Myosin I
A new PIP_3 effector in chemotaxis and phagocytosis

Chun-Lin Chen 1 and Miho Iijima 2,*
1 Department of Biological Science; National Sun Yat-Sen University; Kaohsiung, Taiwan, R.O.C.; 2 Department of Cell Biology; The Johns Hopkins University School of Medicine; Baltimore, MD USA

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*Correspondence to: Miho Iijima; Email: miijima@jhmi.edu

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Phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)_P_3) is a key signaling molecule in chemotaxis, a directed cell migration toward chemotactants. PtdIns(3,4,5)_P_3 is transiently generated by chemotactic stimulation and activates reorganization of the actin cytoskeleton at the leading edge of migrating cells. In a recent study, we demonstrated that PtdIns(3,4,5)_P_3 directly binds to three members of the actin-based motor protein myosin I (myosin ID, IE and IF) in Dictyostelium discoideum and recruits these proteins to the plasma membrane of the leading edge. The PtdIns(3,4,5)_P_3-regulated membrane recruitment of myosin I induced chemotactic-stimulated actin polymerization and was therefore required for chemotaxis. Similarly, human myosin IF was translocated to the plasma membrane through interactions with PtdIns(3,4,5)_P_3 upon chemotactic stimulation in a neutrophil cell line. Interestingly, we also found that the three PtdIns(3,4,5)_P_3-binding myosin I proteins function in phagocytosis, which involves both PtdIns(3,4,5)_P_3 signaling and actin cytoskeleton remodeling. Our findings provide an evolutionarily conserved mechanism by which class I myosin transmits PtdIns(3,4,5)_P_3 signals to the actin cytoskeleton.

Chemotaxis plays important roles in a variety of biological processes, such as embryonic development, axon guidance, wound healing and immune responses. In addition to normal physiology, chemotactic migration is also linked to many pathological conditions. For instance, chemotaxis of tumor cells leads to cancer metastasis, while unwanted immune cell chemotaxis causes chronic inflammatory diseases.1-3 In chemotaxis, chemotactic receptors such as growth factors, insulin and chemokinics bind to their receptors on the cell surface. The majority of chemotactic receptors are either seven-transmembrane G-protein-coupled receptors or receptor tyrosine kinase receptors.6,7 Among downstream mechanisms of these receptors, the lipid molecule PtdIns(3,4,5)_P_3 plays critical roles in intracellular chemotactic signaling. PtdIns(3,4,5)_P_3 is produced in the plasma membrane by PI3-kinase (PI3K) and is turned over by the PI3-phosphatase [phosphatase and tensin homolog (PTEN)] or PI5-phosphatase (SHIP1).9-18 PI3Ks are activated upon chemotactic stimulation and are transiently associated with the plasma membrane at the leading edge of migrating cells. PI3Ks convert phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)_P_2) to PtdIns(3,4,5)_P_3. Conversely, PTEN and SHIP1 degrade PtdIns(3,4,5)_P_3. Therefore, PI3K and PTEN/SHIP1 provide regulation of PtdIns(3,4,5)_P_3 production, with synthesis occurring at the leading edge and degradation at the rear.

To identify novel components that mediate PtdIns(3,4,5)_P_3 signaling in chemotaxis, we used a proteomic approach involving affinity purification of PtdIns(3,4,5)_P_3-binding proteins from Dictyostelium cytosol and their identification using mass spectrometry.5,7 Our experiments identified five PH domain-containing proteins, including two previously characterized proteins, PhdA and PKB, and three novel proteins that we
named PhdB, PhdG, and PhdI. We have shown that PhdB, PhdG and PhdI bind specifically to PtdIns(3,4,5)₃ through PH domains in vitro and in vivo, and that these proteins are functionally important for chemotaxis. In addition to PH domain-containing proteins, we identified three class I myosin proteins, including myosin ID, IE and IF.

In our recent study, we showed that myosin ID, IE and IF are required for chemotaxis using a gene knockout approach.²⁰ Cells lacking these class I myosin proteins, including myosin ID, IE and IF, are defective in chemotaxant-stimulated actin polymerization. Myosin I is a monomeric, actin-based motor protein with ATPase activity and cytoskeletal interactions, including vesicle transport along actin filaments and regulation of plasma membrane tension.²¹-²³

Myosin I molecules also have a tail homology (TH) domain that contains a putative PH domain phosphatidylinositol-binding motif. Previous studies have shown that the TH domain preferentially binds to acidic phospholipids such as phosphatidylserine and PtdIns(4,5)P₂. These phospholipids are relatively abundant in biological membranes and may not change their levels in response to intracellular signaling. In contrast, PtdIns(3,4,5)P₃ levels are highly regulated and function as signaling mechanisms. Our finding that myosin ID, IE and IF interact with PtdIns(3,4,5)P₃ suggests that these myosin molecules are regulated by PtdIns(3,4,5)P₃.

We demonstrated that myosin ID, IE and IF specifically bind to PtdIns(3,4,5)P₃ in lipid dot-blot and liposome binding assays (Fig. 1).²⁰ For these assays, we expressed myosin I as green fluorescent protein (GFP) fusion proteins in Dictyostelium cells. The total cell lysate was incubated with nitrocellulose membranes or liposomes carrying different lipids, and interactions of myosin I-GFP with phospholipids were detected using anti-GFP antibodies. To determine whether these myosin I proteins directly bind to PtdIns(3,4,5)P₃, we immunopurified myosin IE-GFP from Dictyostelium cells and incubated it with fluorescently labeled PtdIns(3,4,5)P₃. In this in vitro binding assay, beads carrying myosin IE-GFP specifically associated with, PtdIns(3,4,5)P₃. The PtdIns(3,4,5)P₃-myosin I interactions mediated by TH1 domain as mutations in this domain abolished the lipid-protein interaction. Furthermore, we showed that human myosin IF, which has been suggested to bind to PtdIns(3,4,5)P₃ in a previous proteomic study,²⁴ also binds to PtdIns(3,4,5)P₃ in a lipid dot-blot assay, and that mutations in the TH domain of human myosin IF blocked its interaction with PtdIns(3,4,5)P₃. These data indicate that the ability of TH-domain-containing myosin I to bind to PtdIns(3,4,5)P₃ is evolutionarily conserved.

In chemotaxing cells, PtdIns(3,4,5)P₃-binding myosin I is located at the leading edge.²⁰ This localization is mediated by interactions with PtdIns(3,4,5)P₃ since mutations that block PtdIns(3,4,5)P₃ interactions inhibited myosin I's localization at the leading edge and function in chemotaxant-stimulated actin polymerization, resulting in chemotaxis defects. Similarly, human myosin IF fused to yellow fluorescent protein (YFP-mycosin IF) was recruited to the plasma membrane in COS-7 cells upon stimulation with epidermal growth factor, which increases PtdIns(3,4,5)P₃ levels in the plasma membrane. When expressed in the human neutrophil cell line HL-60, YFP-mycosin IF was located at the leading edge of migrating cells within 5 min after stimulation with a chemotaxant, N-formyl-methionyl-leucine-phosphatidylserine. These translocations also depend on...
In addition to chemotaxis, we found that PtdIns(3,4,5)P3-binding myosin I is also important for phagocytosis. Like chemotaxis, PtdIns(3,4,5)P3 is generated at phagocytic cups and likely rearsranges the actin cytoskeleton to engulf bacteria and yeast cells. We showed that PtdIns(3,4,5)P3, as well as the PI3-kinase inhibitors 1,2,4,5-tetra-O-acetyl-sn-glycero-3-phosphorylcholine and 1,2,4,5-tetra-O-acetyl-sn-glycero-3-phosphorylcholine, completely blocked the translocation. We also confirmed the localization of endogenous myosin IF using immunofluorescence with anti-myosin IF antibodies in HL-60 cells (Fig. 2).

In chemotaxis and phagocytosis, PtdIns(3,4,5)P3 serves as a conserved signaling molecule which controls the actin cytoskeleton. Although additional, parallel signaling pathways exist, our recent findings and other previous studies suggest that myosin I plays important roles in these dynamic processes (Fig. 3). Considering that only a subset of myosin I binds to PtdIns(3,4,5)P3, different myosin I molecules are likely regulated by different mechanisms and act on distinct steps in intracellular signaling and actin cytoskeleton reorganization. It is of great interest to decipher how myosin I functions in dynamics of biological membranes and the cytoskeleton.

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References

1. Moser B, Leontaritis P. Lymphocyte traffic control by chemokines. Nat Immunol 2001; 2:13-8. PMID: 11179886; http://dx.doi.org/10.1038/94829

2. Murphy PM. Chemotaxis and the molecular basis of cancer metastases. Nat Engl J Med 2001; 345:833-8. PMID:11536386; http://dx.doi.org/10.1096/NEJM2001051034519

3. Braunersreuther V, Mach F. Leukocyte recruitment in inflamed tissue: transient migration for therapeutic approaches. Curr Mol Med 2001; 1:307-88. PMID:11704237; http://dx.doi.org/10.1076/curr.0106172

4. Mrass P, Weninger W. Immune cell migration as a means to control immune privilege: lessons from the CNS and tumors. Immunol Rev 2006; 213:195-212. PMID:16952057; http://dx.doi.org/10.1111/j.1600-065X.2006.00431.x

5. Kofler D, van Melsen J, Hirnstein L, Cozzi E, Segal JE. Cell motility and cytoskeletal regulation in invasiveness and metasization. J Mammary Gland Biol Neoplasia 2007; 12:145-52. PMID:17557195; http://dx.doi.org/10.1007/s10911-007-9046-4

6. Affara N, Wein CJ. Signaling to cytoskeletal dynamics during chemotaxis. Dev Cell 2005; 9:19-34. PMID:16003159; http://dx.doi.org/10.1016/j.devcel.2005.06.014

7. Rørth P. Whence directionality: guidance mechanisms for chemotaxis at a glance. J Cell Sci 2008; 121:2621-4. PMID:18685153; http://dx.doi.org/10.1242/jcs.048014

8. Bagorda A, Parent CA. Eukaryotic chemotaxis at a glance. J Cell Sci 2008; 121:2621-4. PMID:18685153; http://dx.doi.org/10.1242/jcs.048014

9. Nishio M, Watanabe K, Sasaki J, Taya C, Takasuga S, Subramanian KK, Jia Y, Zhou D, Simon RT, Iijima M, Huang YE, Devreotes P. Temporal and spatial regulation of 3-phosphoinositides by PTEN. Mol Cell 2007; 9:36-44; PMID:17173042; http://dx.doi.org/10.1016/j.cell.2006-10-0579

10. Stephens L, Milne L, Hawkins P. Moving towards a better understanding of chemotaxis. Current biology: CB 2008; 18:R485-94. NEJM 2001; 345:1685-9.

11. Iijima M, Devreotes P. Tumor suppressor PTEN mediates sensing of chemoattractant gradients. Dev Cell 2002; 109:411-23. PMID:12062103; http://dx.doi.org/10.1016/S1532-5807(02)00775-5

12. Parent CA, Blacklock BJ, Froehlich WM, Wu J, Devreotes PN. G protein-signaling events are activated at the leading edge of chemotactic cells. Cell 1990; 60:581-91. PMID:20978249; http://dx.doi.org/10.1016/0092-8674(90)90089-8

13. Parent CA, Blacklock BJ, Froehlich WM, Wu J, Devreotes PN. G protein-signaling events are activated at the leading edge of chemotactic cells. Cell 1990; 60:581-91. PMID:20978249; http://dx.doi.org/10.1016/0092-8674(90)90089-8

14. Iijima M, Huang YE, Devreotes P. Spatio-temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN. J Biol Chem 2003; 278:19587-94. PMID:12920542; http://dx.doi.org/10.1074/jbc.M312098200

15. Iijima M, Huang YE, Devreotes P. Spatial and temporal regulation of 3-phosphoinositides by PTEN. J Biol Chem 2004; 279:16606-13. PMID:15177747; http://dx.doi.org/10.1074/jbc.M312098200

16. Meili R, Ellsworth C, Lee S, Reddy TB, Ma H, Firtel RA. Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for completion of phagocytosis. Proc Natl Acad Sci U S A 2010; 107:11829-34; PMID:20725776; http://dx.doi.org/10.1073/pnas.1009521107

17. Stephens L, Milne L, Hawkins P. Moving towards a better understanding of chemotaxis. Current biology: CB 2008; 18:R485-94. NEJM 2001; 345:1685-9.

18. King JS, Insel RH. Chemosensing: finding the way forward with Dictyostelium. Trends Cell Biol 2009; 19:523-7. PMID:19773787; http://dx.doi.org/10.1016/j.tcb.2009.07.004

19. Zhang P, Wang Y, Seasaki H, Iijima M. Phosphatidylinositol-4,5-bisphosphate-binding protein (PI3P) is recruited to phagocytic cups and likely regulated by different mechanisms and act on distinct steps in intracellular signaling and actin cytoskeleton reorganization. It is of great interest to decipher how myosin I functions in dynamics of biological membranes and the cytoskeleton.

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