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# A shortcut from GPCR signaling to Rac-mediated actin cytoskeleton through an ELMO/DOCK complex

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**Chemotaxis, chemoattractant-guided directional cell migration, plays major roles in human innate immunity and in development of a model organism *Dictyostelium discoideum*. Human leukocytes and *D. disscoideum* share remarkable similarities in the molecular mechanisms that control chemotaxis. These cells use G-Protein-Coupled Receptors (GPCRs), such as chemokine receptors, to control a signaling network that carries out chemotactic gradient sensing and directs cell migration. Diverse chemokines bind to their receptors to activate small G protein Rac through an evolutionarily conserved mechanism. Elmo and Dock180 proteins form ELMO/Dock180 complexes functioning as guanine nucleotide exchange factors (GEFs) for Rac activation. However, the linkage between GPCR to Elmo/Dock180 for Rac activation that controls F-actin dynamics remained unclear. Recently, we discovered a novel function of an ELMO protein in *Dictyostelium discoideum* linking GPCR signaling from Gβ to actin dynamics through regulating Rac activation during chemotaxis.**

**Keywords:** chemotaxis, G-protein-coupled receptor, heterotrimeric G protein, Gβ subunit, ELMO protein, small G protein, Rac, guanine nucleotide exchange factor (GEF), the actin cytoskeleton

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The molecular mechanisms underlying chemotaxis in human leukocytes and in *D. discoideum* are evolutionarily conserved. The latter provides a powerful model system to identify new components and to reveal their functions involved in chemotaxis. All these cells utilize G-Protein-Coupled Receptors (GPCRs) signal transduction machinery to carry out chemotactic gradient sensing and cell migration.[1-3] Binding of a chemoattractant to its receptor induces the dissociation of heterotrimeric G-proteins into Gα and Gβγ subunits.[4-6] Free Gα and Gβγ activate their downstream signaling components, such as Rac, to induce the growth of actin filaments.[7] Consequently, activation of GPCRs promotes actin polymerization through Rho/Rac, which plays critical roles in chemotaxis.[8] However, the linkages between a GPCR/GαGβγ machinery and a Rac-controlled actin polymerization machinery have not been fully understood. Recently, we identified an Elmo/Dock complex, which serves as a bridge between GPCR signaling and Rac-regulated actin cytoskeleton during chemotaxis of *D. discoideum* cells.[9]

Activation of Rac proteins promotes the growth of actin filaments that drive cell migration. Rac proteins, like other small GTPases, cycle between GDP- and GTP-bound states. They activate their effectors when they are in their GTP-bounded (active) state. Two large classes of regulatory proteins control the activation state of small GTPases. The GTPase-activating proteins (GAPs) bind to GTP-bound Rac proteins to enhance their GTPase activity, thereby converting them to the inactive GDP-bound state. Conversely, guanine exchange factors (GEFs) promote nucleotide exchange from GDP to GTP on small GTPase. The GEFs for Rho/Rac GTPases can be subdivided into Dbl and Elmo/DOCK (dictator of cytokinesis) families.[10] Active GTP-bound Rac proteins stimulate Arp2/3 complexes promote actin polymerization for cell migration.

Elmo proteins form an evolutionarily conserved and ancient family, which includes members in *D. discoideum*, *C. elegans* and human.[11,12] The first member, *Ced-12*, was discovered in a genetic screen to identify components required for engulfment of dead cells during the development of *C. elegans*. Ced-12 gene encodes a protein that is essential for EnguLfment and cell MOtility, thus named the protein Elmo.[12] Other members of Elmo family were later found in many organisms.[11,13] Extensive studies in *C. elegans* and in mammalian cells showed that Elmo and Dock180 form a complex that serves as a GEF for Rac proteins.[11,12] Although an Elmo protein does not have GEF activity for Rac, the formation of an Elmo/Dock180 protein complex is critical for Rac activation and for Rac-induced cell migration. It appears that Elmo and Dock180 always form a complex, and the Elmo/Dock180 complex exists in two different states, a "closed" state that has little GEF activity for Rac proteins and an "open" state that enables the complex to function as RacGEF.[14] Activation of cell surface receptors, such as GPCRs, stimulates the GEF activity of Elmo/Dock180 complexes, which, in turn, activate Rac proteins for cell migration. However, how a GPCR/G-protein machinery regulates the Elmo/Dock complex is not understood in any organisms.

*D. discoideum* has been established as a key model system for studying GPCR-mediated chemotaxis of eukaryotic cells.[15] This organism utilizes a GPCR, the cAMP receptor (cAR1), coupled with the G-protein Gα2Gβγ to sense the chemoattractant cAMP and mediate directional cell migration.[16] Over the years, many fundamental questions in eukaryotic chemotaxis have been raised and concepts have been developed in studies of chemotaxis using the model system *D. discoideum*. For example, to explain how a cell can persistently migrate in a chemokine gradient, one earlier hypothesis was that the enrichment of chemokine receptors at the leading front of cells is required. However, cAR1 receptors were found to be uniformly distributed in the membrane of chemotaxing *D. discoideum* cells,[17] and this was subsequently showed to the case for chemokine GPCRs of human neutrophils.[18] Therefore, other signaling mechanisms instead of simple localization of GPCRs must account for Rac-controlled actin polymerization at the leading front in chemotaxing cells.

Signaling mechanisms linking activation of GPCR to re-organization of actin cytoskeleton have been extensively studied, and are yet fully understood. In *D. discoideum*, activation of cAR1 leads to the dissociation of the G-proteins into Gα2 and Gβγ subunits,[5] which control several pathways that regulate the actin-based moving apparatus for chemotaxis. The cAR1/G-protein machinery activates small GTPases, Ras proteins, that regulate four signaling components, PI3K, PLA2, TORC2 and sGC.[2] Although each of the four has been shown to contribute to chemotaxis, it is not clear how each of them regulates Rac activity or actin-moving apparatus. In chemotaxing cells, active Rac proteins stimulate Arp2/3 complex that initiate the branching of actin filaments from the existing ones, which results in the growth of the dendritic actin-network pushing the membrane forward in the leading front of cells.[7,19] It is essential to understand the pathways linking the GPCR signaling to the activation of Rac proteins. Activation of Rac proteins requires guanine nucleotide exchange factors (GEFs), and the Elmo/Dock complex is one of the evolutionarily conserved Rac GEFs. Thus, it is important to establish the linkage between chemoattractant GPCRs to the Elmo/Dock complex, which guides the re-organization of the actin-moving apparatus during chemotaxis.

We identified six genes (*elmoA-F*) that encode proteins containing one Elmo-homology domain in *D. discoideum* using a genomic approach.[13,20] Among these six additional members of the Elmo family, we found that ElmoA protein functions as a negative regulator of actin polymerization during phagocytosis and cell migration.[20] Loss of *elmoA* displays an overall increase in phagocytosis, which is dissimilar to the defects in engulfment of dead cells exhibited by the *C. elegans ced-12* (*elmo*) mutant. In addition, *elmoA*-cells have an increased pseudopod formation and an elevated F-actin localization within pseudopods. The mutant is defective in maintaining cell polarity and in suppressing the formation of lateral pseudopods, which are essential for effective chemotaxis. ElmoA associates with cortical actin and myosin II heavy chain, which are known to enhance cell cortical rigidity, thereby ensuring cell polarity.[20] Interestingly, there are six members of Elmo protein in human. In addition to the known functions of Elmo (1–3) and Dock180s as GEFs for Rac proteins, ElmoD2 has been shown to function as a GAP for the small G-proteins Arl and Arfs.[21] Further studies are required for discovering molecular mechanisms involved in these unconventional Elmo proteins. Recently, we found that Elmo and Dock from the Elmo/Dock complexes and serve as a GEF for Rac proteins in *D. discoideum*.[9] There are eight genes to encode Dock-like proteins in *D. discoideum*.[22] Among them, DdDockA-D appear to belong to the subfamily of Dock180-related proteins and DdzizA-D belong to the subfamily of zizimin-related proteins, which include mammalian Dock9 and Dock10.[22] We found that ElmoE associates with DdDockC and DdzziA to from evolutionarily conserved Elmo/Dock complexes that serve as GEFs for small G-protein RacB in *D. discodieum*.

We discovered several proteins associated with ElmoE using proteomic and biochemical analyses.[9] The ElmoE-associating proteins include Gβγ, Dock-like proteins, RacB and the Arp2/3 complex. The association between Gβγ and ElmoE represents the first direct link between the Elmo/Dock complexes to the GPCR signaling. Interestingly, it appears that activation of cAR1 GPCR promotes the association between Gβγ and ElmoE and this association likely occurs at the N-terminal part of ElmoE. Furthermore, activation of cAR1 requires ElmoE to activate RacB, which leads to actin-polymerization for chemotaxis. Although the molecular mechanisms are not well understood, we propose a novel pathway that transduces signals stepwise from GPCR, Gβγ, ElmoE/Dock, RacB, Arp2/3 to actin polymerization for chemotaxis. It is attempting to speculate that this pathway is conserved in other eukaryotic cells, and this pathway may also play a role in chemokine GPCR-controlled cell migration of mammalian cells.

## References


1. Devreotes PN. G protein-linked signaling pathways control the developmental program of Dictyostelium. Neuron 1994; 12:235-41; PMID:8110455; http://dx.doi.org/10.1016/0896-6273(94)90267-4.
2. Jin T. GPCR-controlled chemotaxis in *Dictyostelium discoideum*. Wiley Interdiscip Rev Syst Biol Med 2011.
3. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. Annu Rev Immunol 1994; 12:593-633; PMID:8011292; http://dx.doi.org/10.1146/annurev.iy.12.040194.003113.
4. Jin T, Xu X, Hereld D. Chemotaxis, chemokine receptors and human disease. Cytokine 2008; 44:1-8; PMID:18722135; http://dx.doi.org/10.1016/j.cyto.2008.06.017.
5. Xu X, Meckel T, Brzostowski JA, Yan J, Meier-Schellersheim M, Jin T. Coupling mechanism of a GPCR and a heterotrimeric G protein during chemoattractant gradient sensing in Dictyostelium. Sci Signal 2010; 3:71; PMID:20876874; http://dx.doi.org/10.1126/scisignal.2000980.
6. Xu X, Meier-Schellersheim M, Jiao X, Nelson LE, Jin T. Quantitative imaging of single live cells reveals spatiotemporal dynamics of multistep signaling events of chemoattractant gradient sensing in Dictyostelium. Mol Biol Cell 2005; 16:676-88; PMID:15563608; http://dx.doi.org/10.1091/mbc.E04-07-0544.
7. Sun CX, Magalhães MA, Glogauer M. Rac1 and Rac2 differentially regulate actin free barbed end formation downstream of the fMLP receptor. J Cell Biol 2007; 179:239-45; PMID:17954607; http://dx.doi.org/10.1083/jcb.200705122.
8. Van Haastert PJ, Devreotes PN. Chemotaxis: signalling the way forward. Nat Rev Mol Cell Biol 2004; 5:626-34; PMID:15366706; http://dx.doi.org/10.1038/nrm1435.
9. Yan J, Mihaylov V, Xu X, Brzostowski JA, Li H, Liu L, et al. A Gβγ effector, ElmoE, transduces GPCR signaling to the actin network during chemotaxis. Dev Cell 2012; 22:92-103; PMID:22264729; http://dx.doi.org/10.1016/j.devcel.2011.11.007.
10. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol 2005; 6:167-80; PMID:15688002; http://dx.doi.org/10.1038/nrm1587.
11. Côté JF, Vuori K. GEF what? Dock180 and related proteins help Rac to polarize cells in new ways. Trends Cell Biol 2007; 17:383-93; PMID:17765544; http://dx.doi.org/10.1016/j.tcb.2007.05.001.
12. Reddien PW, Horvitz HR. The engulfment process of programmed cell death in *Caenorhabditis elegans*. Annu Rev Cell Dev Biol 2004; 20:193-221; PMID:15473839; http://dx.doi.org/10.1146/annurev.cellbio.20.022003.114619.
13. Brzostowski JA, Fey P, Yan J, Isik N, Jin T. The Elmo family forms an ancient group of actin-regulating proteins. Commun Integr Biol 2009; 2:337-40; PMID:19721884; http://dx.doi.org/10.4161/cib.2.4.8549.
14. Patel M, Pelletier A, Côté JF. Opening up on ELMO regulation: New insights into the control of Rac signaling by the DOCK180/ELMO complex. Small Gtpases 2011; 2:268-75; PMID:22292130; http://dx.doi.org/10.4161/sgtp.2.5.17716.
15. Jin T, Xu X, Fang J, Isik N, Yan J, Brzostowski JA, et al. How human leukocytes track down and destroy pathogens: lessons learned from the model organism *Dictyostelium discoideum*. Immunol Res 2009; 43:118-27; PMID:18827980; http://dx.doi.org/10.1007/s12026-008-8056-7.
16. Xu X, Meier-Schellersheim M, Yan J, Jin T. Locally controlled inhibitory mechanisms are involved in eukaryotic GPCR-mediated chemosensing. J Cell Biol 2007; 178:141-53; PMID:17606871; http://dx.doi.org/10.1083/jcb.200611096.
17. Xiao Z, Zhang N, Murphy DB, Devreotes PN. Dynamic distribution of chemoattractant receptors in living cells during chemotaxis and persistent stimulation. J Cell Biol 1997; 139:365-74; PMID:9334341; http://dx.doi.org/10.1083/jcb.139.2.365.
18. Servant G, Weiner OD, Herzmark P, Balla T, Sedat JW, Bourne HR. Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. Science 2000; 287:1037-40; PMID:10669415; http://dx.doi.org/10.1126/science.287.5455.1037.
19. Insall RH, Machesky LM. Actin dynamics at the leading edge: from simple machinery to complex networks. Dev Cell 2009; 17:310-22; PMID:19758556; http://dx.doi.org/10.1016/j.devcel.2009.08.012.
20. Isik N, Brzostowski JA, Jin T. An Elmo-like protein associated with myosin II restricts spurious F-actin events to coordinate phagocytosis and chemotaxis. Dev Cell 2008; 15:590-602; PMID:18854143; http://dx.doi.org/10.1016/j.devcel.2008.08.006.
21. Bowzard JB, Cheng D, Peng J, Kahn RA. ELMOD2 is an Arl2 GTPase-activating protein that also acts on Arfs. J Biol Chem 2007; 282:17568-80; PMID:17452337; http://dx.doi.org/10.1074/jbc.M701347200.
22. Meller N, Westbrook MJ, Shannon JD, Guda C, Schwartz MA. Function of the N-terminus of ziziminl: autoinhibition and membrane targeting. Biochem J 2008; 409:525-33; PMID:17935486; http://dx.doi.org/10.1042/BJ20071263.