Two Lipids That Give Direction

Jean-François Côté¹ and Kristiina Vuori²

¹ Institut de Recherches Cliniques de Montréal, Université de Montréal, Montréal, Québec H2W 1R7, Canada

² Burnham Institute for Medical Research, La Jolla, CA 92037, USA

Abstract

Precise and sequential intracellular signaling events involving two phospholipids direct an immune cell toward an attractant molecule gradient.

Neutrophils are highly motile cells of the human immune system that specialize in clearing pathogens from infected tissue. Achievement of this task is no small feat: A neutrophil must relentlessly track its moving target (such as a bacterium) in a full-speed race, abruptly changing direction as needed before closing in on its prey. All this requires that neutrophils sense very small amounts of chemicals, known as chemoattractants, which are released by the escaping pathogens. Receptors on the surface of neutrophils recognize these attractants and initiate cascades of intracellular signaling events that ultimately polarize cell movement in the direction of the pathogen. On page 384, Nishikimi *et al.* (1) report that two phospholipids initiate this cellular polarization.

The morphological changes that allow a neutrophil to alter its direction of movement requires polarized remodeling of the actin cytoskeleton. At the center of these changes is Rac, a member of the Rho family of small guanine nucleotide (GTP)-binding proteins (GTPases), whose activation induces rapid actin polymerization. This event supports physical extension of the cell's plasma membrane (as a pseudopod) toward the pathogen (2). Previous studies have highlighted an important role for the atypical guanine exchange factor (GEF) DOCK2 in neutrophil polarization and migration (3). DOCK2 belongs to a family of Rho GTPase regulators that lack a canonical GEF signaling motif (Dbl-PH). Instead, these DOCK-related proteins use a DOCK homology region-2 (DHR-2) domain to mediate activation of target Rho GTPases (4–6). In addition, all DOCK proteins harbor a DHR-1 domain that binds to the phospholipid phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (7). PIP₃ is generated at membranes by the phosphorylation of phosphatidylinositol 4,5-bisphosphate, a phospholipid component of membranes (8). Both the DHR-1 and -2 domains are required for properly localizing the activation of Rho GTPases by DOCK proteins (9). Kunisaki *et al.* showed that neutrophils lacking DOCK2 demonstrate impaired Rac activation, and consequently, fail both to polarize and display chemotaxis in response to chemoattractant (3).

How do neutrophils initiate polarization? PIP₃ is rapidly produced by phosphoinositide 3-kinases (PI 3-kinases) in response to activated chemoattractant receptors, and accumulates at the site of the plasma membrane that senses the highest concentration of the extracellular

stimulant (8). By directly binding to the DHR-1 domain, PIP₃ recruits DOCK2 to this site of the plasma membrane to activate Rac (3). Although biochemical experiments with PI 3-kinase inhibitors suggest an important contribution of PIP₃ in cell polarization, in vivo experiments with neutrophils lacking PI3K γ (the major isoform in neutrophils) have demonstrated that these cells can nevertheless establish a polarized “leading edge” (region of the cell that extends a pseudopod) toward the chemoattractant (3). Thus, the signaling events leading to neutrophil polarization in the absence of PI 3-kinase activity have remained elusive.

Nishikimi *et al.* report that global membrane recruitment of DOCK2, through DHR-1–PIP₃ interaction, is not sufficient for neutrophil polarization to occur. Instead, the authors demonstrate that an additional phospholipid, phosphatidic acid, narrows and enriches the localization of DOCK2 more precisely at the membrane site that will become the growing leading edge (see the figure). Phosphatidic acid is generated from the hydrolysis of the membrane component phosphatidylcholine by phospholipase D. By using an inhibitor of phospholipase D, the authors show that a signaling pool of phosphatidic acid is responsible for targeting DOCK2 at the leading edge.

What is the mechanism by which phosphatidic acid refines the localization of DOCK2? Nishikimi *et al.* identified a polybasic region at the carboxyl terminus of DOCK2 that interacts directly with this phospholipid. Mutations in these basic residues abrogated DOCK2–phosphatidic acid binding in vitro and also prevented DOCK2 interaction with the plasma membrane at the leading edge in neutrophils. This suggests that binding to phosphatidic acid is responsible for correctly targeting DOCK2. An elegant swapping experiment of the polybasic region of DOCK2 for a polybasic region of a different signaling protein demonstrated that it is indeed phosphatidic acid binding that localizes DOCK2 to the leading edge. Abrogating DOCK2–phosphatidic acid interaction affected both the polarized accumulation of DOCK2 and the ability of neutrophils to migrate rapidly. Thus, Nishikimi *et al.* uncover a two-step mechanism for initiating polarized neutrophil movement: a more global and less specific step that depends on PIP₃ to recruit DOCK2 to the leading edge of the plasma membrane, and a second step that depends on phosphatidic acid to precisely localize DOCK2 to the exact site in the leading edge that will extend the pseudopod. Nishikimi *et al.* also found that other DOCK proteins interact with phosphatidic acid, and thus may function in response to events that alter amounts of phosphatidic acid. Interestingly, these DOCK proteins are implicated in biological processes that require cellular polarization, including myoblast fusion, phagocytosis of apoptotic cells, and neuronal development.

It is not clear how phosphatidic acid accumulates to sufficient amounts at the site of the plasma membrane that senses the highest amount of the chemoattractant. In the case of PIP₃ production, studies have demonstrated the existence of a positive feedback loop, in which PIP₃ recruits a Rac GEF and the Rac-GTP produced in situ can further enhance PIP₃ production by activating PI 3-kinases. After establishing this feedback loop, the cell generates an intracellular lipid gradient, leading to efficient signaling (10). Perhaps a similar feedback loop exists for phosphatidic acid production. Further studies on the regulatory mechanisms governing phospholipase D signaling, such as putative activation by Rac-GTP,

are required to fully explain the signaling events that control cell motility in neutrophils and in other systems.

References

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Figure. Preparing to move

As a nonpolarized neutrophil senses a gradient of chemoattractant (such as a cytokine), signaling events lead to the localization of DOCK2 at the cell's leading edge in two stages, each dependent upon a different phospholipid—PIP₃ and phosphatidic acid (PA). This refinement of DOCK2 localization ensures rapid neutrophil movement toward the chemoattractant gradient.