 0.25% IGEPAL CA-630) with 0.1 mM free calcium or 1 mM EGTA and then centrifuging at 100 × g for 5 min at 4 °C. Purified Myo1b in 250 μl of binding buffer (final concentration of 40 μg/ml) was incubated with the washed beads on a Labquake (Thermo Scientific, Asheville, NC) with gentle mixing for 90 min at 4 °C. The beads were collected at 100 × g for 5 min at 4 °C, followed by washing three times with 500 μl of binding buffer at 100 × g for 3 min each. The supernatant was removed, and the bound protein was eluted with 20 μl of 2× Laemmli sample buffer, followed by analysis by SDS-PAGE and Coomassie Blue staining.

Liposome Pulldown Assay—Myo1b IQ-tail (4 μm) or Myo1c IQ-tail (3.7 μm) was prespun at 355,000 × g (TL100 centrifuge and TLA100 rotor, Beckman Instruments) for 30 min at 25 °C in polycarbonate tubes (7 × 20 mm; Beckman centrifuge tubes 343775) to eliminate any aggregated protein. Then, Myo1b IQ-tail (final concentration of 25 nM) or Myo1c IQ-tail (final concentration of 50 nM) was incubated with various amounts (0–50 μM total lipid for Myo1b and 0–200 μM total lipid for Myo1c) of PolyPIPPoses (65% phosphatidylcholine, 30% phosphatidylethanolamine, and 5% phosphatidylinositol (PI), PIP2, or phosphatidylinositol 3,4,5-triphosphate (PIP3); Echelon Biosciences Inc.) in 200 μl of 30 mM HEPES (pH 7.5), 100 mM KCl, 2 mM MgCl2, 1 mM EGTA, and 0.1 mg/ml BSA for 20 min at room temperature in the same polycarbonate centrifuge tubes pretreated for 1 h with 300 μl of 100 μM phosphatidylcholine to lessen the possibility of absorption of the liposomes to the tubes. The protein-liposome complexes were centrifuged at 350,000 × g for 30 min at 25 °C. The pellets were resuspended in 12.5 μl of 2× Laemmli sample buffer, followed by analysis by SDS-PAGE and staining with Coomassie Brilliant Blue. The gels were scanned and analyzed using an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE). The data are expressed as a percentage of bound Myo1b IQ-tail or Myo1c IQ-tail as a function of total lipid concentration, and the data were fit to hyperbolae with Prism. KIP2 refers to the concentration of total lipid at which 50% of the protein is bound, whereas KIP2PIP2 and KIP3PIP3 are the concentrations of accessible PIP2 and PIP3, respectively ((total lipid × 0.05)/2), at which 50% of the protein is bound.

Immunofluorescence Microscopy—HeLa or COS-7 cells were transfected using FuGENE 6 (Roche Applied Science) and then replated on poly-L-lysine-coated glass coverslips. The cells were fixed in 4% formaldehyde and 0.05% glutaraldehyde 24 h after transfection and were permeabilized and blocked in 0.5% saponin, 5% goat serum, and 2.5% BSA in PBS for 1 h, followed by overnight incubation with the appropriate primary antibodies in 0.1% saponin, 5% goat serum, and 2.5% BSA in PBS. The primary antibodies used in this study were anti-Myc tag polyclonal antibody (Cell Signaling Technology, Danvers, MA), and anti-purified PIP2 mouse monoclonal antibody (Echelon Biosciences Inc.). The cells were then incubated with secondary antibodies (Alexa Fluor 488- and/or Alexa Fluor 594-conjugated goat anti-mouse or anti-rabbit) and/or rhodamine-phalloidin (Invitrogen). The cells were viewed with a Leica TCS SP5 AOBS 405 UV spectral confocal microscopy system. The localization patterns of the Myo1b mutants did not appear different over a wide range of expression levels; nevertheless, cells expressing similar amounts of protein were compared. Images were processed with Leica application suite advanced fluorescence imaging software and Adobe Photoshop.

RESULTS

Specific Binding of Myo1b to PIP2 and PIP3—To determine whether Myo1b interacts with phosphoinositides directly, we used a pulldown assay involving beads coated with various phosphoinositides and purified expressed full-length Myo1b (Fig. 1B). As shown in Fig. 2, full-length Myo1b bound specifically to PIP2 and PIP3 in the presence and absence of Ca2+4. This Ca2+-independent binding suggests that the calmodulin-binding IQ motifs in the neck domain
are not involved in the interaction between Myo1b and phosphoinositides. To determine the phosphoinositide-binding domain of Myo1b, the tail domain without the IQ motifs was used in pulldown assays (Figs. 1B and 2A). The tail domain also showed specific binding to PIP2 and PIP3; binding was not dependent on Ca2+/H11001. Taken together, these results indicate that Myo1b interacts with specific lipids via its tail domain in a Ca2+-independent manner.

A Putative Pleckstrin Homology (PH) Domain Is the Binding Site of Phosphoinositides—Hokanson and Ostap (29) reported that Myo1c interacts with phosphoinositides through a PH domain and proposed that most mammalian class I myosins have a putative PH domain in the tail. Certain PH domains have a common secondary structure, 1-loop-2, in which there are two conserved basic residues that are important for interacting with phosphoinositides (26, 27). The Myo1b tail domain has a putative PH domain containing a 1-loop-2 structure, and the corresponding two basic residues are conserved.
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FIGURE 3. Myo1b IQ-tail binding to liposomes containing PI, PIP₂, or PIP₃ as a function of lipid concentration. Pulldown assays of liposomes and Myo1b IQ-tail (25 nM) were performed with various concentrations of liposomes composed of 5% PI (○), PIP₂ (□), or PIP₃ (□). Data are representative of three experiments for PIP₂ and two experiments for PI and PIP₃. The concentration at which 50% Myo1b IQ-tail bound PIP₂ (Kₚ₂) was 6 nM, and the concentration at which 50% Myo1b IQ-tail bound PIP₃ (Kₚ₃) was 32 nM. Inset, binding of 50 nM Myo1c IQ-tail to liposomes containing 5% PIP₂, at various concentrations. The solid line is the best fit to a hyperbola yielding Kₕₚ₂ = 31.8 μM and Kₚ₂ = 0.8 μM.

FIGURE 4. Localization of Myo1b, Myo1b mutants, and PIP₂ in HeLa cells. HeLa cells were transfected with full-length Myc-tagged wild-type Myo1b (A–C), full-length Myc-tagged Myo1b K966A (D–F), or full-length Myc-tagged Myo1b R977A (G–I) and stained with anti-Myc antibody (A, D, and G; green) and anti-PIP₂ antibody (B, E, and H; red). C, F, and I are merged images of A and B, D and E, and G and H, respectively. PIP₂ staining was observed at the cell periphery and in filopodia. Wild-type Myo1b, but neither of the mutants, colocalized with PIP₂ at the periphery; however, like the wild type, some staining of filopodia was observed for R977A. Scale bars = 10 μm.

Myo1b or Myo1b K966A mutant (Fig. 1C), followed by staining with anti-Myc antibody. Myc-tagged wild-type Myo1b was found at the cell periphery and in filopodia (Fig. 4A), as reported previously for endogenous and Myc-tagged Myo1b (21) and GFP-tagged Myo1b (30, 33) in other cell types. The Myo1b K966A mutant localized diffusely throughout the cytoplasm and was not concentrated at the periphery or in filopodia (Fig. 4).

To test whether the IQ domain contributes to lipid binding, full-length Myo1b K966A (Fig. 1B) was used in pulldown assays with lipid-coated beads (Fig. 2C). Because the tail mutation eliminates lipid binding via the putative PH domain in the tail, any observed lipid binding could potentially result from the IQ domain, which was initially thought to contribute to lipid binding in the case of Myo1c (8, 28). Full-length Myo1b K966A does not bind PIP₂ or PIP₃ in the presence or absence of Ca²⁺, demonstrating that the putative PH domain is the sole lipid-binding domain and that neither the motor nor the IQ domain is involved in lipid binding.

Affinity of Myo1b IQ-Tail for Phosphoinositide—Binding of Myo1b IQ-tail to liposomes containing 5% PIP₂ or PIP₃ as a function of total lipid concentration is shown in Fig. 3. Although binding of Myo1b IQ-tail to PI was limited (open diamonds), Myo1b IQ-tail bound PIP₂ tightly (open circles) and PIP₃ less tightly (closed circles). An accurate Kₚ₂, the concentration of PIP₂ at which 50% of the protein is bound, could not be determined but was estimated at 6 nM. The Kₚ₃ of Myo1b IQ-tail was determined to be 32 nM. More Myo1b (80%) associated with PolyPIPosomes containing 5% PIP₂ than with PolyPIPosomes containing 5% PIP₃ (50%). As the same Myo1b was used in each case, this difference in behavior cannot be attributed to the use of different batches of protein. Furthermore, the protein was prespun to eliminate any aggregates, and in the absence of PolyPIPosomes, little, if any (2–3%), Myo1b pelleted, indicating that the difference is also not due to the quality of the protein. One possibility is that the off-rate is higher for PIP₃ versus PIP₂ and that, during centrifugation, more Myo1b dissociates from PIP₃ than from PIP₂.

The Kₚ₃ of Myo1c was also determined and was estimated to be 0.8 μM, which is close to previously reported values of 0.23 μM (29) and 0.53 μM (10). These results show that Myo1b has a higher affinity for PIP₃ than for PIP₂ and a much higher affinity (>100-fold) for PIP₂, than does Myo1c.

Localization of Myo1b in Filopodia Requires Phosphoinositide Binding—To examine the effect of a mutation in the putative PH domain that abolishes the interaction of Myo1b with phosphoinositides on the cellular distribution of Myo1b, we transfected HeLa cells with Myc-tagged full-length wild-type Myo1b or Myo1b K966A mutant (Fig. 1C), followed by staining with anti-Myc antibody. Myc-tagged wild-type Myo1b was found at the cell periphery and in filopodia (Fig. 4A), as reported previously for endogenous and Myc-tagged Myo1b (21) and GFP-tagged Myo1b (23, 30) in other cell types. The Myo1b K966A mutant localized diffusely throughout the cytoplasm and was not concentrated at the periphery or in filopodia (Fig. 4).

To determine whether these residues are involved in the binding of Myo1b to specific phosphoinositides, we constructed two Myo1b tail mutants, K966A and R977A (Fig. 1B), and examined the effect of these mutations on phospholipid binding using PIP bead pulldown assays. Fig. 2B shows that a single alanine mutation at Lys⁹⁶⁶ or Arg⁹⁷⁷ completely abolished binding to PIP₂ and PIP₃, suggesting that Lys⁹⁶⁶ and Arg⁹⁷⁷ within the putative PH domain play a critical role in the interaction of Myo1b with PIP₂ and PIP₃.
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![FIGURE 5. Co-transfection of Myo1b or Myo1b mutants with PLCδ1-PH-GFP in HeLa cells.](image)

![FIGURE 6. Motor activity is required for localization of Myo1b in filopodia.](image)

4D). Transfected cells were also stained with anti-PIP2 antibodies (Fig. 4, B and E) in addition to anti-Myc antibody. Anti-PIP2 antibodies strongly stained filopodia and the cell perimeter where wild-type Myo1b (Fig. 4C), but not Myo1b K966A (Fig. 4F), localized. These results suggest that phosphoinositide binding is required for localization of Myo1b in PIP2-enriched regions such as filopodia. Surprisingly, the R977A mutant localized diffusely in the cytoplasm like K966A but also localized to filopodia like the wild type (Fig. 4, G–I).

Myo1b and the Phospholipase Cδ1 (PLCδ1) PH Domain Colocalize at the Cell Periphery and in Filopodia—To monitor the Myo1b-PIP2 interaction in cells, HeLa cells were transfected with PIPδ1-PH-GFP (31), a PIP2-specific binding protein, and Myc-tagged full-length Myo1b constructs (Fig. 5). PLCδ1-PH-GFP was found at the cell periphery and in filopodia (Fig. 5, B and F), and wild-type Myo1b colocalized with PLCδ1-PH-GFP (Fig. 5, C and D). On the other hand, the Myo1b K966A mutant did not colocalize with PLCδ1-PH-GFP (Fig. 5, E–H). The Myo1b R977A mutant did not concentrate at the periphery and hence did not colocalize well with PLCδ1-PH (Fig. 5, I–L).

Motor Activity Is Required for Localization in Filopodia—We also examined whether motor activity is required for localization of Myo1b to filopodia, which contain an actin core. Arg165 and Gly390 of Myo1b are located in the switch I and switch II regions, respectively. These sites are strictly conserved among myosins, and replacement with alanine blocks ATP hydrolysis and isomerization, respectively. These myosins are held in the weak binding state in ATP (32, 33). We made Myc-tagged Myo1b constructs in which Arg165 and Gly390 were mutated to alanine to render the Myo1b motors inactive. These constructs were transfected into HeLa cells and stained with anti-Myc and anti-PIP2 antibodies. Both R165A (Fig. 6A) and G390A (Fig. 6D) were concentrated in the cell periphery but not in filopodia, where PIP2 is enriched (Fig. 6, B and E). These mutants were also cotransfected into HeLa cells with PLCδ1-PH-GFP (Fig. 7). Both the R165A and G390A mutants colocalized with PLCδ1-PH-GFP at the cell periphery. These results indicate that, although these mutants should be freely diffusing and can associate with the cell periphery, motor activity is required for Myo1b to enter and/or remain in filopodia.

Localization of Myo1b Relative to PIP2 in Espin-induced Filopodia of COS-7 Cells—Because filopodia of HeLa cells are very thin, making it difficult to see the localization of Myo1b and PIP2 in detail, COS-7 cells were transfected with the actin-bundling protein espin, which induces actin-membrane protrusions (34). PIP2 localized at the membrane of espin-induced filopodia (Fig. 8C and supplemental Fig. S1, B and D), whereas Myo1b localized within the core (Fig. 8, A and D; and supplemental Fig. S1, A, C, G, and I). Actin filaments stained with phalloidin colocalized with Myo1b (Fig. 8, B and D; and supplemental Fig. S1, E, F, H, and I).

DISCUSSION

Ca2+-independent Myo1b-Lipid Binding Is through the Tail Domain—In this study, we have shown direct binding of Myo1b through the putative PH domain in the tail domain to PIP2 and PIPδ1, two phosphoinositides that play major roles in cell signal-
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FIGURE 7. Motor activity is required for colocalization of Myo1b with PLCδ1-PH-GFP in filopodia. Myo1b R165A (A; red) and Myo1b G390A (E; red) were enriched at the cell periphery in HeLa cells that were cotransfected with PLCδ1-PH-GFP (B and F; green). D and H show fluorescence intensity along the white lines in C and G, respectively. PLCδ1-PH-GFP localized at the cell periphery and in filopodia (B and F), but motor-dead Myo1b was not present in filopodia. C and G are merged images of A and B and of E and F, respectively. Scale bars = 10 μm.

FIGURE 8. Localization of Myo1b, PIP2, and actin in COS-7 cells with filopodia induced by espin. COS-7 cells were cotransfected with Myc-tagged wild-type Myo1b and untagged espin. Transfected cells were stained with anti-Myc antibody (A), rhodamine-phalloidin (B), and anti-PIP2 antibody (C). The localization of Myo1b, F-actin, and PIP2 is shown in green, red, and blue, respectively, in the merged image (D). PIP2 localized outside of Myo1b and actin in filopodia. Scale bar = 5 μm. A model of the localization of Myo1b, actin, and PIP2 in filopodia is shown below. Green, Myo1b; red, actin; blue, PIP2. Myo1b binds to actin through its head domain and to PIP2 through its tail domain.

region of Myo1b (35); however, binding of full-length Myo1b to phosphoinositides is not Ca2+-dependent, and the tail domain without the IQ motifs binds both PIP2 and PIP3 (Fig. 2A). That the IQ domain plays no role in lipid binding in the case of Myo1b was further confirmed using the full-length Myo1b tail K966A mutant, which showed no binding to PIP2 or PIP3 in the presence or absence of Ca2+ (Fig. 2C).

Acanthamoeba MyoC (3) and chicken Myo1a (4) bind phosphatidylserine and PIP2; specificity for other phosphoinositides has not been investigated. Mouse Myo1c also binds to PIP3; however, binding is not specific for this particular phosphoinositide because Myo1c also binds to other phosphoinositides with phosphates at positions 4 and 5 of the inositol headgroup with a similar affinity (29). Mutation of the conserved basic residues (K872A and R903A) in the β1-loop-β2 motif of the putative PH domain of mouse Myo1c decreased its affinity for phosphoinositides by >8-fold, whereas the equivalent mutation in Acanthamoeba MyoC (R779A) resulted in only a 2–4-fold decrease in affinity for phosphatidylserine and PIP2.

Both of the homologous mutations in the β1-loop-β2 motif of Myo1b (K966A and R977A) completely abolished binding to PIP2 and PIP3 in vitro (Fig. 2B); moreover, the K966A mutant localized diffusely in the cytoplasm and lost the specific distribution at the cell periphery and in filopodia found for the wild type (Figs. 4 and 5). Although the R977A mutant also localized diffusely in the cytoplasm, it was found in filopodia (Figs. 4G and 5J). The difference in localization between the two tail mutants, neither of which binds PIP2 or PIP3 in vitro, can be explained by small differences in affinity for lipids between the two mutants with the affinity of R977A for PIP2 and/or PIP3 exceeding that of K966A. In the cell, these slightly different affinities for phosphoinositides could lead to differential localization.

Specificity and Affinity of Myo1b-Phosphoinositide Binding—Although Myo1b binds both PIP2 and PIP3 in vitro, its affinity for PIP2 exceeds that for PIP3 (Fig. 3). In addition, the cellular concentration of PIP3 is much less than that of PIP2 (36). Expression in HeLa cells of PLCδ1-PH-GFP, a specific PIP2-binding protein, demonstrates that PIP2 is concentrated at the cell periphery and in filopodia in which Myo1b colocalizes with PIP2 (Fig. 5), whereas Btk-PH-GFP (37), which binds specifically to PIP3, is found throughout the cytoplasm (supplemental Fig. S2). Together, the evidence from in vitro and in vivo studies indicates that Myo1b is more likely to associate with PIP2 than with PIP3 in HeLa cells. Upon stimulation, PIP2 and PIP3 levels dramatically increase in cells (36). Sharma et al. (38) reported that, in stimulated neutrophils, PIP2 and PIP3 localize...
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to the leading edge of cells. Myo1b localization may also change upon stimulation involving an increase in PIP₂ and PIP₃.

The affinity of Myo1b for PIP₂ is substantially higher than that determined for Myo1c with the same method (Fig. 3) or similar methods (10, 29). The high affinity of Myo1b for PIP₂ might explain why Myo1b is more concentrated at the cell periphery compared with Myo1c (21).

**Physiological Role of Myo1b-Lipid Binding—**PIP₂ plays an important role in actin reorganization in cellular protrusions. Many actin-binding proteins that regulate actin dynamics by capping, severing, branching, and bundling actin interact with and are activated by PIP₂ (39). These actin-binding proteins are thought to function in membrane-cytoskeleton interactions (40). Reduction of cellular PIP₂ by expression of PLCβ thought to function in membrane-cytoskeleton interactions might explain why Myo1b is more concentrated at the leading edge of cells. Myo1b localization may also change upon stimulation involving an increase in PIP₂ and PIP₃.

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The affinity of Myo1b for PIP₂ is substantially higher than that determined for Myo1c with the same method (Fig. 3) or similar methods (10, 29). The high affinity of Myo1b for PIP₂ might explain why Myo1b is more concentrated at the cell periphery compared with Myo1c (21).

**Physiological Role of Myo1b-Lipid Binding—**PIP₂ plays an important role in actin reorganization in cellular protrusions. Many actin-binding proteins that regulate actin dynamics by capping, severing, branching, and bundling actin interact with and are activated by PIP₂ (39). These actin-binding proteins are thought to function in membrane-cytoskeleton interactions (40). Reduction of cellular PIP₂ by expression of PLCβ thought to function in membrane-cytoskeleton interactions might explain why Myo1b is more concentrated at the leading edge of cells. Myo1b localization may also change upon stimulation involving an increase in PIP₂ and PIP₃.