17. The lifespan of *hyl-1(lf)* mutants is shown in fig. S3.
18. Y. Mizutani, A. Kihara, Y. Igarashi, *Biochem. J.* **390**, 263 (2005).
19. Y. Mizutani, A. Kihara, Y. Igarashi, *Biochem. J.* **398**, 531 (2006).
20. C. Riebeling, J. C. Allegood, E. Wang, A. H. Merrill Jr., A. H. Futerman, *J. Biol. Chem.* **278**, 43452 (2003).
21. X. Deng *et al.*, *Science* **322**, 110 (2008).
22. Y. A. Hannun, L. M. Obeid, *Nat. Rev. Mol. Cell Biol.* **9**, 139 (2008).
23. B. Zanolari *et al.*, *EMBO J.* **19**, 2824 (2000).
24. We thank F. Solary for help with analysis of aging; Y. Mattenberger and D. Courvoisier from the Clinical Research Center University of Geneva and Geneva University Hospitals for statistical support; S. Herzig for artwork; S. Mitani and the Japanese National Bioresource Project for tm strains; and J. Hodgkin for providing strains CB5670, CB6166, CB5439, and CB5430. Some nematodes were provided by the *Caenorhabditis* Genome Center, which is funded by the NIH National Center for Research Resources. This work was funded by the Swiss National Science Foundation [subsidies 3100A0-109419/2 (J.-C.M.), 3100A0-114189 (H.R.), 3100A0-105907/1 (M.G.), and 3100A0-113889 (M.O.H.)], the Human Frontiers Science Program Organization (H.R.), the Swiss SystemsX.ch initiative (H.R.) evaluated by the Swiss National Science Foundation, the Roche Foundation (V.M.), Ernest Boninchi subsidy for the purchase of the anoxic chamber, the Canton of Zurich (M.O.H.), the Ernst Hadorm Foundation (M.O.H.), and the Geneva Department of Education.

Supporting Online Material
www.sciencemag.org/cgi/content/full/324/5925/381/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 and S2
References

14 November 2008; accepted 25 February 2009
10.1126/science.1168532

Sequential Regulation of DOCK2 Dynamics by Two Phospholipids During Neutrophil Chemotaxis

Akihiko Nishikimi,[1,2] Hideo Fukuhara,[1] Wenjuan Su,[3] Tsunaki Hongu,[4] Shunsuke Takasuga,[5] Hisashi Mihara,[6] Qinhong Cao,[1]* Fumiyuki Sanematsu,[1] Motomu Kanai,[6] Hiroshi Hasegawa,[4] Yoshihiko Tanaka,[1,2] Masakatsu Shibasaki,[6] Yasunori Kanaho,[4] Takehiko Sasaki,[5] Michael A. Frohman,[3] Yoshinori Fukui[1,2]†

During chemotaxis, activation of the small guanosine triphosphatase Rac is spatially regulated to organize the extension of membrane protrusions in the direction of migration. In neutrophils, Rac activation is primarily mediated by DOCK2, an atypical guanine nucleotide exchange factor. Upon stimulation, we found that DOCK2 rapidly translocated to the plasma membrane in a phosphatidylinositol 3,4,5-trisphosphate–dependent manner. However, subsequent accumulation of DOCK2 at the leading edge required phospholipase D–mediated synthesis of phosphatidic acid, which stabilized DOCK2 there by means of interaction with a polybasic amino acid cluster, resulting in increased local actin polymerization. When this interaction was blocked, neutrophils failed to form leading edges properly and exhibited defects in chemotaxis. Thus, intracellular DOCK2 dynamics are sequentially regulated by distinct phospholipids to localize Rac activation during neutrophil chemotaxis.

Chemotaxis regulates a wide range of biological functions, including developmental morphogenesis, wound healing, and immune responses (*1*). During chemotaxis, filamentous actin (F-actin) polymerizes asymmetrically at the leading edge of the cell, providing the force necessary to extend membrane protrusions in the direction of migration (*1*, *2*). This morphologic polarity is regulated by Rac, a member of the small guanosine triphosphatases (GTPases) that cycle between inactive guanosine diphosphate (GDP)–bound and active guanosine triphosphate (GTP)–bound states (*3*). Rac is preferentially activated at the leading edge (*1*, *4*, *5*), which is achieved in part by regulating the subcellular localization of guanine nucleotide exchange factors (GEFs) (*1*, *3*). The GEFs contain a variety of localization motifs such as pleckstrin homology (PH) domains and the DOCK homology region (DHR)–1, both of which bind to phosphatidylinositol 3,4,5-trisphosphate (PIP_3) (*3*, *6*), a lipid product of phosphoinositide 3-kinases (PI3Ks). Upon stimulation, PIP_3 transiently accumulates at the plasma membrane edge facing the highest level of chemoattractant (*1*, *2*). However, a functional leading edge is established even in neutrophils lacking PI3Kγ, the major generator of PIP_3 in this cell type (*7*, *8*). Thus, other factors may alternately suffice to localize Rac GEFs at the leading edge during neutrophil chemotaxis.

DOCK2 is a member of the CDM family of proteins (*Caenorhabditis elegans*, CED-5; mammals, DOCK180; and *Drosophila melanogaster*, Myoblast city) and is predominantly expressed in hematopoietic cells (*9*). Although DOCK2 does not contain the PH and Dbl homology domains typically found in GEFs, DOCK2 can bind to PIP_3 through its DHR-1 domain (*10*) and mediates the GTP-GDP exchange reaction for Rac by means of its DHR-2 domain (*11*, *12*). DOCK2 is a major Rac GEF that controls motility and polarity during neutrophil chemotaxis (*10*). In response to chemoattractants, neutrophils polarize and accumulate DOCK2 at the plasma membrane edge (fig. S1). To explore the mechanism controlling intracellular DOCK2 dynamics, we first analyzed the role of PIP_3 by crossing PI3K$\gamma^{-/-}$ mice with mice that had been made by a "knock-in" strategy to express endogenous DOCK2 as a fusion protein with green fluorescent protein (GFP) (*10*, *13*). When neutrophils from DOCK2-GFP mice were stimulated in suspension with chemotactic factors such as *N*-formyl-Met-Leu-Phe (fMLP) and C5a, DOCK2 rapidly translocated to the plasma membrane at 15 s in the presence, but not in the absence, of PI3Kγ (fig. S2). Thus, the initial membrane translocation of DOCK2 is mediated by PIP_3. However, despite the absence of PI3Kγ expression, DOCK2 and F-actin nonetheless still accumulated preferentially at the pseudopod at later time points (fig. S2).

Phosphatidic acid (PA) is a negatively charged phospholipid that can function as a lipid anchor by binding directly to positively charged sites on effector proteins (*14*). In response to many types of external stimuli, signaling pools of PA are formed through hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) or phosphorylation of diacylglycerol (DAG) by diacylglycerol kinase (DGK) (*14*, *15*). Although PLD has been implicated in migratory responses of *Dictyostelium discoideum*, epithelial cells, and neutrophils (*16*–*18*), the mechanistic basis is largely unknown. To investigate whether subcellular localization of DOCK2 is influenced by PLD, GFP-tagged DOCK2 was expressed in human embryonic kidney (HEK) 293T cells with or without coexpression of PLD2, a PLD isoform that localizes primarily to the plasma membrane (*19*). DOCK2 was localized mainly in the cytosol when expressed alone; however, it readily accumulated at the plasma membrane when PLD2 was coexpressed (Fig. 1, A and B). In contrast, the catalytically inactive PLD2 mutant K758R in which Lys^{758} is replaced by Arg (*20*) failed to alter DOCK2 localization (Fig. 1, A and B). Thus, coexpressing PLD2 induces plasma membrane accumula-

[1]Division of Immunogenetics, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan. [2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Tokyo 102-0075, Japan. [3]Department of Pharmacology, Center for Developmental Genetics, Stony Brook University, Stony Brook, NY 11794–5140, USA. [4]Graduate School of Comprehensive Human Sciences, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki 305-8575, Japan. [5]Division of Microbiology, Department of Pathology and Immunology, Akita University School of Medicine, Akita 010-8543, Japan. [6]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan.

*Present address: College of Biological Sciences, China Agricultural University, Beijing 100193, China.
†To whom correspondence should be addressed. E-mail: fukui@bioreg.kyushu-u.ac.jp

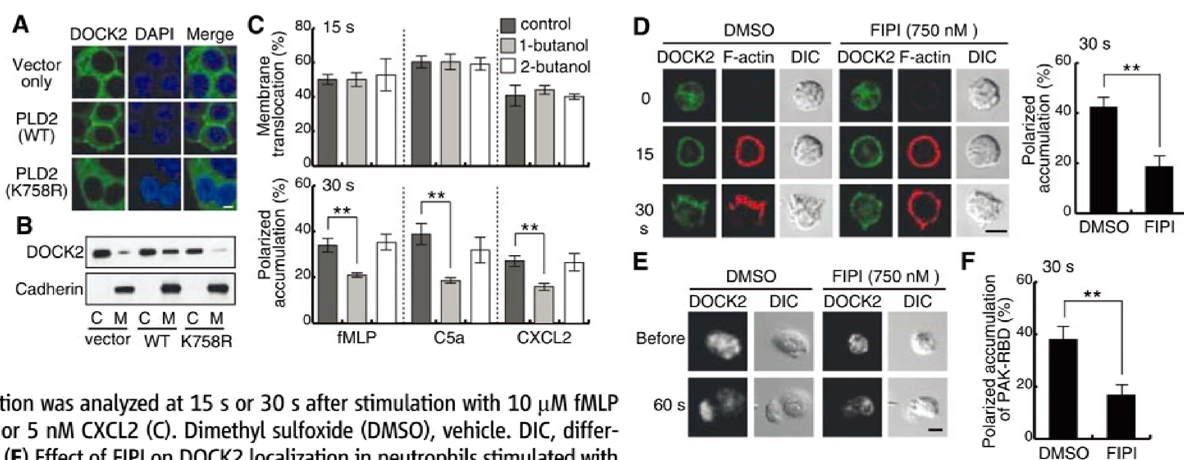


Fig. 1. PA controls DOCK2 localization during neutrophil chemotaxis. (**A** and **B**) Effect of coexpressed PLD2 on localization of GFP-tagged DOCK2 in HEK293T cells. 4′,6′-Diamidino-2-phenylindole (DAPI) was used to stain nuclei. Membrane (M) and cytosol (C) fractions were analyzed by immunoblot. (**C** and **D**) Effect of 0.2% 1-butanol (C) or 750 nM FIPI (D) on DOCK2 localization was analyzed at 15 s or 30 s after stimulation with 10 μM fMLP (C), 25 nM C5a (C and D), or 5 nM CXCL2 (C). Dimethyl sulfoxide (DMSO), vehicle. DIC, differential interference contrast. (**E**) Effect of FIPI on DOCK2 localization in neutrophils stimulated with a micropipette containing 10 μM fMLP. (**F**) Effect of FIPI on localization of GFP-tagged PAK-RBD was analyzed 30 s after stimulation with 25 nM C5a. Data in (C), (D), and (F) are means ± SD of triplicate experiments, in each of which at least 50 cells were analyzed. $^{**}P < 0.01$. Scale bar in (A), (D), and (E), 5 μm.

tion of DOCK2 through a mechanism dependent on its catalytic activity.

Primary alcohols such as 1-butanol compete with water in the hydrolysis of PC by PLD (*15*). To examine whether PLD-generated PA is involved in control of intracellular DOCK2 dynamics, we treated neutrophils of DOCK2-GFP mice with 1-butanol and, as a control, with 2-butanol. In response to fMLP, C5a, and CXCL2, 25 to 35% of neutrophils treated with 2-butanol exhibited polarized morphology with focused distribution of DOCK2. However, treatment with 1-butanol significantly inhibited accumulation of DOCK2 and F-actin at the pseudopods (Fig. 1C and fig. S3). Similar results were obtained when neutrophils were treated with 5-fluoro-2-indolyl des-clorohalopemide (FIPI), a PLD-specific inhibitor (*21*) (Fig. 1D), but not with the DGK inhibitor R59 022 (fig. S4). Although DOCK2 accumulated at the leading edge in control cells in response to a point source of fMLP, the majority of neutrophils treated with 1-butanol or FIPI displayed aberrant morphology with extremely thin lamellae and poorly focused DOCK2 distribution (Fig. 1E and fig. S5). Moreover, treatment with 1-butanol or FIPI impaired localization to the pseudopods of a fluorescent probe that detects activated Rac, the GFP-tagged Rac-binding domain (RBD) of p21-activated kinase (PAK) (*22*) (Fig. 1F and fig. S6). On the other hand, neither 1-butanol nor FIPI inhibited the initial membrane translocation of DOCK2 (Fig. 1, C and D). Thus, PA acts selectively in the late phase to control polarized DOCK2 localization during neutrophil chemotaxis.

Exogenous PA added to culture medium is rapidly incorporated into the plasma membrane and can elicit cellular responses (*23*) (fig. S7). Wild-type (WT) neutrophils treated with PA exhibited polarized morphology with focused distribution of F-actin (Fig. 2A). This morphological change appeared to be mediated by PA itself, because other phospholipids including lysophosphatidic acid (LPA) and DAG, the two major metabolites of PA, failed to induce actin polym-

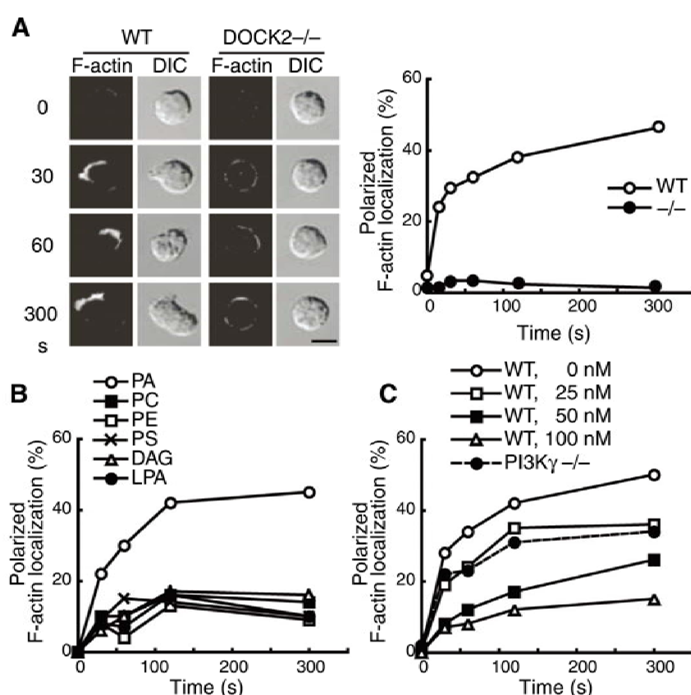


Fig. 2. Exogenous PA induces actin polymerization through a mechanism dependent on DOCK2. (**A**) WT or DOCK2$^{-/-}$ neutrophils were stimulated with PA (10 μg/ml). Scale bar, 5 μm. (**B**) WT neutrophils were stimulated with varied phospholipids (10 μg/ml). (**C**) WT or PI3Kγ$^{-/-}$ neutrophils were stimulated with PA (10 μg/ml) at the indicated concentrations of wortmannin. At least 100 cells were analyzed in each experiment.

erization (Fig. 2B and fig. S8). The effect of PA was insensitive to pertussis toxin, an inhibitor of G_i and G_o proteins (fig. S9), and PA addition did not increase phosphorylation of Akt, a downstream effector of PI3Ks (fig. S10). However, PA-induced DOCK2 accumulation and actin polymerization were attenuated by treating neutrophils with the PI3K inhibitor wortmannin (Fig. 2C and fig. S11), and the ability of PA to stimulate F-actin assembly and morphologic change was almost totally lost for DOCK2$^{-/-}$ neutrophils (Fig. 2A). Thus, PA induces actin polymerization in unstimulated neutrophils and makes "micropolarity" visible in a manner dependent on both basal PI3K activity and DOCK2.

This finding led us to examine whether DOCK2 physically interacts with PA. Although no binding was found when HEK293T cell lysates containing DOCK2 were incubated with lipid vesicles composed solely of PC and phosphatidylethanolamine (PE), DOCK2 bound to PA-containing vesicles in a concentration-dependent manner (Fig. 3A). The PA binding was almost totally abolished by deleting the C-terminal 214 amino acid residues (1615 to 1828) (DOCK2-ΔC) (Fig. 3A), and the C-terminal fragment bound in vitro to lipid vesicles containing PA (Fig. 3B) but not to other acidic phospholipids including PIP_3, phosphatidylserine (PS), and phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] (fig. S12). Thus, the C-terminal region of DOCK2 interacts directly and selectively with PA.

The only motif known for PA-binding proteins is the presence of one or more basic amino acid residues (*14*). Both mouse and human DOCK2 encode the amino acid sequence Ser-Lys-Lys-Arg (residues 1696 to 1699), which mediates PA binding for the yeast protein Opi1p (*24*). When the three basic residues were all mu-

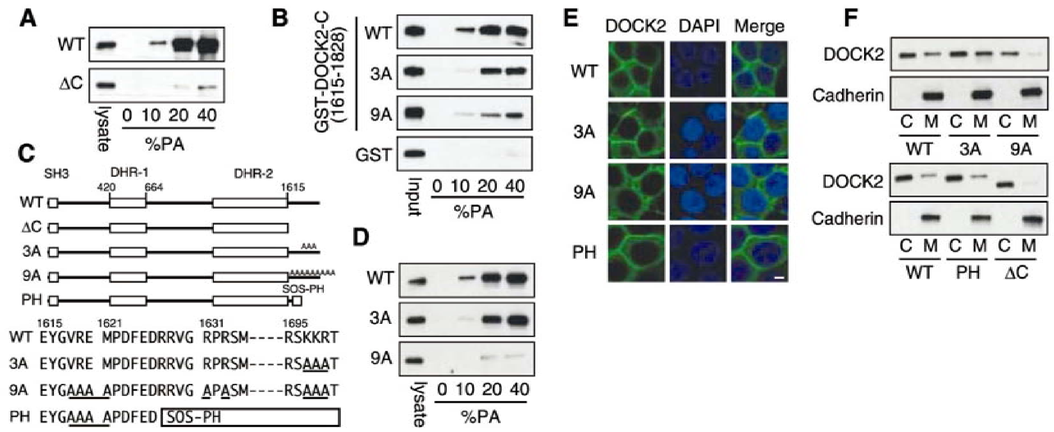


Fig. 3. DOCK2 binds to PA through the C-terminal polybasic amino acid cluster. (**A**) Extracts from HEK293T cells expressing GFP-tagged DOCK2-WT or DOCK2-ΔC were pulled down with PA-containing lipid vesicles. (**B**) Glutathione *S*-transferase (GST) fusion proteins encoding the C-terminal fragment of DOCK2 or its mutants were pulled down with PA-containing lipid vesicles. (**C**) Schematic representation of DOCK2 mutants used in this study (*20*). The mutated amino acid residues are underlined. (**D**) The ability to bind to PA was assayed for DOCK2-WT and its mutants as in (A). (**E** and **F**) Localization of DOCK2-WT or its mutants was visualized in HEK293T cells overexpressing PLD2. Scale bar, 5 μm.

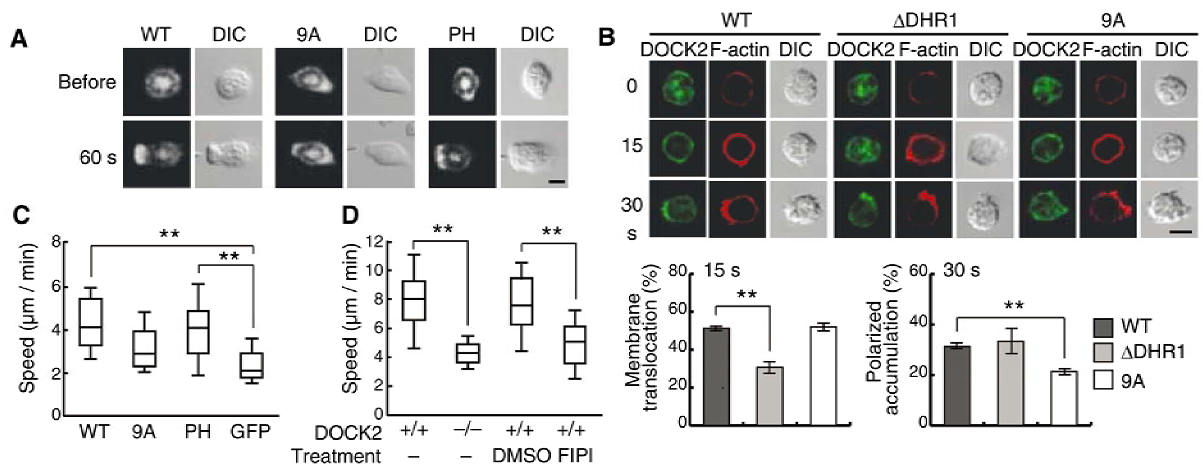


Fig. 4. The DOCK2-PA interaction is required to stabilize the leading edge during neutrophil chemotaxis. (**A**) DOCK2$^{-/-}$ neutrophils expressing GFP-tagged DOCK2 constructs were stimulated with a micropipette containing 10 μM fMLP. (**B**) WT neutrophils expressing GFP-tagged DOCK2 constructs were stimulated with C5a (25 nM). Data are means ± SD of triplicate experiments, in each of which at least 50 cells were analyzed. $^{**}P < 0.01$. (**C**) After expression of GFP-tagged DOCK2 constructs or GFP alone, DOCK2$^{-/-}$ neutrophils were allowed to migrate in an EZ-Taxiscan chamber along an fMLP gradient (0 to 50 μM). The migration speed was analyzed for at least 24 GFP-positive cells that moved more than 20 μm over 15 min. $^{**}P < 0.01$. (**D**) WT or DOCK2$^{-/-}$ neutrophils were allowed to migrate as in (C) in the presence or absence of FIPI (750 nM). The migration speed was analyzed for at least 40 cells. $^{**}P < 0.01$. Scale bar in (A) and (B), 5 μm.

tated to Ala (designated 3A), PA binding was diminished, but only to a modest extent (Fig. 3B). However, by mutating six additional resides to Ala (designated 9A), we found that the binding capacity of the DOCK2 C-terminal fragment to PA decreased to 20% of the WT level (Fig. 3, B and C). Similar results were obtained when full-length DOCK2 protein bearing the 9A mutations (DOCK2-9A) was assayed for PA binding (Fig. 3D). Although the expression of DOCK2-9A in HEK293T cells induced Rac activation fully (fig. S13), the DOCK2-9A failed to localize to the plasma membrane even when PLD2 was coexpressed (Fig. 3, E and F). In contrast, when the C-terminal region of DOCK2 was replaced with a known PA-binding module, the PH domain of Son of sevenless (SOS) (*25*) (Fig. 3C and fig. S14), this chimeric molecule (DOCK2-PH) accumulated effectively at the plasma membrane in the presence of PLD2 (Fig. 3, E and F). Thus, PA controls subcellular localization of DOCK2 by directly binding to the C-terminal polybasic amino acid cluster.

We next examined whether the DOCK2-PA interaction is functionally important by expressing GFP-tagged DOCK2-WT, DOCK2-9A, or DOCK2-PH in DOCK2$^{-/-}$ neutrophils. Although more than 85% of DOCK2$^{-/-}$ neutrophils expressing DOCK2-WT or DOCK2-PH extended lamellipodia containing accumulated DOCK2 toward the fMLP source, the expression of DOCK2-9A only partially restored the leading edge formation (Fig. 4A). Because GFP-tagged SOS-PH localized to the leading edge even in DOCK2$^{-/-}$ neutrophils (fig. S15), this defect appeared to result from the inability of PA to tether DOCK2-9A. Indeed, DOCK2-9A translocated normally to the plasma membrane, but accumulated less effectively at the pseudopods of WT neutrophils than did DOCK2-WT and a DOCK2 mutant lacking DHR-1 domain (DOCK2-ΔDHR1; Fig. 4B). When DOCK2-WT or DOCK2-PH was expressed in DOCK2$^{-/-}$ neutrophils, the migration speed increased by 75% or 65%, respectively, compared with that of the control expressing GFP alone (Fig. 4C). However, the expression of DOCK2-9A failed to significantly increase the motility of DOCK2$^{-/-}$ neutrophils (Fig. 4C). Consistent with this finding, treatment of WT neutrophils with the PLD inhibitor FIPI reduced the migration speed to a level almost comparable to that of the DOCK2$^{-/-}$ neutrophils (Fig. 4D).

We showed here, during neutrophil chemotaxis, that intracellular DOCK2 dynamics were sequentially regulated by two distinct phospholipids: Although the plasma membrane translocation of DOCK2 was initially mediated by PIP_3, PA acted at a late phase to focus DOCK2 localization and to stabilize the leading edge. This two-step regulation fits well with the kinetics of these phospholipids in response to chemoattractants (fig. S16). Lamellipodia formation in epithelial cells is mediated by DOCK180, another GEF, and is inhibited by 1-butanol (*17*, *26*). The C-terminal region of DOCK180 also bound to PA (fig. S17), which suggested a common mechanism for its recruitment. Thus, PA may be involved in spatial regulation for Rac activation in varied biological settings by controlling localization of GEFs.

References and Notes

1. A. J. Ridley *et al.*, *Science* **302**, 1704 (2003).
2. P. Rickert, O. D. Weiner, F. Wang, H. R. Bourne, G. Servant, *Trends Cell Biol.* **10**, 466 (2000).
3. A. Schmidt, A. Hall, *Genes Dev.* **16**, 1587 (2002).

4. V. S. Kraynov *et al.*, *Science* **290**, 333 (2000).
5. E. M. Gardiner *et al.*, *Curr. Biol.* **12**, 2029 (2002).
6. J.-F. Côté, A. B. Motoyama, J. A. Bush, K. Vuori, *Nat. Cell Biol.* **7**, 797 (2005).
7. M. Nishio *et al.*, *Nat. Cell Biol.* **9**, 36 (2007).
8. G. J. Ferguson *et al.*, *Nat. Cell Biol.* **9**, 86 (2007).
9. Y. Fukui *et al.*, *Nature* **412**, 826 (2001).
10. Y. Kunisaki *et al.*, *J. Cell Biol.* **174**, 647 (2006).
11. E. Brugnera *et al.*, *Nat. Cell Biol.* **4**, 574 (2002).
12. J.-F. Côté, K. Vuori, *J. Cell Sci.* **115**, 4901 (2002).
13. Materials and methods are available as supporting material on *Science* Online.
14. C. L. Stace, N. T. Ktistakis, *Biochim. Biophys. Acta* **1761**, 913 (2006).
15. M. McDermott, M. J. O. Wakelam, A. J. Morris, *Biochem. Cell Biol.* **82**, 225 (2004).
16. S. Zouwail *et al.*, *Biochem. J.* **389**, 207 (2005).
17. L. C. Santy, J. E. Casanova, *J. Cell Biol.* **154**, 599 (2001).
18. N. Lehman *et al.*, *Blood* **108**, 3564 (2006).
19. G. Du, P. Huang, B. T. Liang, M. A. Frohman, *Mol. Biol. Cell* **15**, 1024 (2004).
20. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
21. W. Su *et al.*, *Mol. Pharmacol.* **75**, 437 (2009).
22. S. Srinivasan *et al.*, *J. Cell Biol.* **160**, 375 (2003).
23. Y. Fang, M. Vilella-Bach, R. Bachmann, A. Flanigan, J. Chen, *Science* **294**, 1942 (2001).
24. C. J. R. Loewen *et al.*, *Science* **304**, 1644 (2004).
25. C. Zhao, G. Du, K. Skowronek, M. A. Frohman, D. Bar-Sagi, *Nat. Cell Biol.* **9**, 706 (2007).
26. L. C. Santy, K. S. Ravichandran, J. E. Casanova, *Curr. Biol.* **15**, 1749 (2005).
27. This work was supported by grants for Targeted Proteins Research Project, Genome Network Project, Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; Toray Science Foundation; CREST program of Japan Science and Technology Agency; the Global Center of Excellence program of the Japan Society for the Promotion of Science; and NIH award R01GM71520.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1170179/DC1
Materials and Methods
Figs. S1 to S17
References

23 December 2008; accepted 10 March 2009
Published online 26 March 2009;
10.1126/science.1170179
Include this information when citing this paper.

Rare Variants of *IFIH1*, a Gene Implicated in Antiviral Responses, Protect Against Type 1 Diabetes

Sergey Nejentsev,[1,2]* Neil Walker,[1] David Riches,[3] Michael Egholm,[3] John A. Todd[1]

Genome-wide association studies (GWASs) are regularly used to map genomic regions contributing to common human diseases, but they often do not identify the precise causative genes and sequence variants. To identify causative type 1 diabetes (T1D) variants, we resequenced exons and splice sites of 10 candidate genes in pools of DNA from 480 patients and 480 controls and tested their disease association in over 30,000 participants. We discovered four rare variants that lowered T1D risk independently of each other (odds ratio = 0.51 to 0.74; $P = 1.3 \times 10^{-3}$ to 2.1×10^{-16}) in *IFIH1* (*interferon induced with helicase C domain 1*), a gene located in a region previously associated with T1D by GWASs. These variants are predicted to alter the expression and structure of IFIH1 [MDA5 (melanoma differentiation-associated protein 5)], a cytoplasmic helicase that mediates induction of interferon response to viral RNA. This finding firmly establishes the role of *IFIH1* in T1D and demonstrates that resequencing studies can pinpoint disease-causing genes in genomic regions initially identified by GWASs.

Genome-wide association studies (GWASs) of common multifactorial diseases have identified dozens of loci harboring disease-causing sequence variants (*1*, *2*). However, because the human genome contains regions of strong linkage disequilibrium, a disease-associated locus sometimes encompasses several genes and multiple tightly associated polymorphisms, making it difficult to pinpoint the causal variant by association mapping. Moreover, in many instances, the single nucleotide polymorphisms (SNPs) showing the most significant disease association map to genomic regions with no obvious function, thus providing few clues as to how causal variants affect the disease gene.

One way to overcome this limitation is to search for sequence variants that are rare in the population (frequency < 3%) but that reside in exons and other genomic regions of known function to identify polymorphisms that alter expression of the gene and/or function of the protein product (*3*). If rare disease-associated variants with obvious functional effects are found in a candidate gene that harbors a common disease-associated variant, then the gene is likely to be causal. Recent technological advances in high-throughput sequencing (*4*) provide an opportunity to resequence multiple genetic regions in hundreds of participants and discover rare sequence variants (*5*–*7*). We used 454 sequencing (*8*) to search for rare variants in 10 candidate genes and to study their association with type 1 diabetes (T1D), previously known as insulin-dependent diabetes mellitus (IDDM). T1D is a common disorder that develops as a result of a complex interaction of genetic and environmental factors leading to the immune-mediated destruction of the insulin-producing pancreatic β cells. To date, 15 loci associated with T1D have been identified in the human genome (*9*–*13*).

Of the 10 genes that we selected, 6 contain common T1D-associated polymorphisms: *PTPN22*, *PTPN2*, *IFIH1*, *SH2B3*, *CLEC16A*, and *IL2RA* (*10*, *11*, *14*–*16*). We also studied two genes that contain rare mutations causing monogenic syndromes that may include immune-mediated diabetes: (i) *FOXP3*, which is responsible for X-linked syndrome of immunodysregulation-polyendocrinopathy-enteropathy [Online Mendelian Inheritance in Man (OMIM) 304790], and (ii) *AIRE*, which is responsible for the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (OMIM 240300). Finally, we studied *KCNJ11* because mutations in this gene cause permanent neonatal diabetes, an insulin-dependent diabetes of the non-immune etiology that can be misdiagnosed as T1D in young children (*17*). We also studied *IAN4L1* because the ortholog of this gene is associated with immune-mediated diabetes in the rat model of T1D (*18*, *19*).

We resequenced 144 target regions that covered exons and regulatory sequences of the 10 genes, 31 kb in total (table S1 and T1DBase: www.t1dbase.org/page/PosterView/454Resequencing), in DNA of 480 T1D patients and 480 healthy controls from Great Britain arranged in 20 DNA pools (*20*). We generated 9.4 million reads with an average length of 250 bases and identified a total of 212 SNPs (*20*). We classified 33 of them as common because their estimated minor allele frequency (MAF) was >3% (table S2), and we categorized 179 as rare because their estimated MAF was <3%. Of the 179 rare SNPs, 156 were previously unseen (table S3). In the pooled samples, it was impossible to distinguish rare insertion/deletion polymorphisms from sequencing errors; thus, we studied nucleotide substitutions only.

Our goal was not only to discover previously unseen rare variants but also to test their association with T1D in the same experiment, comparing allele frequency in DNA pools of patients and controls. Therefore, it was important that sequence reads generated from the DNA pools estimated accurately allele frequency among individuals that contributed DNA to these pools. To test this, we analyzed eight SNPs from the sequenced regions that had been genotyped previously. We found good correlation between allele frequency in the individual samples and its estimate in the DNA pools (correlation coefficient r = 0.99) (fig. S1), demonstrating that high-throughput sequencing of

[1]Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, CB2 0XY, UK. [2]Department of Medicine, University of Cambridge, CB2 2QQ, UK. [3]454 Life Sciences, Branford, CT 06405, USA.

*To whom the correspondence should be addressed. E-mail: sn262@cam.ac.uk

References and Notes

1. L. Evans, *Traffic Safety* (Science Serving Society, Bloomfield Hills, MI, 2004).
2. T. A. Dingus *et al.*, "The 100-Car Naturalistic Driving Study, Phase II—Results of the 100-Car Field Experiment," DOT HS 810 593 (Virginia Tech Transportation Institute, Blacksburg, VA, 2006).
3. S. G. Klauer, T. A. Dingus, V. L. Neale, J. D. Sudweeks, D. J. Ramsey, "The Impact of Driver Inattention on Near-Crash/Crash Risk: An Analysis Using the 100-Car Naturalistic Driving Study Data," DOT HS 810 594 (National Highway Traffic Safety Administration, Washington, DC, 2006).
4. M. A. Recarte, L. M. Nunes, *J. Exp. Psychol. Appl.* **9**, 119 (2003).
5. J. S. McCarley *et al.*, *Human Factors* **3**, 424 (2004).
6. T. W. Victor, J. L. Harbluk, J. A. Engstrom, *Transport. Res. F* **8**, 167 (2005).
7. W. J. Horrey, M. F. Lescha, A. Garabeta, *Accident Anal. Prevent.* **40**, 675 (2008).
8. O. Svenson, *Acta Psychol.* **47**, 143 (1981).
9. A. E. Waylen, M. S. Horswill, J. L. Alexander, F. P. McKenna, *Transport. Res. F* **7**, 323 (2004).
10. S. Rajalin, H. Summala, *Accident Anal. Prevent.* **29**, 277 (1997).
11. M. P. White *et al.*, *Risk Anal.* **24**, 323 (2004).
12. J. D. Lee, D. V. McGehee, T. L. Brown, M. L. Reyes, *Human Factors* **44**, 314 (2002)
13. D. V. McGehee, M. Raby, C. H. Carney, M. L. Reyes, J. D. Lee, *J. Safety Res.* **38**, 15 (2007).
14. T. Tomer, T. Lotan, *Transport. Res. Rec.* **1953**, 112 (2006).
15. B. Donmez, L. N. Boyle, J. D. Lee, *Accident Anal. Prevent.* **40**, 776 (2008).

16. The work presented is based in part on the SAVE-IT program [Safety Vehicle(s) Using Adaptive Interface Technology] sponsored by the National Highway Traffic Safety Administration, U.S. Department of Transportation.

10.1126/science.1168085

CELL BIOLOGY

Two Lipids That Give Direction

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

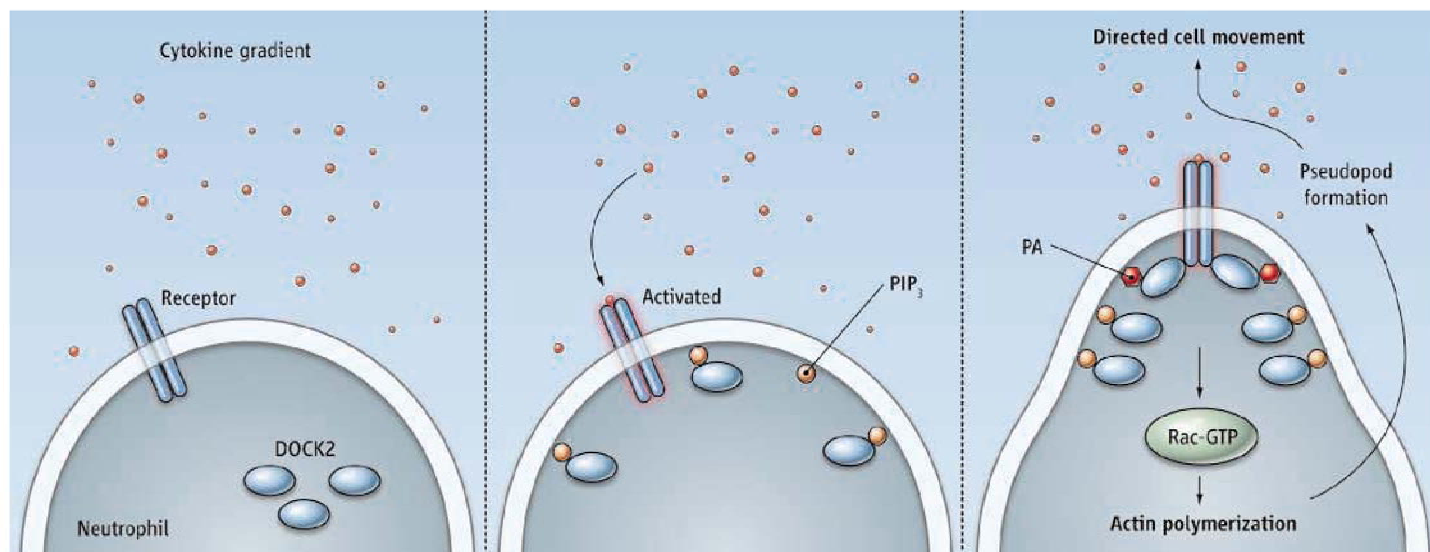
Jean-François Côté[1] and Kristiina Vuori[2]

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.

[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. E-mail: jean-francois.cote@ircm.qc.ca, kvuori@burnham.org

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 (PIP_3) (*7*). PIP_3 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? PIP_3 is rapidly produced by phosphoinositide 3-kinases (PI 3-kinases) in response to activated chemoattractant receptors, and accu-



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—PIP_3 and phosphatidic acid (PA). This refinement of DOCK2 localization ensures rapid neutrophil movement toward the chemoattractant gradient.

CREDIT: C. BICKEL/SCIENCE

References and Notes

1. L. Evans, *Traffic Safety* (Science Serving Society, Bloomfield Hills, MI, 2004).
2. T. A. Dingus *et al.*, "The 100-Car Naturalistic Driving Study, Phase II—Results of the 100-Car Field Experiment," DOT HS 810 593 (Virginia Tech Transportation Institute, Blacksburg, VA, 2006).
3. S. G. Klauer, T. A. Dingus, V. L. Neale, J. D. Sudweeks, D. J. Ramsey, "The Impact of Driver Inattention on Near-Crash/Crash Risk: An Analysis Using the 100-Car Naturalistic Driving Study Data," DOT HS 810 594 (National Highway Traffic Safety Administration, Washington, DC, 2006).
4. M. A. Recarte, L. M. Nunes, *J. Exp. Psychol. Appl.* **9**, 119 (2003).
5. J. S. McCarley *et al.*, *Human Factors* **3**, 424 (2004).
6. T. W. Victor, J. L. Harbluk, J. A. Engstrom, *Transport. Res. F* **8**, 167 (2005).
7. W. J. Horrey, M. F. Lescha, A. Garabeta, *Accident Anal. Prevent.* **40**, 675 (2008).
8. O. Svenson, *Acta Psychol.* **47**, 143 (1981).
9. A. E. Waylen, M. S. Horswill, J. L. Alexander, F. P. McKenna, *Transport. Res. F* **7**, 323 (2004).
10. S. Rajalin, H. Summala, *Accident Anal. Prevent.* **29**, 277 (1997).
11. M. P. White *et al.*, *Risk Anal.* **24**, 323 (2004).
12. J. D. Lee, D. V. McGehee, T. L. Brown, M. L. Reyes, *Human Factors* **44**, 314 (2002)
13. D. V. McGehee, M. Raby, C. H. Carney, M. L. Reyes, J. D. Lee, *J. Safety Res.* **38**, 15 (2007).
14. T. Tomer, T. Lotan, *Transport. Res. Rec.* **1953**, 112 (2006).
15. B. Donmez, L. N. Boyle, J. D. Lee, *Accident Anal. Prevent.* **40**, 776 (2008).

16. The work presented is based in part on the SAVE-IT program [Safety Vehicle(s) Using Adaptive Interface Technology] sponsored by the National Highway Traffic Safety Administration, U.S. Department of Transportation.

10.1126/science.1168085

CELL BIOLOGY

Two Lipids That Give Direction

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

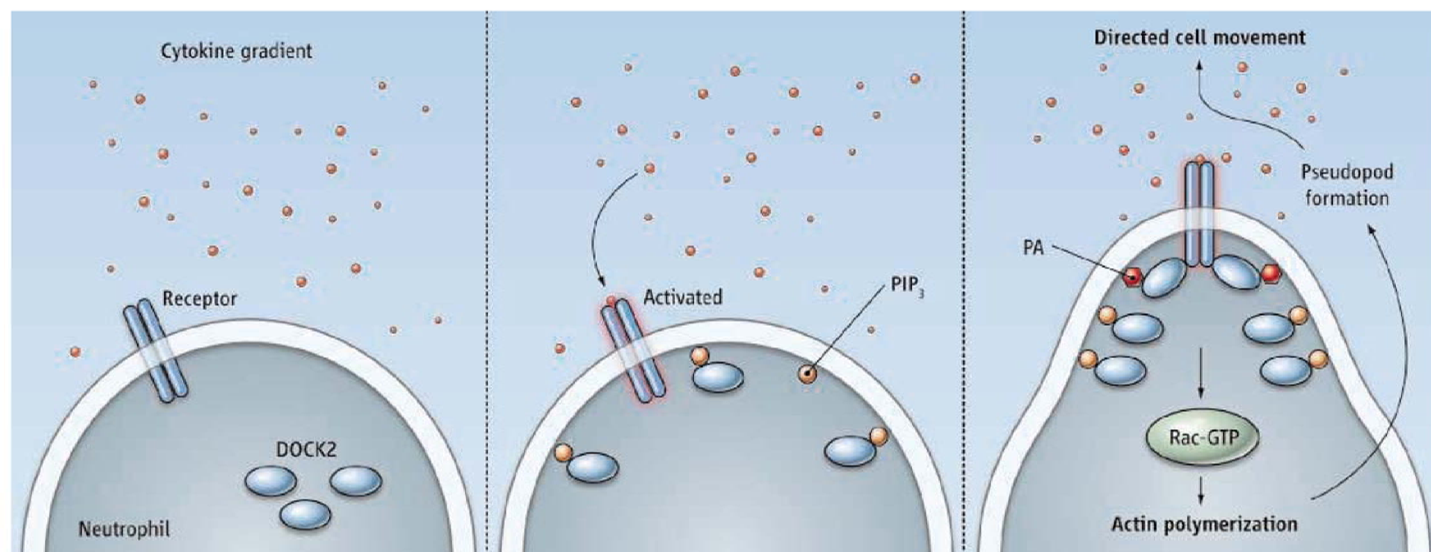
Jean-François Côté[1] and Kristiina Vuori[2]

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.

[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. E-mail: jean-francois.cote@ircm.qc.ca, kvuori@burnham.org

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 (PIP_3) (*7*). PIP_3 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? PIP_3 is rapidly produced by phosphoinositide 3-kinases (PI 3-kinases) in response to activated chemoattractant receptors, and accu-



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—PIP_3 and phosphatidic acid (PA). This refinement of DOCK2 localization ensures rapid neutrophil movement toward the chemoattractant gradient.

CREDIT: C. BICKEL/SCIENCE