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CHEMOTAXIS: SIGNALLING THE WAY FORWARD

Peter J. M. Van Haastert and Peter N. Devreotes‡*

During random locomotion, human neutrophils and *Dictyostelium discoideum* amoebae repeatedly extend and retract cytoplasmic processes. During directed cell migration — chemotaxis — these pseudopodia form predominantly at the leading edge in response to the local accumulation of certain signalling molecules. Concurrent changes in actin and myosin enable the cell to move towards the stimulus. Recent studies are beginning to identify an intricate network of signalling molecules that mediate these processes, and how these molecules become localized in the cell is now becoming clear.

NEUTROPHIL
A phagocytic cell of the myeloid lineage that has an important role in the inflammatory response. It undergoes chemotaxis towards sites of infection or wounding.

FLAGELLUM
The cell-motility apparatus in swimming bacteria.

LEADING EDGE
The thin margin of a lamellipodium that spans the area of the cell from the plasma membrane to about 1 µm back into the lamellipodium.

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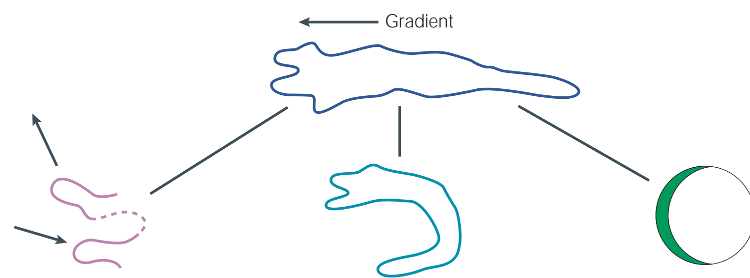
Far from being static, some types of cell are highly motile. They can sense the presence of extracellular signals and guide their movement in the direction of the concentration gradient of these signals. This process, called chemotaxis, has a role in diverse functions such as the sourcing of nutrients by prokaryotes, the formation of multicellular structures in protozoa, the tracking of bacterial infections by NEUTROPHILS, and the organization of the embryo in metazoa[1–4]. To orientate its movements, theoretically, a cell might use the spatial aspect of the chemoattractant gradient or the temporal signals that are generated when it moves in the static gradient. Prokaryotes can only use the temporal component — they are too small (1–2 µm) to process spatial information. They undergo a 'random walk' with movement steps in all directions that are interrupted by tumbles. When they encounter an increase in chemoattractant concentration — that is, when they move up the gradient — the frequency of tumbling decreases and movement in one direction is prolonged[5,6]. Eukaryotic chemotactic cells are larger (10–20 µm in diameter), which allows them to process both spatial and temporal information. They can measure the difference in chemoattractant concentration between the ends of the cell, and then move up this gradient[7]. Eukaryotic cells can respond to differences in chemoattractant concentration that are as small as 2–10% between the front and the back of the cell, whether the average level of receptor occupancy is small or large. The ability to sense and respond to shallow gradients of extracellular signals is remarkably similar in simple amoebae such as *Dictyostelium discoideum* and mammalian neutrophils[8–11]. Although both organisms effectively sense and respond to a spatial gradient, for optimal chemotactic sensitivity they combine spatial and temporal information. In the natural habitat, chemotactic gradients fluctuate, either because the bacterial source moves, or because cells signal to each other using waves of chemoattractant. Cells are usually more sensitive to a spatial gradient when the mean concentration is constant or increases with time. Cells therefore respond better to 'new' gradients than to 'dissipating' gradients.

Chemotaxis is essential for survival — if cells fail to reach their proper destinations they die, or the organism dies — so it is expected that the mechanisms for processing chemotactic signals have been optimized during evolution. In prokaryotes, transduction of chemotactic signals is mediated by a single pathway that involves just six proteins, and the response that is elicited is the clockwise or counterclockwise rotation of the FLAGELLUM[6,12]. In eukaryotes, the outcome of transducing information regarding the spatial gradient is the extension of the cell membrane — in the form of a pseudopod — at the LEADING EDGE. At the same time, pseudopod formation is suppressed elsewhere, and the UROPOD at the rear of the cell retracts[8,9,13–15]. As the time spent by cells moving in a spatial gradient of chemoattractants increases, their polarity increases, the leading edge becomes more



Self-organizing pseudopodia
- Periodic
- Uniform chemoattractant
- G-protein independent
- PtdIns(3,4,5)P_3 enhanced
- Actin/myosin required

Polarized sensitivity
- Persistent
- Uniform chemoattractant
- G-protein dependent
- PtdIns(3,4,5)P_3 enhanced
- Actin/myosin required

Directional sensing
- Persistent
- Gradient of chemoattractant
- G-protein dependent
- PtdIns(3,4,5)P_3 independent
- Actin/myosin independent

Figure 1 | **Chemotaxis involves three interrelated phenomena.** Chemotaxis is comprised of three interrelated phenomena: the formation of periodic self-organizing pseudopodia, polarization and directional sensing. The pseudopodia have a relatively well-defined size, shape, and duration and are extended at roughly 60-second intervals at sites that are biased by the gradient of chemoattractant. Polarization is evident in an elongate cell shape as well as in the tendency of a cell to turn towards a new gradient because its anterior is more sensitive than its posterior. Directional sensing is observed even in cells that have been immobilized by treatment with the inhibitor of actin-filament polymerization latrunculin A. This is indicated by the accumulation of pleckstrin-homology (PH)-domain-containing proteins on the membrane towards the side of the cell that is experiencing the higher end of the gradient. These phenomena have overlapping, but distinct, properties, as listed here and described in the text. PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate.

sensitive to chemoattractant, and the frequency of formation of lateral pseudopods is suppressed[16,17]. To accomplish these tasks, a complex network of interactions between different signalling components and cytoskeletal and regulatory elements has evolved, which, together, provide the compass to orientate cells effectively in chemotactic gradients[14]. These networks are remarkably similar with respect to gradient sensing, and regulation of myosin and the actin cytoskeleton in neutrophils and in *D. discoideum* — which diverged about 800 million years ago[18]. In this review, we discuss how pseudopod formation is biased in chemotactic gradients, how signalling molecules modulate actin and myosin, and how actin and myosin filaments direct cell locomotion.

Three phenomena and a forward movement

Chemotaxis can be broken down into three interrelated phenomena (FIG. 1). These include the periodic extension of stereotypical pseudopodia, the establishment of polarity, which enhances the relative sensitivity of the cell at its anterior compared to its posterior end[15], and directional sensing, which constantly links the response to the external gradient.

Pseudopod formation. Unstimulated neutrophils lack polarity and fail to produce protrusions in the absence of chemoattractants. But growing, undifferentiated *D. discoideum* cells, even those that lack cyclic AMP receptors or G-protein signalling — components that detect and transduce the presence of the chemoattractant — extend pseudopodia and are motile[19–22]. Starved, differentiated *D. discoideum* cells[7] are more like neutrophils in that chemoattractant induces polarity and increased motility[23]. In the absence of a gradient of chemoattractant, unpolarized *D. discoideum* cells extend pseudopodia in more or less random directions (FIG. 1). Pseudopodia are periodically induced at points on the cell periphery, as if driven by an internal oscillator, and then follow a specific sequence of events[19,24]. First, actin filaments are formed, and a dynamic pseudopod, which lasts for about 60 seconds[25,26], is extended. Then, the pseudopod is either retracted or it attaches to the underlying substratum and pulls the remainder of the cell body towards it. Without directional cues, *D. discoideum* cells can have one or two pseudopods, as new extensions are formed about every 30 to 60 seconds in a nearly random direction. Although chemoattractants increase the probability that a pseudopod is formed at the leading edge, and suppress the formation of lateral pseudopodia, they have lesser effects on the properties of the pseudopod, such as boundary, size, frequency and lifetime. The relative uniformity of pseudopodia supports the notion that they are self-organizing structures[26–32]. The main advantage of a switchable self-organizing element in motility and chemotaxis is the ease with which it can be locally triggered, which thereby simplifies the problems of integrating cues from all over the cell[26].

Polarization. When chemotactic cells become biochemically and functionally polarized[33], they usually have only one pseudopod at a time, which is formed close to the previous one and in the same direction. The front of the cell contains actin filaments, which induce the formation of new pseudopodia, whereas at the back of the cell the retracting uropod is enriched in myosin filaments. As noted above, neutrophils and starved, differentiated *D. discoideum* cells[16,23,34] can have strong polarity. In a uniform concentration of chemoattractant both cell types are elongated and move in a persistent, but random, direction. Changing the position of the source of the chemoattractant usually leads to the formation of additional pseudopodia at the old front of the cell, which the cell then uses to make a U-turn towards the new chemoattractant source[7]. In *D. discoideum*, polarity is also gradually strengthened during prolonged chemotaxis[33]. Polarization might improve chemotaxis by enhancing the persistence of movement towards the source, but it would also make the cells less able to respond to rapid changes in gradient direction.

Directional sensing. Directional sensing refers to the bias that is seen in the sites of pseudopod formation in the presence of a chemotactic gradient. In such a gradient, more pseudopodia are produced at the side of the cell that faces the source of the chemoattractant — that is, the side that is exposed to the highest chemoattractant concentration. Retraction of a new pseudopod is rarely observed; instead, the back of the cell contracts. Even when movement and polarity are absent, eukaryotic cells can still sense and amplify the spatial gradient. This was evident in early experiments that

UROPOD
A slender appendage that is formed at the trailing, rear edge of fast-migrating cells such as amoebae, neutrophils or lymphocytes.

were conducted in neutrophils, which are rounded in the absence of chemoattractant[7], or electropermeabilized *D. discoideum* cells in Ca^{2+}-free buffers[35]. When a chemotactic gradient was applied, both cell types extended the first pseudopod in the 'correct' direction towards the higher concentration. Furthermore, cells treated with inhibitors of actin polymerization such as latrunculin A become rounded, stop moving and lose polarity, yet continue to sense the direction of chemotactic gradients nearly as well as untreated cells. As described in further detail below, this is evident from the accumulation of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3), and the enzymes that regulate it, at distinct places on the membrane relative to the gradient[36] (FIG. 2).

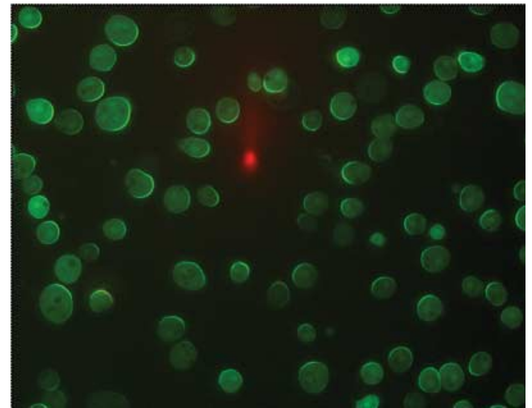


Figure 2 | **Directional sensing in immobilized cells.** The image shows a field of wild-type *Dictyostelium discoideum* cells that are expressing the pleckstrin-homology (PH) domain of cytosolic regulator of adenylyl cyclase (Crac) fused to green fluorescent protein (GFP). The cells are rounded and immobilized owing to treatment with the actin-polymerization inhibitor, latrunculin A. A micropipette that releases Cy3-labelled cyclic AMP (red) was positioned among the cells to provide a chemoattractant gradient. The green crescents that are seen to align on the edges of the cells that are facing the pipette are representative of steady-state levels of phosphatidylinositol-3,4,5-trisphosphate in the membrane. Note that cells that are closer or further away from the pipette, and that are therefore experiencing gradients of higher or lower midpoints, respond equally well. Image courtesy of C. Janetopoulos, Johns Hopkins University School of Medicine, Baltimore, USA.

Signalling events that set direction

Receptors and heterotrimeric G proteins. *D. discoideum* undergoes directed cell migration in response to nutrients, platelet-activating factor (PAF) and cAMP, whereas neutrophils respond to formylated Met-Leu-Phe (fMLP), the complement factor PAF, and chemokines that are released from sites of inflammation (TABLE 1). These chemoattractants are detected by SEVEN-TRANSMEMBRANE-SPANNING 'SERPENTINE' RECEPTORS, which couple to HETEROTRIMERIC G PROTEINS and also signal directly to downstream events[37–41]. In *D. discoideum*, four cAMP receptors have been identified. They can all support chemotaxis, but they have different affinities for cAMP[42,43]. Lower affinity is conferred when a few residues in the second extracellular loop are substituted with charged amino acids. The affinity of the receptors for the ligand and certain aspects of the behaviour of the receptor itself — such as trafficking within the cell — are also regulated by robust chemoattractant-induced phosphorylation on the divergent cytoplasmic tails. These phosphorylations, however, have little direct influence on chemotaxis[44]. Furthermore, the chemokine receptors of neutrophils undergo chemoattractant-induced phosphorylation, but this modification also does not seem to be important in chemotaxis[45].

Chemoattractant receptors in both *D. discoideum* and neutrophils signal through similar G proteins. *D. discoideum* contains one G-protein β and one γ subunit, and this βγ-complex is absolutely essential for chemotaxis. Although there are at least 11 α subunits, which are about 45% identical to each other and to mammalian $G\alpha_i$[21,46], genetic studies and fluorescence resonance energy transfer (FRET) experiments imply that it is mainly $G\alpha_2$ that is linked to the cAMP receptors. However, the signalling downstream is mediated by βγ[47]. In neutrophils, chemoattractant receptors are predominantly linked to $G\alpha_i$ and $G\alpha_{12/13}$ (REF. 48). Signalling through $G\alpha_i$ also seems to be mediated by βγ, but $G\alpha_{12/13}$ might directly signal to events that occur at the rear of the cell. Although heterotrimeric G proteins are essential for chemotaxis, chemoattractant–receptor binding also induces responses that do not depend on functional G proteins — for example, receptor phosphorylation, the opening of Ca^{2+} channels, and the activation of mitogen-activated protein kinases (MAPKs) and signal transducer and activator of transcription (STAT) kinases[49–51]. Although they are typically associated with gene expression, it is conceivable that some of these G-protein-independent responses contribute to chemotaxis.

Surprisingly, none of these upstream components of the signal pathways shows a specific localization in chemotaxing or polarized cells (TABLE 2). In *D. discoideum* and neutrophils, the chemoattractant receptors are uniformly distributed along the cell perimeter and, in *D. discoideum*, all of the G-protein subunits mirror this localization. There is no modulation of receptor affinity along the length of the cell; receptor occupancy in *D. discoideum* reflects the shallow external gradient of the chemoattractant. Furthermore, FRET analysis of $G\alpha_2$ indicates that G-protein activation is proportional to receptor occupancy. The upstream components of the sensory system therefore remain distributed along the membrane — poised to allow the cell to respond rapidly to changes in the external gradient.

Phosphoinositide signalling. During chemotaxis in both *D. discoideum* and neutrophils, several proteins that contain PLECKSTRIN-HOMOLOGY (PH) DOMAINS translocate from the cytosol to the plasma membrane at the leading edge of the cell (TABLES 1,2)[36,52,53]. These proteins include the cytosolic regulator of adenylyl cyclase (CRAC) and protein kinase B (PKB; also known as Akt). Binding to the membrane of fusion proteins that comprise the PH domain of these proteins joined to green fluorescent protein (GFP) reflects the local accumulation of

SEVEN-TRANSMEMBRANE-SPANNING RECEPTOR
A receptor that contains seven membrane-spanning helices and usually transmits signals to the inside of a cell by activating heterotrimeric G proteins.

HETEROTRIMERIC G PROTEIN
A protein complex of three proteins (Gα, Gβ, and Gγ). Whereas Gβ and Gγ form a tight complex, Gα is part of the complex in its inactive, GDP-bound, form but dissociates in its active, GTP-bound, form. Both Gα and Gβγ can transmit downstream signals after activation.

FRET
(fluorescence resonance energy transfer). A method to identify the proximity of two proteins, each of which is labelled with a different fluorescent group.

PLECKSTRIN-HOMOLOGY (PH) DOMAIN
A sequence of 100 amino acids that is present in many signalling molecules and binds to lipid products of phosphatidylinositol 3-kinase. Pleckstrin is a protein of unknown function that was originally identified in platelets. It is a principal substrate of protein kinase C.

Table 1 | **Chemotaxis components in *Dictyostelium discoideum* and mammalian neutrophils**

Component	Sensory molecules of *Dictyostelium discoideum*	Sensory molecules of neutrophils	References
Attractants	Nutrients, cAMP, PAF	fMLP, C5a, chemokines, PAF	
Receptors	FAR*, PAFR*, cARs	fMLPR, C5aR, PAFR, chemokine receptors	37–41
G-proteins	G_2, βγ	G_i, $G_{12/13}$, βγ	21,46,48
G-protein signalling	βγ	βγ (for G_i), $\alpha_{12/13}$ (for $G_{12/13}$)	48,98
PI3K	PI3K1–PI3K3,	$PI3K_\gamma$, $PI3K_\alpha$, $PI3K_\delta$	54,55,99
PTEN	PTEN orthologue	PTEN	57,73,100
SHIPS	Dd5P2, Dd5P3	SHIP1	58,59
PAK	PakA	PAK	73,85
Rho	NI	Rho	48
Rac	15 Rac-family members	Rac1, Rac2	71
Cdc42	NI	Cdc42	67–70
Messengers elevated	PtdIns(3,4,5)P_3 cGMP cAMP $InsP_3$ NI Ca^{2+} Receptor phosphorylation	PtdIns(3,4,5)P_3 cGMP not elevated cAMP $InsP_3$ DAG Ca^{2+} Receptor phosphorylation	56,101 89 102,103 104,105 105 105,106 45,107

*Genes for these receptors have not been identified. C5a, complement factor C5a; cAMP, cyclic adenosine monophosphate; cAR, cAMP receptor; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; Dd5P, *D. discoideum* PI-5 phosphatase; FAR, hypothetical folic acid receptor; fMLP, formylated Met-Leu-Phe peptide; $InsP_3$, inositol-1,4,5-trisphosphate; NI, not identified; PAF, platelet-activating factor; PAFR, hypothetical PAF receptor; PAK, p21-activated kinase; PI3K, phosphatidylinositol 3-kinase; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue; R, receptor; SHIP, Src-homology-2 (SH2)-domain-containing PI5-phosphatase.

phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P_2) and, in particular, PtdIns(3,4,5)P_3. (See also the article by Paul A. Janmey and Uno Lindberg in this issue.) PtdIns(3,4,5)P_3 is generated from PtdIns(4,5)P_2 by phosphatidylinositol 3-kinases (PI3Ks), including PI3K1 and PI3K2 in *D. discoideum*, and by $PI3K_\gamma$ as well as other class-I PI3Ks in neutrophils[54–56]. It is degraded by the lipid 3-phosphatase PTEN (phosphatase and tensin homologue)[57] and by several 5-phosphatases[58,59]. In *D. discoideum* cells that lack Pten, the absence of 3-phosphatase activity leads to an increase in the proportion of the membrane area to which PH_{Crac}–GFP binds, and simultaneous expansion of the region from which filamentous (F)-actin-filled pseudopodia are extended. This indicates that PtdIns(3,4,5)P_3 enhances actin polymerization and pseudopod formation. In a gradient of chemoattractant, cells that lack Pten extend two or three pseudopods simultaneously, which impairs progress towards the chemoattractant[57]. Normally when *D. discoideum* cells are exposed to a gradient, PI3K1 and PI3K2 translocate from the cytosol to the front of the cell, whereas Pten detaches from the anterior membrane and becomes localized to the back of the cell. This provides a robust mechanism to generate PtdIns(3,4,5)P_3 locally at the leading edge[57,60]. These gradient-induced localizations of PI3K and Pten to the front and back of the cell, respectively, do not depend on the activities of the enzymes, the actin cytoskeleton or the absolute intracellular level of PtdIns(3,4,5)P_3, which indicates that events controlling the localization of PI3K and Pten occur upstream of phosphoinositide signalling[60,61]. It is unknown which protein or lipid components of the membrane bind PI3K or Pten. The internal gradients of these molecules are steeper than the external chemoattractant gradient and the distributions readjust dynamically when the stimulus is repositioned. In neutrophils and other cells, PtdIns(3,4,5)P_3 also accumulates at the leading edge but the mechanisms of localization have not been determined. The localization of PtdIns(3,4,5)P_3 is expected to depend on the distributions or activities of PI3Ks, PTEN, and 5-phosphatases, but might also depend on the availability of the substrate PtdIns(4,5)P_2.

D. discoideum cells that are stimulated with a uniform increase in cAMP undergo biphasic PH–GFP localization, cell-shape changes, and actin-polymerization responses (FIG. 3). First, there is a uniform translocation of PH–GFP to the cell boundary, which is followed by a second prolonged translocation of PH–GFP to smaller patches on the membrane[26,34]. During the first phase, the cell 'cringes' or rounds and, during the second, it extends pseudopodia. The patches of PH–GFP mark the pseudopodia and have essentially the same properties with respect to size, lifetime and intensity as those formed on pseudopodia at the leading edge of cells that have been exposed to a concentration gradient. The uniform stimulus increases their number[26] and the patches occur around the entire periphery of the cell. In suspensions of *D. discoideum* cells, uniform concentrations of chemoattractant induce two phases of actin polymerization: a very brief large spike, and a more prolonged smaller peak (FIG. 3). These two phases undoubtedly correspond to the 'cringe' and 'patch/extension' responses that are seen in individual cells. Disruption

Table 2 | **Localization of signalling components and events in chemotaxing cells**

Component	Localization in *Dictyostelium discoideum*	Localization in neutrophils	References
Receptors	Uniform (cARs)	Uniform (C5a)	108,109
G-protein subunits	Uniform (α, β, γ)	NL	98
G-protein activation	Similar to gradient	NL	47
Small G proteins	NI	Anterior membrane (Rac, Cdc42)	68,69
PI3K	Anterior membrane	NL	60
PTEN	Posterior membrane	NL	57,60,61
PtdIns(3,4,5)P_3	Anterior membrane	Anterior membrane	36,52,53
F-actin	Anterior cortex	Anterior cortex	15
Myosin	Posterior cortex	Posterior cortex	78,79
PAK	Posterior	Anterior	73,85

C5a, complement factor, C5a; cAR, cyclic AMP receptor; NI, not identified; NL, not localized; PAK, p21-activated kinase; PI3K, phosphatidylinositol 3-kinase; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homologue.

of Pten and elevation of PtdIns(3,4,5)P_3 preferentially increase the second phase of the response. Moreover, treatment with the PI3K inhibitor LY294002, or simultaneous deletion of PI3K1 and PI3K2 completely block the second phase of actin polymerization but have little effect on the initial spike.

These data imply that a new pseudopod is preferentially made at a position on the membrane that contains a patch of PtdIns(3,4,5)P_3, which might also subtly enhance the size or lifetime of the pseudopod. Considering these strong links between PtdIns(3,4,5)P_3, actin polymerization and pseudopod extension, it is surprising that the chemotactic defect of *D. discoideum pi3k1⁻/pi3k2⁻* or mammalian *PI3Kγ*-null cells is not more severe. Both types of mutant cell show reduced directionality and speed of movement, although the chemotactic response still occurs. These modest defects are also observed in wild-type cells that have been treated with moderate concentrations of the PI3K inhibitors LY294002 and wortmannin[26,34,56,57,60,62]. Apparently, in the near absence of the PI3K pathway, cells rely on underlying mechanisms for gradient sensing to induce a chemotactic response. These unknown biochemical events might trigger the initial spike in actin polymerization in the absence of PtdIns(3,4,5)P_3 and might also support rudimentary chemotaxis in gradients.

Organizing the cytoskeleton

Localizing actin filaments. Actin filaments have an inherent polarity, which is used to drive membrane protrusions (see also the article by Daniel Louvard and colleagues in this issue). This is probably not by elongation of the actin filaments *per se*, but by an elastic Brownian-motion mechanism — thermal energy bends the filament and thereby stores energy, whereas unbending against the leading edge of the cell provides the driving force for protrusion[15]; or alternatively, the membrane might bulge, allowing the filament to elongate and support the extension. *In vitro* studies indicate that actin polymerization is probably mediated by the ARP2/3 COMPLEX, which binds to the sides of pre-existing filaments and induces the formation of branches. Activation of the Arp2/3 complex is induced by Wiskott–Aldrich syndrome protein (WASP) and the related WAVE/SCAR (suppressor of cAR (cAMP receptor)) proteins[63], and recent evidence indicates that activation of Wave/Scar proteins in *D. discoideum* enhances the production of actin-filled pseudopods[64–66]. Surprisingly, however, although elimination of Wave/Scar might alter pseudopod formation, it does not effect the ability of chemoattractant to regulate actin polymerization in *D. discoideum*[65,66], which is in contrast to the strong effects that eliminating WAVE/SCAR has in mammalian cells[63].

It is thought that RHO-FAMILY GTPases[13,15,67] activate WAVE/SCAR, and so the chemotactic signal that biases sites of pseudopod formation might localize the activity of exchange factors for these small G proteins[68–70]. Both *D. discoideum* and neutrophils contain tens of small GTP-binding proteins[67,71,72] (TABLE 1). Most prominent are members of the Rac subfamily — Rac and Cdc42 — although the precise orthologues have not been identified in *D. discoideum*[13,48]. Cdc42 seems to function as a master regulator of polarity in eukaryotic cells, from yeast to humans, and *in vivo* assays have shown that Cdc42 is active towards the front of migrating neutrophils[69]. A feedback loop that could determine where pseudopodia are formed in neutrophils consists of p21-activated kinase-1 (PAK1), a target of Cdc42, which, in turn, stimulates Cdc42 (REF. 73). The key event that determines where actin polymerization occurs seems to be defining where Rac is active in the cell. This is probably achieved by locally activating GUANINE NUCLEOTIDE-EXCHANGE FACTORS (GEFs) for Rac or by delivering activated Rac GEFs, either by locally produced PtdIns(3,4,5)P_3 or by direct receptor activation[74]. PREX1 and PAK-interacting exchange factor (PIX) are two Rac GEFs that are regulated by PtdIns(3,4,5)P_3 in mammalian cells[75,76]. Once Rac activation is initiated, several feedback loops have been identified that help to amplify and maintain local Rac activation and subsequent membrane protrusion. Rac might stimulate or recruit PI3K at the membrane, which produces PtdIns(3,4,5)P_3, which, in turn, locally activates an

Arp2/3 COMPLEX
A complex that consists of two actin-related proteins, Arp2 and Arp3, along with five smaller proteins. When activated, the Arp2/3 complex binds to the side of an existing actin filament and nucleates the assembly of a new actin filament. The resulting branch structure is Y-shaped.

RHO-FAMILY GTPases
Ras-related small GTPases that are involved in controlling the polymerization of actin.

GUANINE NUCLEOTIDE-EXCHANGE FACTOR (GEF)
A protein that facilitates the exchange of GDP for GTP in the nucleotide-binding pocket of a GTP-binding protein.

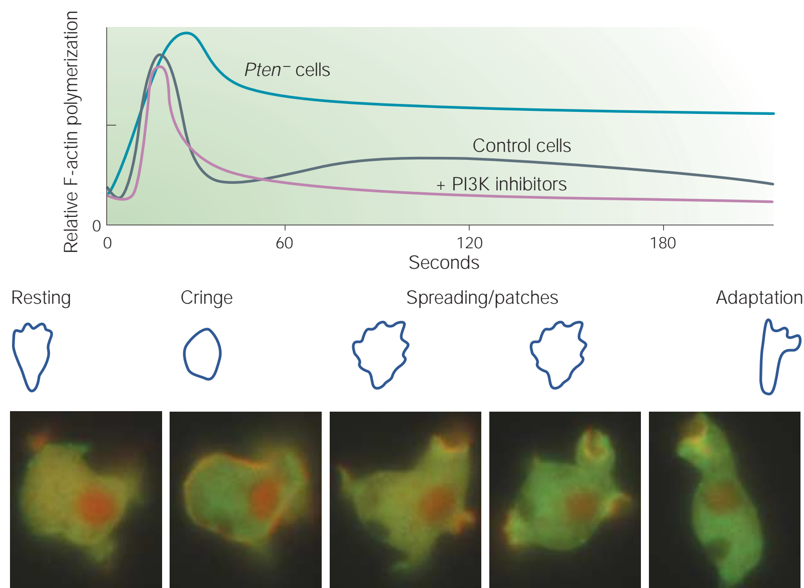


Figure 3 | **Uniform chemoattractant increments trigger biphasic responses.** Actin-polymerization responses in suspensions of *Dictyostelium discoideum* cells. Cells that are suddenly exposed to a uniform increase in chemoattractant show a biphasic increase in actin polymerization. The initial phase peaks in 4–8 seconds and is followed by a round of depolymerization. During this time, the cell freezes motion and rounds up or 'cringes'. The second, broad, low phase peaks at 90 seconds. During this time the cell is seen extending filamentous (F)-actin-filled pseudopodia in restricted regions along the perimeter. A schematic representation of the changes in morphology of the *D. discoideum* cells during the response is depicted and corresponding images of wild-type *D. discoideum* cells during the response to a uniform increase in cyclic AMP are shown below these diagrams. The changes in morphology from the resting state through to when the cells adapt are clearly visible. The red staining shows the relative distribution of phosphatidylinositol-3,4,5-trisphosphate, which was measured using red fluorescent protein fused to the pleckstrin-homology domain of Crac (cytosolic regulator of adenylyl cyclase). The green staining, which is generated by green fluorescent protein fused to coronin, shows the relative distribution of actin. The images are reproduced with permission from REF. 110 © (2003) The American Society for Cell Biology. Pten, phosphatase and tensin homologue; PI3K, phosphatidylinositol 3-kinase.

DOMINANT-NEGATIVE
A defective protein that retains interaction capabilities and so distorts or competes with normal proteins.

as-yet-unknown Rac GEF[74,77]. In addition, actin and myosin filaments at the leading and trailing edges, respectively, might stabilize the association of PI3K and Pten with the membrane and thereby stimulate PtdIns(3,4,5)P_3 production in *D. discoideum* (P.N.D., unpublished observations).

In contrast to the well-defined roles of Rac and Cdc42 in neutrophil chemotaxis, so far, genetic studies in *D. discoideum* have failed to uncover a specific small GTP-binding protein that mediates chemotaxis. Instead, studies that use cells with DOMINANT-NEGATIVE or constitutively active GTP-binding proteins or GEFs, or cells in which these proteins have been deleted, indicate that several small GTP-binding proteins are involved[71]. This situation might reflect the level at which these proteins function: heterotrimeric G proteins function at the beginning of the sensory transduction pathway, whereas small GTP-binding proteins are important further downstream after bifurcation of the pathway. In *D. discoideum*, identification of the pathways between heterotrimeric G proteins and effector enzymes such as PI3K and Pten should help to understand how these enzymes become localized and activated.

Localizing myosin filaments. In randomly moving *D. discoideum* cells, myosin-II filaments are present predominantly at the cortex in the back and at the sides of the cells, as well as in retracting pseudopodia[78,79]. In a chemotactic gradient, however, new pseudopods are generally not retracted, and myosin-II filaments are rarely observed in pseudopodia (FIG. 4). Furthermore, more myosin-II filaments are present in the uropod in a gradient of chemoattractant than are present in randomly moving cells. Both observations indicate that a gradient of chemoattractant regulates the back as well as the front of a moving cell. Finally, the region of the cell that contains myosin-II filaments extends more along the sides of the cell in a gradient of chemoattractant, and thereby provides the mechanism to suppress lateral pseudopodia in chemotaxing cells — both in *D. discoideum* and in neutrophils[48,78–81]. The myosin-II filaments at the back of migrating cells therefore provide the power to retract the uropod. The myosin-II filaments at the sides of these cells suppress the formation of lateral pseudopodia, which otherwise would induce deviations of the cell trajectory. And filaments at both locations support cell polarity.

Early observations indicated that the intracellular cyclic GMP that is produced on chemotactic stimulation mediates the formation of myosin filaments in *D. discoideum* (TABLE 1)[82]. The genes that encode guanylyl cyclases (which generate cGMP), phosphodiesterases (which hydrolyse cGMP) and proteins that are targets of cGMP have recently been identified, and have been used to resolve the function of cGMP in regulating myosin[81,83,84]. Cells that lack two cGMP phosphodiesterases have very high levels of cGMP and increased levels of myosin filaments in the cortex, and lateral pseudopodia are suppressed[81,83,84]. Cells that lack guanylyl cyclases or cGMP targets have less myosin filaments in the cortex, which results in the formation of many lateral pseudopodia and poor chemotaxis[81,85]. Elevated levels of cGMP also activate myosin-light-chain kinase A (Mlcka), which phosphorylates myosin regulatory light chain (Mlc) and thereby increases the motor activity of myosin II (REF. 86).

In *D. discoideum*, chemoattractants also induce a reaction that counteracts the formation of myosin-II filaments — the phosphorylation of myosin II at three threonine residues in the tail region, which bends the coiled-coil structure and depolymerizes the filament[86]. It is thought that this chemoattractant-induced phosphorylation of myosin II reflects the increased turnover of myosin-II filaments that occurs in retracting uropods. Alternatively, it has been proposed that the rapidly diffusing cGMP induces the formation of myosin-II filaments throughout the cell whereas phosphorylation by myosin-heavy-chain kinase A and subsequent depolymerization is restricted to the leading edge, thereby coordinating chemotaxis[14,87].

In neutrophils, the formation of myosin filaments is regulated in a similar way, but other signalling molecules are involved. The function of myosin-II is regulated by MLC phosphorylation through MLCK that is activated by Ca^{2+} and by a Rho-stimulated kinase

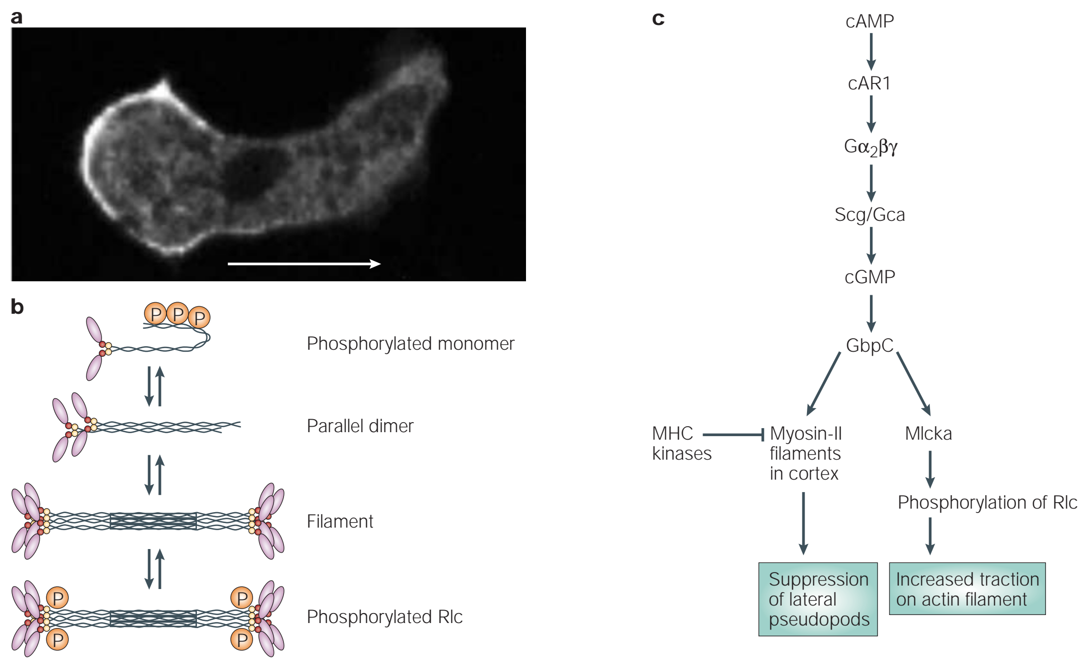


Figure 4 | **Myosin-II filaments in chemotaxis. a** | The image shows the localization of green fluorescent protein (GFP)-tagged myosin II in the posterior of a *Dictyostelium discoideum* cell that is moving in the direction of the arrow. **b** | The myosin-II protein exists in different forms. Phosphorylated (P) myosin II is bent, which inhibits dimerization and filament formation. Phosphorylated regulatory light chain (RLC) increases the motor activity of myosin II. **c** | Regulation of myosin function by cyclic GMP in *D. discoideum*[81]. Activation of the cyclic-AMP receptor cAR1 by the chemoattractant cAMP signalling through $G\alpha_2\beta\gamma$ activates two guanylyl cyclases — soluble guanylate cyclase (Sgc) and guanylyl cyclase A (Gca) . The resulting cGMP that is generated activates a complex target protein GbpC (cGMP-binding protein C), which mediates two functions. First, the formation of myosin-II filaments in the cortex at the rear of the cell inhibits pseudopod formation here. Second, phosphorylation of Rlc through the activity of myosin-light-chain kinase A (Mlcka) enhances the traction force of myosin filaments, which thereby causes the uropod to retract. Filament formation is inhibited by several myosin-II-heavy-chain (MHC) kinases. In *D. discoideum*, cGMP is a main regulator of myosin-II function, but p21-activated kinase a (Paka) also controls it[85]. The myosin-II filaments have a similar role in neutrophil chemotaxis, but are regulated by Rho kinases[48,88] rather than by cGMP.

(ROCK), which are both stimulated by chemoattractant receptors; ROCK also inhibits an MLC phosphatase[48,88]. Although these pathways are very different in neutrophils and *D. discoideum*, the end result is essentially identical — myosin filaments are formed at the back and at the sides of the cell, and this mediates the contraction of the posterior and inhibits the formation of lateral pseudopodia.

Adaptation responses

Many chemoattractant-mediated responses undergo adaptation. Cells respond only to increments in the chemoattractant concentration. In *D. discoideum*, chemoattractant-induced PtdIns(3,4,5)P_3 accumulation, actin polymerization, cGMP formation and myosin-filament formation are regulated in this way[44,89–91] (FIG. 3). These regulatory events could be very important for chemotaxis. As long as cells encounter increases in the concentration of chemoattractants, or are exposed to a gradient, responses are likely to be persistently elevated (or depressed in cases where a stimulus induces a downward response). When the chemoattractant concentration no longer changes temporally or spatially, or when the gradient reverses, the responses subside. This adaptive component of chemotactic signalling in *D. discoideum* is similar to the adaptation that is seen in the temporal mechanism of chemotaxis in prokaryotes[5]. For eukaryotic cells, adaptation is used to sense the spatial gradient as well as temporal signals.

The molecular mechanism of adaptation in eukaryotes is poorly understood. In prokaryotes, adaptation takes place at the level of receptor methylation[92]. In eukaryotes, adaptation of a signalling cascade might be the result of several reactions. For example, in *D. discoideum*, chemoattractant-stimulated cGMP formation is negatively controlled by receptor phosphorylation. This leads to a reduced affinity of the receptor for the ligand[93], inhibition of guanylyl cyclases by receptor-stimulated influx of Ca^{2+} and by the cGMP-occupied target protein[94,95], and activation of cGMP phosphodiesterases by cGMP[96]. All these reactions lead to a decline of the cGMP response after prolonged stimulation, but still might not be sufficient to explain the observed perfect adaptation[97]. Adaptation of chemoattractant-induced formation of PtdIns(3,4,5)P_3 obviously involves the chemoattractant-induced movements of PI3K and PTEN onto and off the membrane, but the regulation of these events is still under investigation.

Conclusions

Knowledge from different systems such as mammalian neutrophils and protozoan amoebae indicates that gradient sensing during chemotaxis does not depend on a single molecular mechanism, but consists of several modules of interconnected signalling networks. These networks achieve periodic pseudopod extension, directional sensing and polarization. They direct actin polymerization at the anterior, and cortical-myosin formation at the posterior, of the cell. The PtdIns(3,4,5)P_3 system probably functions as a strong amplifier, sensitizer, and director of chemoattractant-induced pseudopod formation. When a new pseudopod is to be formed, it is preferentially induced at places with elevated PtdIns(3,4,5)P_3 levels. In a chemoattractant gradient, PtdIns(3,4,5)P_3 is highly localized towards the edge of the cell that is exposed to the highest concentration of chemoattractant owing to reciprocal distributions of PI3K and PTEN. These signalling molecules help to shape the actin-filament system at the leading edge, although the initial asymmetry is determined upstream. The protrusion at the leading edge would not be very effective unless the back of the cell was retracted and pseudopodia at the sides of the cell were suppressed. This is mediated by myosin filaments that are formed in these regions of the cell. In neutrophils and *D. discoideum* this occurs in a similar way, with essentially the same functions, but probably arises by the use of very different signalling molecules — ROCK and a cGMP-dependent kinase, respectively, both of which activate an MLCK.

Research into the molecular basis of chemotaxis has progressed quickly over the past few years. Intricate networks of key regulatory molecules have been identified. However, there are still many unresolved issues regarding how and where pseudopodia are formed, how the spatially and temporally segregated molecular processes are integrated, and especially how a shallow gradient of chemoattractant can be processed to generate the initial localized response at the leading edge. Investigation of these issues requires methods to visualize the dynamics of activated components of larger complexes in moving cells. Ultimately we will have to accumulate quantitative kinetic data with sufficient temporal and spatial resolution to develop models that can integrate the various aspects of chemotaxis.

1. Baggiolini, M. Chemokines and leukocyte traffic. *Nature* **392**, 565–568 (1998).
2. Campbell, J. J. & Butcher, E. C. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr. Opin. Immunol.* **12**, 336–341 (2000).
3. Crone, S. A. & Lee, K. F. The bound leading the bound: target-derived receptors act as guidance cues. *Neuron* **36**, 333–335 (2002).
4. Iijima, M., Huang, Y. E. & Devreotes, P. Temporal and spatial regulation of chemotaxis. *Dev. Cell* **3**, 469–478 (2002).
5. Berg, H. C. A physicist looks at bacterial chemotaxis. *Cold Spring Harb. Symp. Quant. Biol.* **53**, 1–9 (1988).
6. Bourret, R. B. & Stock, A. M. Molecular information processing: lessons from bacterial chemotaxis. *J. Biol. Chem.* **277**, 9625–9628 (2002).
7. Zigmond, S. H. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J. Cell Biol.* **75**, 606–616 (1977).
8. Devreotes, P. N. & Zigmond, S. H. Chemotaxis in eukaryotic cells: a focus on leukocytes and *Dictyostelium*. *Annu. Rev. Cell Biol.* **4**, 649–686 (1988).
9. Weiner, O. D. Regulation of cell polarity during eukaryotic chemotaxis: the chemotactic compass. *Curr. Opin. Cell Biol.* **14**, 196–202 (2002).
10. Chemoattractant-induced phosphatidylinositol 3,4,5-trisphosphate accumulation is spatially amplified and adapts, independent of the actin cytoskeleton. *Proc. Natl Acad. Sci. USA* **101**, 8951–8956 (2004).
11. Mato, J. M., Losada, A., Nanjundiah, V. & Konijn, T. M. Signal input for a chemotactic response in the cellular slime mold *Dictyostelium discoideum*. *Proc. Natl Acad. Sci. USA* **72**, 4991–4993 (1975).
12. Stock, J. Sensitivity, cooperativity and gain in chemotaxis signal transduction. *Trends Microbiol.* **7**, 1–4 (1999).
13. Ridley, A. J. *et al.* Cell migration: integrating signals from front to back. *Science* **302**, 1704–1709 (2003).
14. Postma, M., Bosgraaf, L., Loovers, H. M. & Van Haastert, P. J. M. Chemotaxis: signalling modules join hands at front and tail. *EMBO Rep.* **5**, 35–40 (2004).
The authors present a concept for chemotaxis based on the diffusion properties of signalling molecules, and their organization in several modules that regulate actin and myosin.
15. Pollard, T. D. & Borisy, G. G. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453–465 (2003).
An excellent review on the regulation of actin filaments in motile cells by the Arp2/3 complex, profilin, capping proteins, WASP and WAVE/SCAR.
16. Swanson, J. A. & Taylor, D. L. Local and spatially coordinated movements in *Dictyostelium discoideum* amoebae during chemotaxis. *Cell* **28**, 225–232 (1982).
17. Varnum-Finney, B., Edwards, K. B., Voss, E. & Soll, D. R. Amebae of *Dictyostelium discoideum* respond to an increasing temporal gradient of the chemoattractant cAMP with a reduced frequency of turning: evidence for a temporal mechanism in ameboid chemotaxis. *Cell Motil. Cytoskeleton* **8**, 7–17 (1987).
18. Baldauf, S. L., Roger, A. J., Wenk-Siefert, I. & Doolittle, W. F. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**, 972–977 (2000).
19. Wessels, D., Vawter-Hugart, H., Murray, J. & Soll, D. R. Three-dimensional dynamics of pseudopod formation and the regulation of turning during the motility cycle of *Dictyostelium*. *Cell Motil. Cytoskeleton* **27**, 1–12 (1994).
20. Postma, M. *et al.* Uniform cAMP stimulation of *Dictyostelium* cells induces localized patches of signal transduction and pseudopodia. *Mol. Biol. Cell* **14**, 5019–5027 (2003).
21. Wu, L., Valkema, R., Van Haastert, P. J. & Devreotes, P. N. The G protein β subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. *J. Cell Biol.* **129**, 1667–1675 (1995).
22. Insall, R. H., Soede, R. D., Schaap, P. & Devreotes, P. N. Two cAMP receptors activate common signaling pathways in *Dictyostelium*. *Mol. Biol. Cell* **5**, 703–711 (1994).
23. Kriebel, P. W., Barr, V. A. & Parent, C. A. Adenylyl cyclase localization regulates streaming during chemotaxis. *Cell* **112**, 549–560 (2003).
24. Soll, D. R., Wessels, D., Heid, P. J. & Zhang, H. A contextual framework for characterizing motility and chemotaxis mutants in *Dictyostelium discoideum*. *J. Muscle Res. Cell Motil.* **23**, 659–672 (2002).
25. Soll, D. R. The use of computers in understanding how animal cells crawl. *Int. Rev. Cytol.* **163**, 43–104 (1995).
26. Postma, M. *et al.* Sensitization of *Dictyostelium* chemotaxis by PI3-kinase mediated self-organizing signalling patches. *J. Cell Sci.* **117**, 2925–2935 (2004).
27. Varnum, B. & Soll, D. R. Effects of cAMP on single cell motility in *Dictyostelium*. *J. Cell Biol.* **99**, 1151–1155 (1984).
28. Verkhovsky, A. B., Svitkina, T. M. & Borisy, G. G. Self-polarization and directional motility of cytoplasm. *Curr. Biol.* **9**, 11–20 (1999).
29. Bretschneider, T. *et al.* Dynamic actin patterns and Arp2/3 assembly at the substrate-attached surface of motile cells. *Curr. Biol.* **14**, 1–10 (2004).
30. Misteli, T. The concept of self-organization in cellular architecture. *J. Cell Biol.* **155**, 181–185 (2001).
31. Nicolis, G. & Prigogine, I. *Self-organization in nonequilibrium systems: from dissipative structures to order through fluctuations.* (John Wiley & Sons, New York, 1977).
32. Bourne, H. R. & Weiner, O. A chemical compass. *Nature* **419**, 21 (2002).
33. Devreotes, P. & Janetopoulos, C. Eukaryotic chemotaxis: distinctions between directional sensing and polarization. *J. Biol. Chem.* **278**, 20445–20448 (2003).
The authors define directional sensing as the ability of a cell to detect an asymmetric extracellular cue and generate an internal amplified response, whereas polarization is defined as the propensity of the cell to assume an asymmetric shape with a defined anterior and posterior. With these definitions many models for chemotaxis are evaluated.
34. Chen, L. *et al.* Two phases of actin polymerization display different dependencies on PI(3,4,5)P_3 accumulation and have unique roles during chemotaxis. *Mol. Biol. Cell* **14**, 5028–5037 (2003).
35. Van Duijn, B. & Van Haastert, P. J. Independent control of locomotion and orientation during *Dictyostelium discoideum* chemotaxis. *J. Cell Sci.* **102**, 763–768 (1992).
36. Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B. & Devreotes, P. N. G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* **95**, 81–91 (1998).
37. Klein, P. S. *et al.* A chemoattractant receptor controls development in *Dictyostelium discoideum*. *Science* **241**, 1467–1472 (1988).
38. Saxe, C. L. 3rd, Johnson, R., Devreotes, P. N. & Kimmel, A. R. Multiple genes for cell surface cAMP receptors in *Dictyostelium discoideum*. *Dev. Genet.* **12**, 6–13 (1991).
39. Youn, B. S., Mantel, C. & Broxmeyer, H. E. Chemokines, chemokine receptors and hematopoiesis. *Immunol. Rev.* **177**, 150–174 (2000).
40. Le, Y., Murphy, P. M. & Wang, J. M. Formyl-peptide receptors revisited. *Trends Immunol.* **23**, 541–548 (2002).
41. Maghazachi, A. A. G protein-coupled receptors in natural killer cells. *J. Leukoc. Biol.* **74**, 16–24 (2003).
42. Kim, J. Y., Borleis, J. A. & Devreotes, P. N. Switching of chemoattractant receptors programs development and morphogenesis in *Dictyostelium*: receptor subtypes activate common responses at different agonist concentrations. *Dev. Biol.* **197**, 117–128 (1998).
43. Dormann, D., Kim, J. Y., Devreotes, P. N. & Weijer, C. J. cAMP receptor affinity controls wave dynamics, geometry and morphogenesis in *Dictyostelium*. *J. Cell Sci.* **114**, 2513–2523 (2001).
44. Kim, J. Y. *et al.* Phosphorylation of chemoattractant receptors is not essential for chemotaxis or termination of G-protein-mediated responses. *J. Biol. Chem.* **272**, 27313–27318 (1997).
45. Richardson, R. M., Marjoram, R. J., Barak, L. S. & Snyderman, R. Role of the cytoplasmic tails of CXCR1 and CXCR2 in mediating leukocyte migration, activation, and regulation. *J. Immunol.* **170**, 2904–2911 (2003).

46. Zhang, N., Long, Y. & Devreotes, P. N. Gγ in *Dictyostelium*: its role in localization of Gβγ to the membrane is required for chemotaxis in shallow gradients. *Mol. Biol. Cell* **12**, 3204–3213 (2001).
47. Janetopoulos, C., Jin, T. & Devreotes, P. Receptor-mediated activation of heterotrimeric G-proteins in living cells. *Science* **291**, 2408–2411 (2001).
48. Xu, J. *et al.* Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* **114**, 201–214 (2003).
Expression of constitutively active and dominant-negative versions of various G proteins and GTPases in neutrophils indicate that chemoattractant-mediated signals segregate into two mutually exclusive pathways: G_i–PtdIns(3,4,5)P_3–Rac-dependent formation of F-actin at the front of cells and $G_{12/13}$–RhoA-dependent formation of myosin-II filaments at the back.
49. Araki, T. *et al.* Developmentally and spatially regulated activation of a *Dictyostelium* STAT protein by a serpentine receptor. *EMBO J.* **17**, 4018–4028 (1998).
50. Milne, J. L., Wu, L., Caterina, M. J. & Devreotes, P. N. Seven helix cAMP receptors stimulate Ca^{2+} entry in the absence of functional G proteins in *Dictyostelium. J. Biol. Chem.* **270**, 5926–5931 (1995).
51. Maeda, M. & Firtel, R. A. Activation of the mitogen-activated protein kinase ERK2 by the chemoattractant folic acid in *Dictyostelium. J. Biol. Chem.* **272**, 23690–23695 (1997).
52. Meili, R. *et al.* Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in *Dictyostelium. EMBO J.* **18**, 2092–2105 (1999).
53. Servant, G. *et al.* Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. *Science* **287**, 1037–1040 (2000).
54. Zhou, K., Takegawa, K., Emr, S. D. & Firtel, R. A. A phosphatidylinositol (PI) kinase gene family in *Dictyostelium discoideum*: biological roles of putative mammalian p110 and yeast Vps34p PI 3-kinase homologs during growth and development. *Mol. Cell. Biol.* **15**, 5645–5656 (1995).
55. Hirsch, E. *et al.* Central role for G protein-coupled phosphoinositide 3-kinase γ in inflammation. *Science* **287**, 1049–1053 (2000).
56. Huang, Y. E. *et al.* Receptor mediated regulation of PI3Ks confines PI(3,4,5)P_3 to the leading edge of chemotaxing cells. *Mol. Biol. Cell* **14**, 1913–1922 (2003).
57. Iijima, M. & Devreotes, P. Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell* **109**, 599–610 (2002).
This paper and reference 60 characterize in detail the function and cellular localization of PI3K and Pten in *D. discoideum* cells. During chemotaxis PI3K is enriched at the leading edge and Pten accumulates at the posterior membrane of the cell.
58. Kalesnikoff, J. *et al.* The role of SHIP in cytokine-induced signaling. *Rev. Physiol. Biochem. Pharmacol.* **149**, 87–103 (2003).
59. Loovers, H. *et al.* A diverse family of inositol 5-phosphatases playing a role in growth and development in *Dictyostelium discoideum. J. Biol. Chem.* **278**, 5652–5658 (2002).
60. Funamoto, S., Meili, R., Lee, S., Parry, L. & Firtel, R. A. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**, 611–623 (2002).
61. Iijima, M., Huang, Y. E., Luo, H. R., Vazquez, F. & Devreotes, P. N. Novel mechanism of PTEN regulation by its phosphatidylinositol 4,5-bisphosphate binding motif is critical for chemotaxis. *J. Biol. Chem.* **279**, 16606–16613 (2004).
62. Wang, F. *et al.* Lipid products of PI(3)Ks maintain persistent cell polarity and directed motility in neutrophils. *Nature Cell Biol.* **4**, 513–518 (2002).
63. Millard, T. H., Sharp, S. J. & Machesky, L. M. Signalling to actin assembly via the WASP (Wiskott–Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem. J.* **380**, 1–17 (2004).
64. Machesky, L. M. & Insall, R. H. Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* **8**, 1347–1356 (1998).
65. Blagg, S. L., Stewart, M., Sambles, C. & Insall, R. H. PIR121 regulates pseudopod dynamics and SCAR activity in *Dictyostelium. Curr. Biol.* **13**, 1480–1487 (2003).
66. Bear, J. E., Rawls, J. F. & Saxe, C. L. 3rd. SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. *J. Cell Biol.* **142**, 1325–1335 (1998).
67. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. *Nature* **420**, 629–635 (2002).
68. Gardiner, E. M. *et al.* Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis. *Curr. Biol.* **12**, 2029–2034 (2002).
69. Itoh, R. E. *et al.* Activation of rac and cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. *Mol. Cell Biol.* **22**, 6582–6591 (2002).
70. Wedlich-Soldner, R., Altschuler, S., Wu, L. & Li, R. Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. *Science* **299**, 1231–1235 (2003).
71. Chubb, J. R. & Insall, R. H. *Dictyostelium*: an ideal organism for genetic dissection of Ras signalling networks. *Biochim. Biophys. Acta* **1525**, 262–271 (2001).
72. Lim, C. J., Spiegelman, G. B. & Weeks, G. Cytoskeletal regulation by *Dictyostelium* Ras subfamily proteins. *J. Muscle Res. Cell Motil.* **23**, 729–736 (2002).
73. Li, Z. *et al.* Directional sensing requires Gβγ-mediated PAK1 and PIXα-dependent activation of Cdc42. *Cell* **114**, 215–227 (2003).
74. Welch, H. C., Coadwell, W. J., Stephens, L. R. & Hawkins, P. T. Phosphoinositide 3-kinase-dependent activation of Rac. *FEBS Lett.* **546**, 93–97 (2003).
75. Welch, H. C. *et al.* P-Rex1, a PtdIns(3,4,5)P_3- and Gβγ-regulated guanine-nucleotide exchange factor for Rac. *Cell* **108**, 809–821 (2002).
76. Park, H. S. *et al.* Sequential activation of phosphatidylinositol 3-kinase, βPix, Rac1, and Nox1 in growth factor-induced production of H_2O_2. *Mol. Cell Biol.* **24**, 4384–4394 (2004).
77. Srinivasan, S. *et al.* Rac and Cdc42 play distinct roles in regulating PI(3,4,5)P_3 and polarity during neutrophil chemotaxis. *J. Cell Biol.* **160**, 375–385 (2003).
78. Levi, S., Polyakov, M. V. & Egelhoff, T. T. Myosin II dynamics in *Dictyostelium*: determinants for filament assembly and translocation to the cell cortex during chemoattractant responses. *Cell Motil. Cytoskeleton* **53**, 177–188 (2002).
79. Moores, S. L., Sabry, J. H. & Spudich, J. A. Myosin dynamics in live *Dictyostelium* cells. *Proc. Natl Acad. Sci. USA* **93**, 443–446 (1996).
80. Eddy, R. J., Pierini, L. M., Matsumura, F. & Maxfield, F. R. Ca^{2+}-dependent myosin II activation is required for uropod retraction during neutrophil migration. *J. Cell Sci.* **113**, 1287–1298 (2000).
81. Bosgraaf, L. *et al.* A novel cGMP signalling pathway mediating myosin phosphorylation and chemotaxis in *Dictyostelium. EMBO J.* **21**, 4560–4570 (2002).
Using mutants with deletions of guanylyl cyclases, cGMP-phosphodiesterases or cGMP-binding targets, the authors characterize the function of cGMP in *D. discoideum* as an inducer of myosin-II filaments in the cortex at the back of the cell, leading to suppression of lateral pseudopodia.
82. Liu, G. & Newell, P. C. Role of cyclic GMP in signal transduction to cytoskeletal myosin. *Symp. Soc. Exp. Biol.* **47**, 283–295 (1993).
83. Goldberg, J. M., Bosgraaf, L., Van Haastert, P. J. M. & Smith, L. Identification of four candidate cGMP targets in *Dictyostelium. Proc. Natl Acad. Sci. USA* **99**, 6749–6754 (2002).
84. Roelofs, J., Smith, J. L. & Van Haastert, P. J. M. cGMP signalling: different ways to create a pathway. *Trends Genet.* **19**, 132–134 (2002).
85. Chung, C. Y., Potikyan, G. & Firtel, R. A. Control of cell polarity and chemotaxis by Akt/PKB and PI3 kinase through the regulation of PAKa. *Mol. Cell* **7**, 937–947 (2001).
86. De La Roche, M. A., Smith, J. L., Betapudi, V., Egelhoff, T. T. & Cote, G. P. Signaling pathways regulating *Dictyostelium* myosin II. *J. Muscle Res. Cell Motil.* **23**, 703–718 (2002).
87. Steimle, P. A. *et al.* Recruitment of a myosin heavy chain kinase to actin-rich protrusions in *Dictyostelium. Curr. Biol.* **11**, 708–713 (2001).
88. Riento, K. & Ridley, A. J. ROCKs: multifunctional kinases in cell behaviour. *Nature Rev. Mol. Cell Biol.* **4**, 446–456 (2003).
89. Van Haastert, P. J. M. & Van der Heijden, P. R. Excitation, adaptation, and deadaptation of the cAMP-mediated cGMP response in *Dictyostelium discoideum. J. Cell Biol.* **96**, 347–353 (1983).
90. Berlot, C. H., Spudich, J. A. & Devreotes, P. N. Chemoattractant-elicited increases in myosin phosphorylation in *Dictyostelium. Cell* **43**, 307–314 (1985).
91. Zhang, H. *et al.* Phosphorylation of the myosin regulatory light chain plays a role in motility and polarity during *Dictyostelium* chemotaxis. *J. Cell Sci.* **115**, 1733–1747 (2002).
92. Stock, A. M. & Mowbray, S. L. Bacterial chemotaxis: a field in motion. *Curr. Opin. Struct. Biol.* **5**, 744–751 (1995).
93. Caterina, M. J., Devreotes, P. N., Borleis, J. & Hereld, D. Agonist-induced loss of ligand binding is correlated with phosphorylation of cAR1, a G protein-coupled chemoattractant receptor from *Dictyostelium. J. Biol. Chem.* **270**, 8667–8672 (1995).
94. Valkema, R. & Van Haastert, P. J. Inhibition of receptor-stimulated guanylyl cyclase by intracellular calcium ions in *Dictyostelium* cells. *Biochem. Biophys. Res. Commun.* **186**, 263–268 (1992).
95. Kuwayama, H. & Van Haastert, P. J. Regulation of guanylyl cyclase by a cGMP-binding protein during chemotaxis in *Dictyostelium discoideum. J. Biol. Chem.* **271**, 23718–23724 (1996).
96. Bosgraaf, L. *et al.* Identification and characterization of two unusual cGMP-stimulated phoshodiesterases in *Dictyostelium. Mol. Biol. Cell* **13**, 3878–3889 (2002).
97. Valkema, R. & Van Haastert, P. J. M. A model for cAMP-mediated cGMP response in *Dictyostelium discoideum. Mol. Biol. Cell* **5**, 575–585 (1994).
98. Jin, T., Zhang, N., Long, Y., Parent, C. A. & Devreotes, P. N. Localization of the G protein βγ complex in living cells during chemotaxis. *Science* **287**, 1034–1036 (2000).
99. Sadhu, C., Masinovsky, B., Dick, K., Sowell, C. G. & Staunton, D. E. Essential role of phosphoinositide 3-kinase δ in neutrophil directional movement. *J. Immunol.* **170**, 2647–2654 (2003).
100. Sulis, M. L. & Parsons, R. PTEN: from pathology to biology. *Trends Cell Biol.* **13**, 478–483 (2003).
101. Traynor-Kaplan, A. E. *et al.* Transient increase in phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol trisphosphate during activation of human neutrophils. *J. Biol. Chem.* **264**, 15668–15673 (1989).
102. Roos, W., Scheidegger, C. & Gerish, G. Adenylate cyclase activity oscillations as signals for cell aggregation in *Dictyostelium discoideum. Nature* **266**, 259–261 (1977).
103. Jackowski, S. & Sha'afi, R. I. Response of adenosine cyclic 3′,5′-monophosphate level in rabbit neutrophils to the chemotactic peptide formyl-methionyl-leucyl-phenylalanine. *Mol. Pharmacol.* **16**, 473–481 (1979).
104. van Haastert, P. J. & van Dijken, P. Biochemistry and genetics of inositol phosphate metabolism in *Dictyostelium. FEBS Lett.* **410**, 39–43 (1997).
105. Berridge, M. J. & Irvine, R. F. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**, 315–321 (1984).
106. Bumann, J., Wurster, B. & Malchow, D. Attractant-induced changes and oscillations of the extracellular Ca^{++} concentration in suspensions of differentiating *Dictyostelium* cells. *J. Cell Biol.* **98**, 173–178 (1984).
107. Klein, P., Vaughan, R., Borleis, J. & Devreotes, P. The surface cyclic AMP receptor in *Dictyostelium.* Levels of ligand-induced phosphorylation, solubilization, identification of primary transcript, and developmental regulation of expression. *J. Biol. Chem.* **262**, 358–364 (1987).
108. Xiao, Z., Zhang, N., Murphy, D. B. & Devreotes, P. N. Dynamic distribution of chemoattractant receptors in living cells during chemotaxis and persistent stimulation. *J. Cell Biol.* **139**, 365–374 (1997).
109. Servant, G., Weiner, O. D., Neptune, E. R., Sedat, J. W. & Bourne, H. R. Dynamics of a chemoattractant receptor in living neutrophils during chemotaxis. *Mol. Biol. Cell* **10**, 1163–1178 (1999).
110. Chen, L. *et al.* Two phases of actin polymerization display different dependencies on PI(3,4,5)P_3 accumulation and have unique roles during chemotaxis. *Mol. Biol. Cell.* **14**, 5028–5037 (2003).

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Competing interests statement

The authors declare no competing financial interests.

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