Signaling pathways controlling cell polarity and chemotaxis

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Many important biological processes, including chemotaxis (directional cell movement up a chemoattractant gradient), require a clearly established cell polarity and the ability of the cell to respond to a directional signal. Recent advances using *Dictyostelium* cells and mammalian leukocytes have provided insights into the biochemical and molecular pathways that control chemotaxis. Phosphoinositide 3-kinase plays a central and possibly pivotal role in establishing and maintaining cell polarity by regulating the subcellular localization and activation of downstream effectors that are essential for regulating cell polarity and proper chemotaxis. This review outlines our present understanding of these pathways.

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Directional cell movement, in part, involves the differential polymerization of filamentous actin (F-actin) at the front (leading edge or pseudopod) of a cell, leading to protrusion of the membrane surface and 'forward' movement[1,2]. This is followed by contraction of the cell's posterior, which often results from myosin II-mediated contraction (Fig. 1). Chemotaxis consists of directional cell movement up a chemoattractant gradient. Chemoattractants are generally small, water-soluble molecules that function as ligands for cell-surface receptors activating signaling pathways leading to localized F-actin assembly. Directional cell movement requires a defined cell polarity in which cytoskeletal components are differentially localized at two poles of a cell; this localization can be an intrinsic property of a cell or arise in response to directional signals. Intrinsic asymmetry can be defined by the biased distribution of the cytoskeletal elements necessary for cell movement. Alternatively, cell polarity can be established by spatially amplifying an extracellular, directional signal at the site in the membrane closest to the chemoattractant source, as is the case for leukocytes and *Dictyostelium* cells responding to chemoattractant gradients (see following text).

Intrinsic asymmetry of cytoskeletal and regulatory components in different parts of the cell confers an innate ability to rapidly respond by locally activating signaling events and changes in the cytoskeleton when the cell perceives a chemoattractant. For example, preassembly of the components necessary to assemble F-actin at one pole of the cell would promote extension of the pseudopod at that site (Fig. 2). This ability is seen most dramatically in neutrophils, which increase their rate of persistent cell movement (movement without changing direction) when a chemoattractant is detected, even in the absence of a concentration gradient[3,4]. Other cell types such as *Dictyostelium* cells, which can become highly polarized in response to directional and/or developmental signals[5], require a chemoattractant gradient to increase their rate of persistent cell movement. Impressively, polarized *Dictyostelium* cells and leukocytes can detect and respond to a shallow chemoattractant gradient with a chemoattractant concentration difference of 2% between the anterior and posterior of the cell[6]. A change in the direction of a shallow chemoattractant gradient elicits a turning response in which the cell's F-actin-enriched leading edge (pseudopod) turns to reorient itself in the direction of the new gradient, suggesting that, once established, a cell's polarity is intrinsically resistant to change, enabling cells to respond to very small differences in the chemoattractant concentration between the front and rear of the cell. *Dictyostelium* cells can also respond to a strong chemotaxis gradient, such as that emitted from a micropipette placed near a cell, by retracting the old pseudopod and extending a new pseudopod from the side of the cell at the position closest to the micropipette tip[7] (Fig. 1). The ability to use genetic and biochemical approaches to examine the underlying mechanisms controlling cell polarity and directional movement has revealed new components in the pathways and provided unifying models for the establishment of the anterior of a chemotaxing polarized cell.

Asymmetric organization of the cytoskeleton

Dictyostelium cells and leukocytes possess intrinsic cell polarity, which could play a role in their inherent ability to rapidly respond to chemoattractants[1,6–26]. Even in the absence of a chemoattractant gradient, both cell types exhibit a strong differential subcellular localization of F-actin and assembled myosin II (Fig. 1). The highest concentration of F-actin is found at the presumptive leading edge and the lower concentration at the cell's posterior in the rear body[27]. Assembled myosin II is preferentially found in the rear body and along the lateral sides in a decreasing posterior-to-anterior gradient[10]. Other cytoskeletal proteins and cellular components that regulate the cytoskeleton (e.g. Arp2/3 and *Dictyostelium* PAKa) exhibit a strong differential localization within the cell between the leading edge and the rear-body[11,12].

F-actin assembly at the leading edge

The first step in chemotaxis involves a regulated increase in actin nucleation and branching activity

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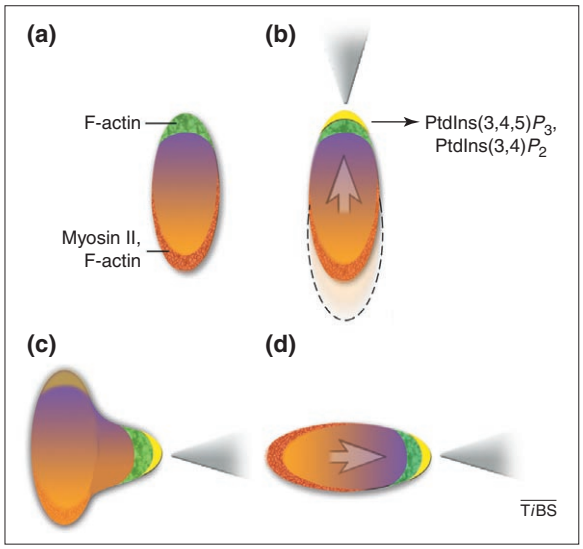

Fig. 1. Model for chemotaxis of polarized cells to a strong directional stimulus. (a) Polarized cells exhibit a differential localization of F-actin and myosin II as shown. PH-domain-containing proteins are not localized to the front of unstimulated cells. (b) When the cells sense a chemoattractant (in this case in the same direction of the cell's intrinsic polarization), there is a rapid activation of PI3K at the leading edge, leading to production of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, PH domain localization and movement up the chemoattractant concentration gradient. (c) When the direction of the chemoattractant gradient is changed by placing a micropipette containing a chemoattractant near the side of a cell (producing a strong chemoattractant gradient), there is a rapid loss of the localized concentration of PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2 at the old leading edge as indicated by a loss of PH domain localization and the formation of a new PH-domain-enriched area on the membrane closest to the micropipette. There is a slower loss of F-actin from the old leading edge and myosin II from the old posterior. A new leading edge containing new F-actin forms at the position of the PH domain localization. A new posterior enriched in myosin II forms subsequently (d). The cell now moves in the direction of the new chemoattractant gradient. This model is based on analyses of chemotaxing *Dictyostelium* cells. Abbreviations: F-actin, filamentous actin; PH, pleckstrin homology; PI3K, phosphatidylinoside 3-kinase; PtdIns(3,4)P_2, phosphatidylinositol (3,4) bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5) trisphosphate.

that is correlated with an increase in actin polymerization occurring seconds after chemotactic stimulation at the leading edge of the cell[2,6]. Actin filaments at the leading edge are linked into a network by Y-shaped branches, in which the pointed end of each filament is attached to the side of another filament and the rapidly growing barbed end faces forward[1]. Forward extension of cells is driven by the protrusion of two F-actin-rich structures, lamellipodia and filopodia. Although the pathways are complex, members of the Rho family of small G proteins are key regulators of the actin and myosin II cytoskeleton[13] in all systems that have been examined. Rac1 is essential for the general reorganization of the actin cytoskeleton at the leading edge during lamellipod extension and cell migration in leukocytes and *Dictyostelium* cells, whereas Cdc42 appears to control the directionality of movement in mammalian cells, possibly by localizing F-actin assembly[14,15]. Because of space restrictions, the complex roles of the Rac-family GTPases in controlling this process will not be discussed in detail.

The Wiskott–Aldrich syndrome protein (WASP) and related proteins have emerged as key downstream components converging on multiple signaling pathways to F-actin polymerization. Recent studies show that the C-terminal acidic motif of WASP and its relatives N-WASP and Scar, interact with the Arp2/3 complex and activate the ability of the proteins to nucleate actin filaments[16]. The actin-monomer-binding V domain and the Arp2/3-binding A domain from the C-terminus of N-WASP and Scar are required for Arp2/3 complex formation, which initiates the barbed-end growth of actin filaments by enhancing nucleation[17]. The Arp2/3 complex enhances actin nucleation and causes branching and crosslinking of actin filaments *in vitro*; *in vivo* it is thought to initiate the formation of new actin filaments from the sides of older filaments and the formation of lamellipodia[18]. The catalytic activity of many PAKs (p21-activated kinases) is regulated by the binding of $Rac1^{GTP}$ or $Cdc42^{GTP}$ to a highly conserved CRIB (Cdc42/Rac interactive binding) domain. PAK1 colocalizes with F-actin in membrane ruffles and to lamellipodia of motile cells, where it helps mediate actin assembly[4,19,20]. PAK can phosphorylate LIM kinase and stimulate its kinase activity[21]. Activated LIM kinase then phosphorylates and inactivates cofillin, inhibiting cofillin's actin-depolymerizing and severing activity. PAK1 also functions as an adaptor by binding Rac1/Cdc42 exchange factors (GEFs) such as PIX or Trio, and adaptor proteins such as Nck or Dock, which can link PAK1 to phosphotyrosine residues on receptors[22–24]. PAK1 might thus play an important role in bringing the necessary Rac/Cdc42 GEFs to the site where F-actin is to be assembled. In addition, myosin I, which controls actin polymerization through its interactions with WASP and Arp2/3, localizes to the leading edge and is regulated by a PAK-family kinase in some cell types, including *Dictyostelium* and *Acanthamoeba*[25,26,28].

Myosin II

To maintain persistent motility, cells must release adhesive contacts with the substratum at their posterior and retract the rear body or uropod, a process that depends on the motor activity of myosin II (Ref. 29). In addition, assembled myosin II is necessary for cortical tension along the lateral sides of cells, which is important in the prevention of lateral pseudopod formation[8,29]. *Dictyostelium* myosin II is the best-studied conventional non-muscle myosin and has a molecular structure very similar to that of mammalian myosin II (Ref. 30). The C-terminal coiled-coil regions of two myosin II monomers associate to form a bipolar dimer. Assembly of these dimers into myosin filaments is regulated, in part, by phosphorylation of three threonine residues in the tail region by myosin heavy chain kinase, with phosphorylation resulting in filament disassembly and dephosphorylation in assembly[31]. In chemotaxing

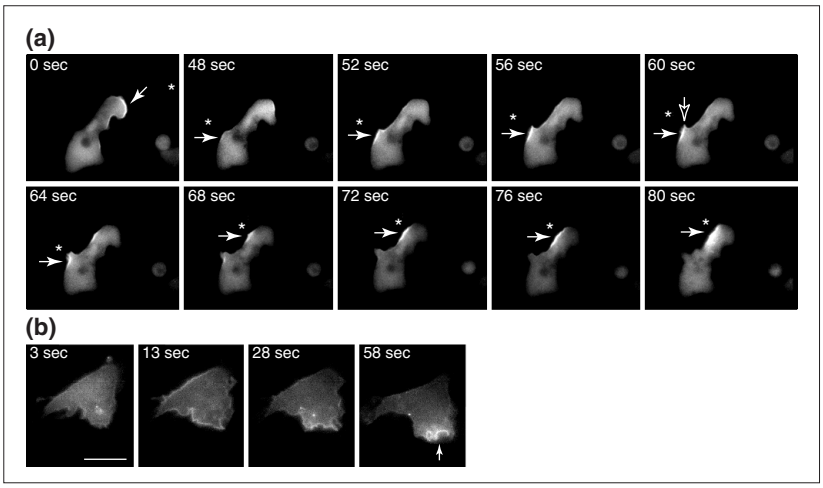

Fig. 2. Translocation of PH-domain-containing proteins to the leading edge during migration. (a) Translocation of the PI3K effector PhdA–GFP to the leading edge of migrating cells. A cell expressing PhdA fused to GFP was stimulated locally with a micropipette filled with cAMP. (The asterisks indicate the position of the tip of the micropipette containing the cAMP solution.) A distinct localization of PhdA-GFP at the position of the cell closest to the cAMP source is seen (arrow). As the position of the micropipette changes, the PhdA–GFP rapidly translocates to the area where local activation occurs. The formation of a new pseudopod follows the translocation of PhdA–GFP. This pseudopod rapidly retracts on removal of the micropipette (see Ref. 53 for details.) The translocation of this protein is indistinguishable from the translocation of the *Dictyostelium* Akt/PKB-PH-domain GFP fusion[58]. (b) Localization of mammalian Akt/PKB PH domain fusion (PHAKT–GFP) to the leading edge of neutrophil-differentiated HL60 cells. Cells were stimulated with the chemoattractant fMLP at time zero and then images were taken at the times indicated. The arrow points to the leading edge. Scale bar = 10 μm. Reproduced, with permission, from Ref. 63, and S. Funamoto and R.A. Firtel, unpublished. Abbreviations: Akt/PKB, protein kinase B; fMLP, formyl-Met-Leu-Phe; GFP, green fluorescent protein; PH, pleckstrin homology; PhdA, PH domain-containing protein; PI3K, phosphatidylinoside 3-kinase.

Dictyostelium cells, myosin II aligns along the posterior lateral edges, forming a C-shaped domain[10,29]. Contraction of the myosin cap facilitates detachment of the posterior of the cell from the substratum, allowing the posterior to retract towards the leading edge. In myosin II (*myoII*) null cells, the retraction of the posterior of the cell during chemotaxis is defective and there is a loss of the normal lateral cortical tension, leading to the formation of lateral pseudopodia and inefficient chemotaxis[29]. In fibroblasts and neutrophils, myosin II is also localized in the uropod and involved in uropod retraction[32–35].

The PAK kinase family members also regulate the assembly and motor activity of myosin II. *Dictyostelium* PAKa, a putative PAK, is a key component of chemoattractant-mediated myosin II assembly and of myosin assembly in the contractile ring during cytokinesis in *Dictyostelium*[36]. PAKa is thought to negatively regulate myosin II heavy chain kinase A (MHCKA), thus preventing myosin II phosphorylation, which leads to myosin II assembly[37]. Cells null for the gene encoding PAKa (*paka*) lack chemoattractant-mediated myosin II assembly, no longer preferentially localize myosin II to the cell's posterior, and have phenotypes similar to those of *myoII* null cells. The role of PAKs in regulating myosin II assembly and contractility is not restricted to *Dictyostelium*. In PC12 cells, the myosin II heavy chain is phosphorylated in a yet-to-be-defined, Rac1-dependent pathway that involves PAK1 and takes part in controlling the contractility of these cells[35].

In endothelial cells, PAK1 is involved in coordinating the formation of new adhesions at the leading edge (including the disassembly of focal adhesions and stress fibers) with contraction and detachment at the trailing edge, probably via regulating the contractability of myosin II filaments[38]. PAK1 also phosphorylates the myosin regulatory light chain kinase (MLCK), decreasing the phosphorylation and activation of the regulatory light chain of myosin and thus inhibiting myosin II-based contractility[35,39]. In so doing, PAK1 acts antagonistically towards the RhoA pathway that promotes myosin II assembly and contractility through Rho kinase and MLCK (Ref. 35).

Role of lipid signaling: phosphoinositide 3-kinase (PI3K)

A central question is how cells recognize a spatial gradient and thus assemble F-actin at the leading edge and myosin II at the posterior, resulting in cell polarization. In leukocytes and *Dictyostelium* cells, chemotaxis is mediated by diverse G-protein-coupled, seven-transmembrane-containing chemotaxis/chemokine receptors[6,7,40]. Mammalian leukocytes and other cell types respond to several growth factors and cytokines such as granulocyte/macrophage colony stimulating factor (GM-CSF) and platelet-derived growth factor (PDGF) via tyrosine kinase receptors, migrating up a chemoattractant gradient[41–45]. Growing evidence suggests that cells locally activate and amplify distinct sets of signaling pathways in the anterior of the cell, creating an asymmetrical internal signal at the leading edge and establishing cell polarity that results in directional movement (Fig. 3). This localized, asymmetrical activation is not a consequence of a preferential localization of the chemoattractant receptors at the leading edge as studies demonstrate that green fluorescent protein (GFP)-tagged, G-protein-coupled chemoattractant receptors (*Dictyostelium* cAMP or mammalian C5a receptors) are uniformly distributed along the plasma membrane[46,47]. However, in highly polarized *Dictyostelium* cells, there is a shallow anterior–posterior gradient of membrane-associated Gβγ subunits of heterotrimeric G proteins, raising the possibility that differential distribution of Gβγ subunits that directly couple to chemoattractant receptors could help determine the chemotactic sensitivity of polarized cells[48].

Recent studies in *Dictyostelium* cells, leukocytes and fibroblasts indicate that PI3K plays a central role in the amplification of internal signaling asymmetry and thus helps establish cell polarity and define the leading edge of the cell. The product of PI3K, phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3], is produced by two classes of PI3K, class I_A (PI3Kα, PI3Kβ and PI3Kδ) and class I_B (PI3Kγ) (Fig. 4). Class I_A PI3Ks, consisting of a regulatory and a catalytic subunit, are activated through tyrosine kinase receptors and cytosolic tyrosine kinases[49]. PI3Kγ also consists of a catalytic

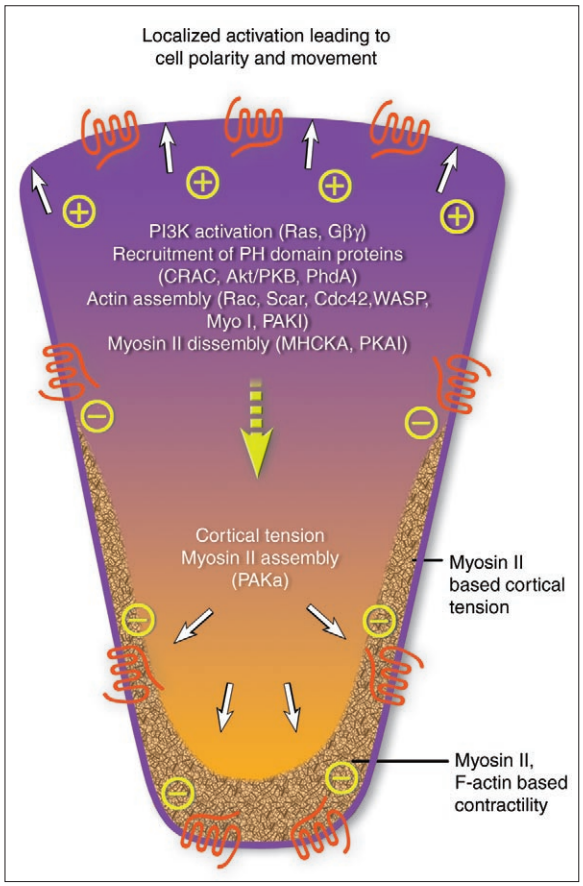

Fig. 3. The cartoon and model for the formation of an activation domain at the leading edge in chemotaxing cells via G-protein-coupled receptors (GPCRs). GPCRs are uniformly distributed around the cells. Localized activation of PI3K leads the accumulation of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 (not shown), forming a gradient of lipid products that is steeper than the chemoattractant gradient. PI3K is activated by Ras and/or Gβγ subunits depending on the PI3K isoform. Amplification of this initial temporal signal by positive feedback loops at the front of cells (indicated by '+'), in combination with inhibition of pathways along the lateral sides of the cells (indicated by '–'), could lead to the establishment of robust signaling networks preferentially at the presumptive leading edge. The preferential localization of multiple signaling proteins, including PH-domain-containing proteins, at the leading edge would produce an activation domain. F-actin assembly is activated by the activation of small GTPases (Rac1/Cdc42) and their downstream effectors, including WASP, myosin I kinase and PAK1 in mammalian cells. In *Dictyostelium*, myosin II heavy chain kinase A (MHCKA) gets recruited to the activation domain by an unknown mechanism and phosphorylates myosin II tails, resulting in myosin II disassembly at the leading edge. This function might be controlled by a PAK1-mediated activity in mammalian cells. The ability of cells to translate the shallow chemoattractant gradient to a steep lipid gradient is consistent with a model for a global inhibition mechanism to prevent the formation of an activation domain in other regions of the cell. Building cortical tension by assembling myosin II in the trailing cell body helps prevent the formation of lateral pseudopodia. In *Dictyostelium*, activation and localization of PAKa in the trailing edge by Akt/PKB leads to the assembly of myosin II in the trailing edge. Abbreviations: Akt/PKB, protein kinase B; F-actin, filamentous actin; PAK, p21-activated kinase; PH, pleckstrin homology; PI3K, phosphatidylinoside 3-kinase; PtdIns(3,4)P_2, phosphatidylinositol (3,4) bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5) trisphosphate; WASP, Wiskott–Aldrich syndrome protein.

subunit and a separate adaptor protein, and is activated by heterotrimeric G protein βγ subunits[49]. Earlier studies using the PI3K inhibitors Wortmannin and LY294002 demonstrated that PI3Ks play a pivotal role in chemotaxis (Table 1). The first indication that specific PI3Ks regulate mammalian cell migration and the cytoskeleton came from studies using antibodies against specific class I_A p110 PI3K isoforms. Microinjection of antibodies against the δ or β PI3K but not against p110α inhibited CSF-1-mediated actin reorganization and cell migration[50]. Furthermore, introduction of inhibitory antibodies to PI3Kγ, but not antibodies to PI3Kα, into permeabilized natural killer cells inhibited chemokine-induced chemotaxis[51]. Additional evidence supports the involvement of PI3K in PDGF-mediated chemotaxis[43].

In *Dictyostelium*, cells lacking the two class I_A-related PI3Ks, PI3K1 and PI3K2 (*pi3k1/2* null cells), or wild-type cells treated with LY294002, exhibit impaired F-actin organization and a lower increase in F-actin levels in response to chemoattractant stimulation and chemotaxis[52,53]. These cells cannot effectively polarize when placed in a chemoattractant gradient and lack chemoattractant-mediated myosin II assembly[12,53]. Studies of leukocytes from mice homozygous for a knockout of PI3Kγ reveal that PI3Kγ is involved in chemoattractant-mediated motility and cell migration (Table 1). In addition, neutrophils and macrophages purified from PI3Kγ-null mice display impaired motility and reduced chemoattractant-mediated migration. The results are consistent with a possible impairment of directionality[54–56]. Further studies on neutrophils lacking PI3Kγ suggest that these cells are defective in polarizing in a chemoattractant gradient and exhibit impaired directionality, supporting an involvement of PI3K in controlling directional movement, a key component in the persistence or effectiveness with which cells move up a chemoattractant gradient[57]. In fibroblasts migrating towards a shallow gradient of PDGF, a strong spatial correlation between the polarized production of 3′ phosphoinositides and rapid membrane spreading has been demonstrated[43], implying that 3′ phosphoinositide lipids are direct mediators of polarized migration in receptor tyrosine kinase pathways. These studies are consistent with the model in which PtdIns(3,4,5)P_3 production might be a necessary step to produce and amplify the internal asymmetries in cell polarity necessary for chemotaxis. Figure 5 outlines the pathways in leukocytes and *Dictyostelium* downstream from G-protein-coupled receptors leading to chemotaxis, with a focus on a central role for PI3K.

In both *Dictyostelium* cells and leukocytes, chemoattractant stimulation leads to a rapid, transient, PI3K-dependent activation of Akt/PKB (Refs 54,58), an essential effector of PI3K in promoting cell survival[49,59]. Genetic studies in *Dictyostelium* indicate that chemoattractant-mediated Akt/PKB activation is required for cell polarity and proper chemotaxis. Akt/PKB (*pkbA*) null cells cannot polarize properly when placed in a chemotactic gradient and the cells move slowly[58].

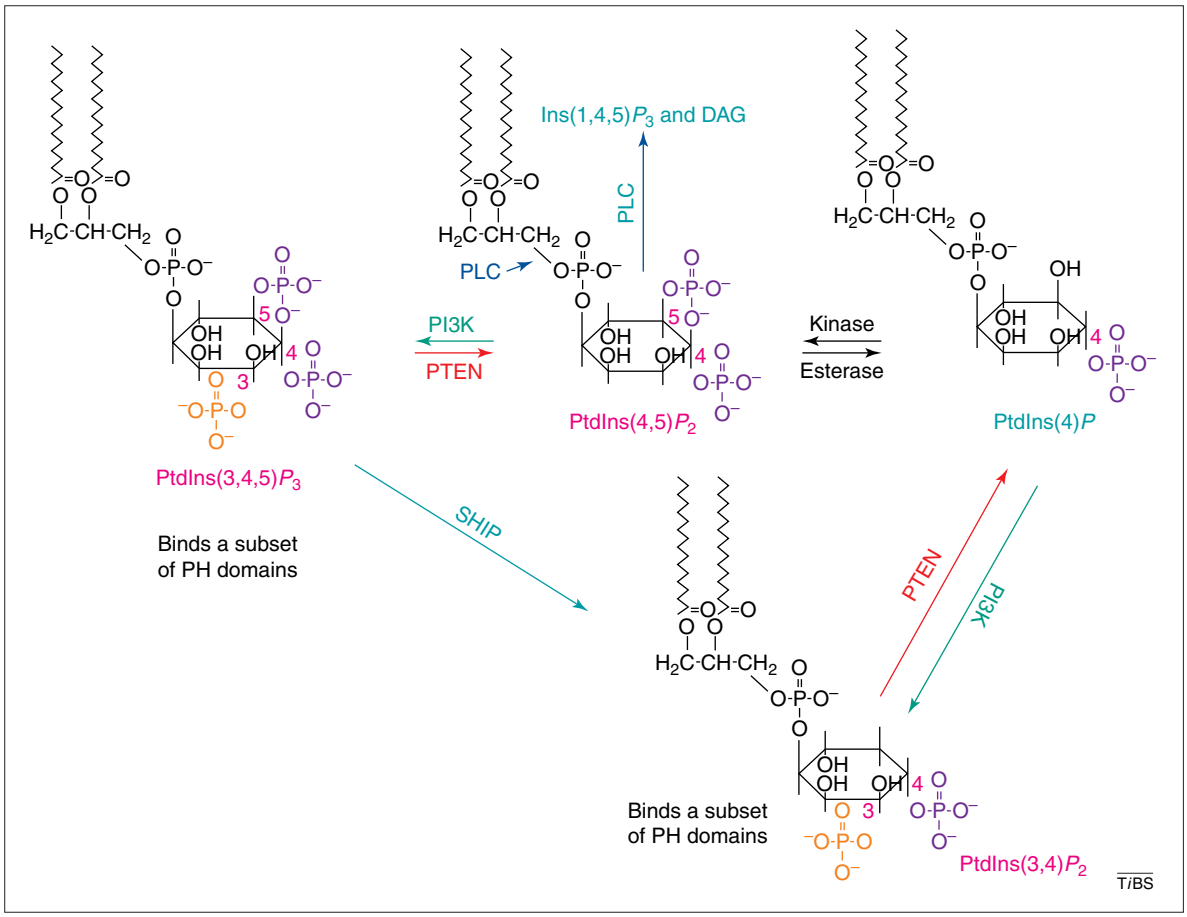

Fig. 4. PI3K pathway. The figure shows that the PI3K pathway is thought to play a role in controlling chemotaxis. The major substrate for PI3K, PtdIns(4,5)P_2, is also the substrate for PLC. Activation of PI3K results in the phosphorylation of PtdIns(4,5)P_2 on the 3′ position of the inositol ring, generating PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 can be dephosphorylated at the 5′ position by the phosphatidyl inositol 5′ phosphatase SHIP, generating PtdIns(3,4)P_2, which can also be produced by PI3K by phosphorylating the 3′ position of the inositol ring of PtdIns(4)*P* (Ref. 70). Both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 can function as binding sites for a variety of PH domains including those of Akt – a PI3K effector that is activated by chemoattractants. Differences in binding specificities of various PH domains can cause differential activation of effectors. For example, some PH domains but not others might preferentially bind to PtdIns(3,4,5)P_3 over PtdIns(3,4)P_2 (Ref. 60). SHIP is a point of regulation that could differentiate between two downstream PI3K-dependent pathways by distinguishing between different classes of PH domains. PI3K pathways are thought to be negatively regulated by 3′ phosphatases, including the tumor suppressor PTEN (Ref. 70). Loss of the 3′ phosphate would preclude further binding of the PH domains to the phosphoinositol lipids. Abbreviations: DAG, diacylglycerol; PH, pleckstrin homology; PI3K, phosphatidylinoside 3-kinase; PLC, phospholipase C; PtdIns(4)*P*, phosphatidylinositol (4) monophosphate; PtdIns(3,4)P_2, phosphatidylinositol (3,4) bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5) trisphosphate; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SHIP, SH2-containing inositol 5-phosphatase.

Insights into the possible mechanisms by which PI3K mediates chemotaxis derive from experiments using GFP fusions of several PH (pleckstrin homology) domain-containing proteins or isolated PH domains, a subclass of which bind to the PI3K products PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 (Refs 60,61). The PH-domain-containing proteins include *Dictyostelium* CRAC, *Dictyostelium* and mammalian Akt/PKB, and *Dictyostelium* PhdA (Refs 43,53,58,62,63; Fig. 2). *Dictyostelium* CRAC and PhdA are novel proteins with an N-terminal PH domain that shows weak homology to the C-terminal domain. CRAC is required for receptor activation of adenylyl cyclase, whereas PhdA is required for proper F-actin assembly in response to chemoattractant stimulation[53,62]. *Dictyostelium* and mammalian Akt/PKB PH domain fusions, CRAC and PhdA differentially translocate to the plasma membrane in response to global stimulation by a chemoattractant and to the leading edge in response to directional chemoattractant stimulation[43,53,58,62,63]. These localizations do not occur in *pi3k1/2* null cells nor in wild-type cells treated with LY294002 (Ref. 53). This suggested dependency on PI3K is supported by studies showing that mutant PH domains unable to bind the lipid products of PI3K do not translocate in response to chemoattractant stimulation[53,61]. Such a mutation in Akt/PKB prevents the activation of Akt/PKB in response to chemoattractants, presumably because this protein cannot be phosphorylated and activated by its upstream activator PDK1, another PH-domain-containing kinase[53,58,64]. These studies led to a model in which a localized activation of PI3K recruits PH-domain-containing proteins to specific regions of the plasma membrane enriched in the PI3K products PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, resulting in a localized activation of downstream effectors[6,7,40] (Fig. 3).

Table 1. Effects of PI3K inhibition on cell motility and chemotaxis[a]

Cell type	PI3K inhibitors	Effects on chemotaxis, cell migration and motility[b]	Chemoattractant
Dictyostelium	30 μM LY	50–60% inhibition	cAMP
Hepatic stellate cell	WM	Inhibition	IGF
	LY	Inhibition	
Megakaryocytic cell	10 μM LY	43% inhibition	SDF-1
Swiss 3T3 cell	100 nM–10 μM LY	No effect	PDGF
Vascular smooth muscle cell	1 nM–1 μM WM	No effect	PDGF
Vascular smooth muscle cell	200 nM WM	50% inhibition	uPA
	50 μM LY	80% inhibition	
Vascular smooth muscle cell	100 μM LY	85–89% inhibition	TSP-1, Fn and Vn
Rat osteoclast	100 or 500 nM WM	Inhibition	M-CSF
	100 μM LY	Inhibition	
Neutrophil	10–100 nM WM	Inhibition	C1q
	0.5–10.0 μM LY	Inhibition	
C2C12 myoblast	10 μM LY	No effect	bFGF, HGF and IGF-I
PC cell	50 nM WM	70% inhibition	EGF
RBL-2H3 cell	100 nM WM	Inhibition	LTB_4, fMLP and PAF
Peritoneal mast cell	50 μM LY	73% inhibition	NGF
Jurkat cell	100 nM WM	Inhibition	SDF-1
Normal T lymphocyte	100 nM WM	Inhibition	SDF-1
Neutrophil	WM	Inhibition	PDGF, TGFβ
CHO cell	100 nM WM	Inhibition	fMLP
Cell type	**Gene knockouts**	**Effects on chemotaxis, cell migration, motility**	**Chemoattractant**
Neutrophil	*pi3kγ*$^{-/-}$ mice	50% inhibition	fMLP
Macrophage	*pi3kγ*$^{-/-}$ mice	50–70% inhibition	SDF-1 and C5a
Dictyostelium	*pi3k1/2*$^{-/-}$	50–60% inhibition	cAMP, folate
Cell type	**PI3K neutralizing antibody**	**Effects on cell migration**	**Chemoattractant**
Macrophage	p110β/p110δ	30–50% inhibition	CSF-1

[a]Abbreviations: bFGF, basic fibroblast growth factor, C1q, first component of complement system; C5a, fifth component of complement system; CSF-1, colony-stimulating factor 1; EGF, epidermal growth factor; fMLP, formyl-Met-Leu-Phe; Fn, fibronectin; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; LTB_4, leukotriene B_4; LY, LY294002; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PI3K, phosphotidylinoside 3-kinase; *pi3k*, gene encoding PI3K; SDF-1, stromal-derived factor 1; TGFβ, transforming growth factor β; TSP-1, thrombospondin 1; uPA, urokinase-type plasminogen activator; Vn, vitronectin; WM, Wortmannin.

[b]See text for discussion on effect of inhibitors and mutations on chemotaxis and/or motility. References for this table could not be included because of reference limitations. The table is reproduced with full referencing at http://www-biology.ucsd.edu/~firtel/

The kinetics of these responses in *Dictyostelium* and mammalian cells are impressively rapid; the GFP–PH domain protein fusions exhibit a peak of localization at 5–6 seconds in response to global stimulation, consistent with the model hypothesizing that PH domain translocation could be one of the first steps in initiating pathways leading to cell movement and might be crucial for localizing components to the leading edge. The adaptation or turning off of this response is equally rapid in *Dictyostelium* and slightly slower in neutrophils, occurring within 12 seconds in *Dictyostelium*. This adaptation is thought to result from a loss of binding sites through hydrolysis of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 by the phosphatidyl inositol 3′-specific phosphatase PTEN or other phosphatases (Fig. 4). PTEN homologs, which have a substrate specificity and structure similar to those of mammalian PTEN, have been identified in *Dictyostelium* (R. Meili, J. Dixon, and RAF, unpublished). The rapid kinetics of PI3K-dependent PH domain membrane localization are consistent with membrane localization occurring before CRAC activates adenylyl cyclase and before Akt/PKB is activated[58,62]. The transient localization of PH-domain-containing proteins to the leading edge, but not to the lateral sides or posterior, of chemotaxing cells might represent one of the underlying mechanisms by which cells generally produce a pseudopod only in the direction of the chemoattractant source and the mechanism that confers the ability of cells to respond rapidly to a newly formed gradient[6,7,40]. Although global stimulation results in only a transient plasma membrane localization of the PH domains, cells chemotaxing up a chemoattractant gradient show a

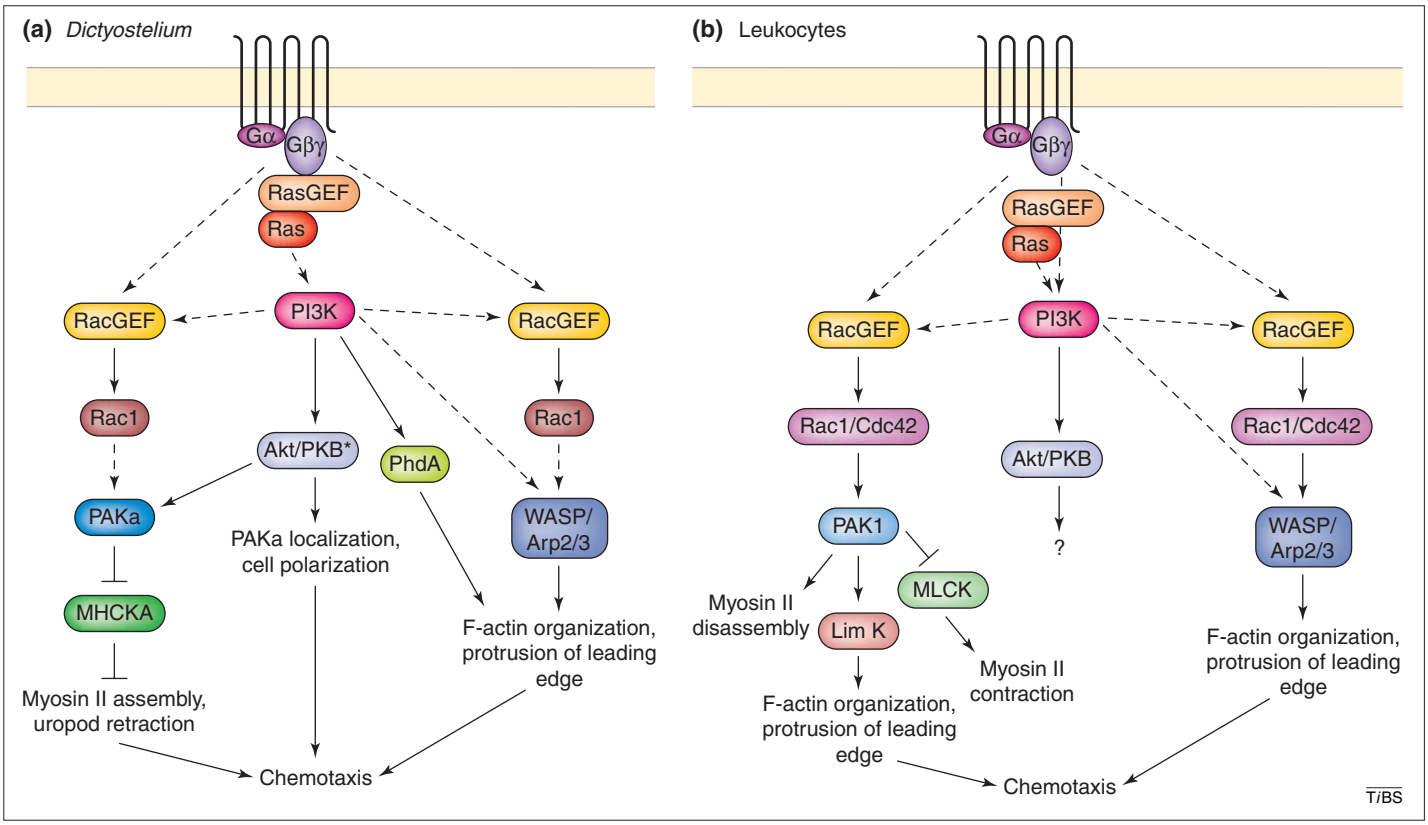

Fig. 5. Model of pathways regulating coordinate control of chemotaxis via G-protein-coupled receptors (GPCRs). The diagram shows the interconnection of signaling pathways that regulate chemotaxis by GPCRs in leukocytes and *Dictyostelium* cells. The pathways described focus on a central role of PI3K. As described in the text, PI3K might be involved in activating Rac GDP/GTP exchange, which can also be activated by PI3K-independent pathways. (a) In *Dictyostelium*, PI3K is necessary for membrane localization of PH-domain-containing proteins involved in chemotaxis including Akt/PKB and PhdA. PhdA is required for proper F-actin organization. Akt/PKB is directly involved in PAKa activation, which stimulates myosin assembly. PAKa localizes to the posterior of the cell and leads to myosin assembly through the inhibition of MHCKA in this region and, possibly, via a direct activation of myosin assembly. MHCKA localizes to the leading edge and controls myosin disassembly in this region of the cell by direct phosphorylation. PAKa has a $Rac1^{GTP}$-binding (CRIB) domain and might require $Rac1^{GTP}$-binding for activation. Other PAKs, such as myosin I heavy chain kinase, control myosin I function (not shown in the cartoon) in *Dictyostelium*[29]. PhdA and the PI3K effector Akt/PKB are thought to control different branches of the pathway leading to cell polarity and chemotaxis. From the phenotypes of the various null strains, we predict that Akt/PKB must have other effectors in addition to PAKa. Similarly, we expect that other PI3K effectors, in addition to PhdA, will regulate the actin cytoskeleton. In *Dictyostelium*, the mechanism of PI3K activation is not known, but PI3K1 and PI3K2 have essential Ras^{GTP}-binding domains. (b) PI3K is essential for chemotaxis in leukocytes, as it is in *Dictyostelium*. In leukocytes, G-protein activation leads to activation of Rac1 and Cdc42, which activate the WASP family proteins Scar and WASP and, through these, the Arp2/3 complex and PAK1. As discussed in the text, PI3K might regulate Rac1/Cdc42 activation via several mechanisms in these cells. In addition, Rac1/Cdc42 is activated by PI3K-independent pathways. PAK1 controls myosin II function by two independent pathways. PAK1 is involved directly or indirectly in myosin disassembly by direct phosphorylation of myosin II tails by a pathway that is not yet completely defined, as well as through the regulation of MLCK. This leads to myosin II disassembly in leukocytes, as it does in *Dictyostelium*. As PAK1 localizes to the leading edge, PAK1 might disassemble myosin II in this part of the cell in the same way that MHCKA disassembles myosin at the leading edge in *Dictyostelium* cells. Other pathways, including the Rho-dependent kinase, could also play a role in uropod contraction in mammalian cells (not shown in the cartoon). In addition, PAK1 directly phosphorylates and activates Lim kinase, which is involved in F-actin organization. Chemoattractants lead to activation of Akt/PKB. However, the possible roles of Akt/PKB in chemotaxis in leukocytes are not known. Dashed lines indicate possible pathways that have not been fully demonstrated in these cells. The lipid phosphatases that downregulate the pathway and/or change the lipid composition (which could alter the ability of PH domains to bind) are not shown. Abbreviations: Akt/PKB, protein kinase B; F-actin, filamentous actin; GEF, guanine nucleotide exchange factor; Lim K, Lim kinase; MHCKA, myosin heavy chain kinase A; MLCK, myosin light chain kinase; PAK, p21-activated kinase; PH, pleckstrin homology; PhdA, PH domain-containing protein A; PI3K, phosphatidylinoside 3-kinase; WASP, Wiskott–Aldrich syndrome protein.

more persistent leading edge localization of the PH domains. We expect that, as cells move up a chemoattractant gradient, the concentration of chemoattractant perceived by the leading edge increases and leads to an increase in the fraction of the receptors being occupied, resulting in an increase in the downstream signal, an increase in PI3K activity, and PH domain localization. This model suggests that the cell integrates both spatial and temporal signals to control directionality and sense gradients. A careful analysis of chemotaxing cells is consistent with a cyclical activation/adaptation of these pathways at the leading edge as the cells differentially activate signaling pathways at the leading edge and uropod to coordinate aspects of cell movement. Similarities of the pathways in *Dictyostelium* cells and leukocytes outlined in Fig. 5 suggest that the mechanism of creating internal signaling asymmetry in these distinct cell types is conserved[53,58,62,63].

Regulation of actin and myosin II by PI3K-dependent effector pathways

Regulation of F-actin assembly at the leading edge

PI3K is also important for regulating F-actin assembly during chemotaxis. *Dictyostelium pi3k1/2* null cells or wild-type cells treated with LY294002 exhibit a modest 30% decrease in chemoattractant-mediated F-actin assembly with kinetics that are

slower than in wild-type cells[53]. Probably the most important consequence of a defect in F-actin assembly is a significant reduction in the ability of these cells to respond to changes in the direction of the chemoattractant gradient and to re-establish polarity. When the position of a chemoattractant-emitting micropipette is changed, coronin-GFP, an evolutionarily conserved F-actin binding protein and a marker for F-actin assembly[65], is rapidly lost from the old leading edge and quickly accumulates as the new leading edge forms. However, in *pi3k1/2* null cells, this relocalization is significantly delayed[53]. In mammalian REF-52 cells, activity of the Rac exchange factor Vav is controlled by substrates and products of PI3K (Ref. 66). The PI3K substrate PtdIns(4,5)P_2 inhibits Vav activation by the tyrosine kinase Lck, whereas the PI3K product PtdIns(3,4,5)P_3 enhances Lck-mediated Vav phosphorylation and activation. Furthermore, the lipid products of PI3K bind to Rac1 and RhoA and facilitate GDP exchange[67], thus providing the means by which PI3K regulates F-actin assembly at the leading edge. Additionally, inhibition of PI3K abrogates PAK1 activation in fibroblasts and membrane ruffling during induced wound healing, consistent with a role for this factor in Rac1/Cdc42 activation[20]. Rac1-mediated formation of lamellipodia can also be activated by PI3K-independent pathways, suggesting that multiple signaling pathways downstream of receptors can mediate this process[68].

Genetic and biochemical studies indicate that the PH-domain-containing protein PhdA might have a role in the PI3K-mediated control of chemoattractant-induced F-actin assembly in *Dictyostelium*[53]. Cells null for *phdA* have the same F-actin assembly defects as *pi3k1/2* null cells[53]. Structure–function studies using mutations that separately abrogate the function of the PhdA PH C-terminal domains suggest that PhdA could act as an adaptor protein that mediates the localization of yet-to-be-identified signaling components required for efficient F-actin assembly at the leading edge.

Signaling pathways regulating uropod contraction

Recently, *Dictyostelium* PAKa was found to be a key effector of Akt/PKB, linking PI3K to the regulation of myosin II assembly and axial polarity. PAKa kinase activity is not stimulated in response to chemoattractants in *pkbA* or *pi3k1/2* null cells[12], which lack an axial polarity. PAKa is uniformly distributed around the cell's cortex, except in the location of new pseudopod formation[12]. *In vitro* and *in vivo* analyses indicate that PAKa is a substrate of Akt/PKB, and that Akt/PKB phosphorylation on Thr579 of PAKa is required for chemoattractant-mediated stimulation of PAKa kinase activity and the subcellular localization of PAKa in the rear cell body[12]. The dynamic relocalization of PAKa from the detergent-soluble to the detergent-insoluble cytoskeletal fraction in response to chemoattractant stimulation is not seen with a Thr579Ala PAKa mutant, indicating that Akt/PKB is vital for regulating changes in the cytoskeleton. The regulation of PAKa via direct phosphorylation by Akt/PKB suggests a direct regulation of signaling events and cytoskeletal reorganizations (PAKa activation and myosin II assembly) at the cell's posterior by events (Akt/PKB activation) initiated at the leading edge. The chemotaxis phenotypes of cells null for *akt/pkb* are more severe than those of *paka* null cells, suggesting that Akt/PKB has substrates in addition to PAKa that are necessary for proper chemotaxis[12,53]. As mammalian Akt/PKB is activated by chemoattractants[69], we expect that it is involved in chemotaxis of leukocytes, although downstream effectors in this pathway are not known for these cells.

How is cell polarity first established?

Multiple scenarios of how polarity is first established are consistent with available data. We suggest that when randomly moving cells first perceive a chemoattractant gradient, there is an initial activation of PI3K (and possibly other pathways) at the edge of the cell, where the chemoattractant first activates the receptors. Amplification of this initial temporal signal by feedback loops[40], in combination with inhibition of pathways along the lateral sides of the cells, could lead to the establishment of robust signaling networks at the presumptive leading edge, including the localization of PH-domain-containing proteins. These proteins and other signaling molecules could be components of an autoregulatory loop that differentially amplifies the signal at the leading edge[40]. Inhibitory pathways might preferentially act on the lateral sides of the cell to block the activation of PI3K and other signaling pathways. One such negative regulatory pathway could include PTEN, which might be constitutively active and function in unstimulated cells to maintain low background levels of PtdIns(3,4,5)P_3. Some components, such as PI3K, might be limiting, and their localization at the leading edge might preclude further stimulation of pathways away from the leading edge. We suggest that a combination of these mechanisms function in concert to produce a polarized cell in response to a chemoattractant gradient.

If this model is correct, inhibition of PI3K by LY294002 could inhibit cell polarization and disrupt established cell polarity. This appears to be the case in *Dictyostelium* as the addition of LY294002 leads to a rapid loss of F-actin and coronin-GFP from the leading edge and a redistribution of the proteins throughout the cell[12]. Similar studies indicate a redistribution of GFP–PAKa and myosin II from the posterior of the cell to a uniform cortical or cytosolic localization, respectively. The redistribution of PAKa in response to the inhibition of PI3K is very similar to that observed when cell polarity is disrupted by treating cells globally with a chemoattractant[12,36]. This finding suggests that, at least in *Dictyostelium* in which these

Acknowledgements
We thank O. Weiner, G. Servant and H. Bourne (UCSF) for generously supplying pictures of Akt-PH domain localization to the leading edge in HL-60 cells and B. Errede (UNC) for the bases of the PI3K pathway figure. We thank members of the Firtel lab for helpful suggestions and Jennifer Roth for critically reading the manuscript. C.Y.C. was funded, in part, by a grant from The Leukemia and Lymphoma Society (#3965-01). This work was supported by USPHS research grants to R.A.F. We apologize for the omission of some references because of space restrictions.

pathways are best understood, PI3K controls axial polarity and cytoskeletal reorganization during chemotaxis by controlling aspects of myosin II assembly and F-actin polymerization. As PI3K is also required for chemotaxis in leukocytes, we expect that the results from *Dictyostelium* can be generalized to many other cell types. Although evidence indicates that PI3K is an important component for establishing cell polarity and chemotactic movement, there appear to be PI3K-independent pathways that regulate directional cell movement. *Dictyostelium pi3k1/2* null cells or cells treated with LY294002 move directionally towards a chemoattractant source, albeit more slowly and less efficiently than wild-type cells[52,53]. Results with mammalian cells are consistent with this observation[40,43,50,57].

In conclusion, the results from leukocytes and *Dictyostelium* suggest a highly conserved mechanism that mediates both the establishment of cell polarity and the cytoskeletal responses required for chemotaxis. An intrinsic cell polarity potentially aids a cell's responsiveness to chemoattractants and thus could enhance a cell's ability to respond to weak gradients; by contrast, cell polarity can also be remodeled by strong chemoattractant signals. Although some signaling components are polarized in these cells in the absence of directional signals, others such as PH-domain-containing proteins are not. Which of these signaling components are required for random amoeboid movement and which are required only for directional movement are not fully understood. Moreover, we do not know how conserved the details of the signaling pathways controlling cell polarity and directional cell movement are between *Dictyostelium* cells and leukocytes, and whether non-ameboid vertebrate cells with directional cell movement use the same pathways as leukocytes. However, the evidence indicates that PI3K plays a central role in the establishment and maintenance of intrinsic cell polarity and chemotaxis in both *Dictyostelium* cells and leukocytes.

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Dynamic protein interactions in the bacteriophage T4 replisome

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The bacteriophage T4 DNA replisome is a complex dynamic system employing a variety of proteins to orchestrate the synthesis of DNA on both the leading and lagging strands. Assembly of the protein complexes responsible for DNA synthesis and priming requires the coordination of transient biomolecular interactions. This interplay of proteins has been dissected through the use of small molecules including fluorescent probes and crosslinkers, enabling the development of a complex dynamic structural and kinetic model for DNA polymerase holoenzyme assembly and primosome formation.

DNA replication is a process requiring the interactions of multiple proteins to form a functional machine, with many of these proteins possessing structures and functions conserved throughout evolution. One of the more elementary replication systems is that of bacteriophage T4, which involves eight proteins in the formation and propagation of the replication fork (reviewed in Refs 1,2). The core of this replication complex is the DNA polymerase (gp43), which forms the holoenzyme in conjunction with interacting accessory proteins. Gp43 catalyzes the incorporation of nucleotides in the 5′→3′ direction, and maintains replication fidelity through a 3′-exonuclease activity[3]. The accessory proteins are the sliding clamp [gp45; a ring-shaped homotrimeric processivity factor with an internal diameter large enough to encircle DNA (Refs 4,5)] and the clamp loader (gp44/62; a 4:1 complex of the gp44 and gp62 proteins, respectively). Gp45 is responsible for the processivity of the holoenzyme[6], and gp44/62 acts catalytically to load the clamp onto DNA (Refs 7,8) and then chaperone the polymerase to the gp45–DNA complex in the formation of the holoenzyme[9,10].

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