PHYSIOLOGY 19: 300–308, 2004; 10.1152/physiol.00017.2004

Moving Forward: Mechanisms of Chemoattractant Gradient Sensing

Jonathan Franca-Koh
and Peter N. Devreotes
Department of Cell Biology, Johns Hopkins University
School of Medicine, Baltimore, Maryland 21205
pnd@jhmi.edu

Cells use an internal compass to sense the direction of chemoattractant gradients. This is used to bias pseudopod extension at the front of the cell and to orient cell polarization. Recent studies have highlighted the important roles played by phosphoinositide-3,4,5-triphosphate and small G proteins, but many questions remain.

Cell migrations are without doubt one of the most dramatic and fascinating aspects of cell biology, as well as one of the most important. Cell movements give shape and form to developing embryos and bring about the many connections and interactions between the cells of our nervous system during development (9). Later in life, cell movements are required for tissue maintenance and repair, whereas cells of our immune system migrate from the bloodstream toward sites of infection. In addition to its roles in normal physiology, inappropriate migration is the basis for several pathological conditions, including metastasis and chronic inflammatory diseases (25, 31). In many cases, concentration gradients of small molecules act as extracellular cues to guide and direct movement in space and time, a process known as chemotaxis.

Studies of *Dictyostelium discoideum*, a social amoeba, have provided many of the key insights into the mechanisms of chemotaxis, and these are largely conserved in mammalian cells such as neutrophils (18, 32, 38). Both systems share many of the core components, such as seven-transmembrane receptors and heterotrimeric G proteins, and employ largely similar downstream pathways. In *Dictyostelium*, chemotaxis plays a critical role in all stages of its life cycle. During vegetative growth, the individual amoebae sense gradients of metabolites secreted by the bacteria and yeast that they feed on. In response to starvation, *Dictyostelium* cells secrete a chemoattractant of their own, cAMP, which directs both sexual and asexual developmental programs. The sexual program is triggered by moisture and darkness and involves the aggregation of cells around a single zygote, resulting in the formation of a macrocyst (41). Brighter, dryer conditions favor the asexual program where the cells aggregate into mounds of ~100,000 cells that proceed to develop into multicellular fruiting bodies containing spores (1). It is in this context that chemotaxis in *Dictyostelium* has mainly been studied.

Directional Sensing Orients Cell Migration and Polarization

Cell migration is a complex process that requires the coordinated regulation of the cytoskeleton and cell adhesion. Actin polymerization at the cell cortex generates filaments that produce pseudopods and other membrane extensions that provide forward drive (34). In unstimulated *Dictyostelium* cells, pseudopods are formed at random positions independently of receptors and G proteins (FIGURE 1). To produce migration rather than ruffling or spreading, actin polymerization needs to be restricted to a defined region of the cell and extension of the leading edge must be synchronized with retraction of the cell's rear. This is accomplished through contractile force generated by myosin motor proteins and their interactions with actin filaments at lateral and posterior regions of the cell cortex (10, 46, 49, 55). In neutrophils and highly developed *Dictyostelium* cells, the efficiency and speed of movement is enhanced by polarization. For the purposes of this review, the term polarization refers to the elongated morphology along the anterior-posterior axis that is acquired by these cells. This process is dependent on actin polymerization and is thought to be established by positive feedback loops that occur at the leading edge (50, 51). In the presence of a chemoattractant concentration gradient, cells use a directional sensing system to produce "front"-specific responses at the region experiencing the highest levels of signal and "rear"-specific responses where stimulation is lowest (11, 17, 33). This system amplifies the shallow directionality of the gradient into sharp internal differences most clearly demonstrated by the relocalization of certain proteins to either the front or back of cells and can occur independently of the cytoskeleton (FIGURES 1 AND 2). Directional sensing serves to bias pseudopod formation toward the source of chemoattractant and uropod retraction in the rear and thus orients the direction of cell movement according to the direction of the chemoattractant gradient. Readers are directed to

the previous review from our laboratory (8) for a further discussion on the differences between polarization and directional sensing.

Knowing the scale at which these processes operate provides an appreciation of how sensitive this directional sensing "compass" is during chemotaxis. *Dictyostelium* amoebae have a length of ~10 μm, and in shallow gradients the difference in receptor occupancy at the front compared with the back can be as little as 2%. Even under these conditions, however, the cells are able to perform chemotaxis. Recent studies have begun to dissect the mechanisms of how this is achieved and have highlighted important roles for phosphoinositides and members of the Rho family of small GTPases.

The Role of Phosphoinositides in Directional Sensing

The development of fluorescent proteins and the ability to study the subcellular localizations of specific proteins in vivo has been an essential tool for much of the recent progress. The initial breakthrough that suggested an important role for phosphoinositides was the discovery that a pleckstrin homology (PH) domain-containing protein called cytosolic regulator of adenylyl cyclase (CRAC) labeled the front of cells performing chemotaxis (33). In unstimulated cells, CRAC is uniformly distributed throughout the cytoplasm, but in a gradient, it translocates to the membrane at the front of the cell (FIGURE 2). The PH domain was found to direct the localization of CRAC, and biochemical studies demonstrated that, as with a number of other known PH domains, the PH domain of CRAC bound to phosphoinositide-3,4-bisphosphate (PIP_2) and phosphoinositide-3,4,5-triphosphate (PIP_3). Other PH domain-containing proteins, such as PKB and PHD, that have similar lipid-binding properties were subsequently found to behave equivalently in *Dictyostelium* and neutrophils (12, 30, 43). In both cell types, chemoattractant stimulation induces PIP_3 synthesis, so together these findings support the conclusion that this lipid is generated and concentrated specifically at the front of the cell, where it mediates the recruitment of PH domain-containing proteins.

Recent studies have demonstrated how the coordinated regulation of phosphoinositol 3-kinase (PI3K), which synthesizes PIP_3 from PIP_2, and phosphatase and tensin homolog (PTEN), which catalyses the reverse reaction, achieves this striking localization pattern (11, 17). In *Dictyostelium*, PI3K is recruited from the cytosol to the plasma membrane in response to chemoattractants, whereas stimulation results in the dissociation of PTEN from the membrane. In amoebae exposed to a uniform stimulus of cAMP, these responses are transient and biphasic. A rapid initial response peaks by ~10–15 s after stimulation and is terminated after ~30 s. This is followed by a second, slower phase of activity that peaks at 1–2 min and then slowly subsides (5). During this time, PH domains localize to discrete patches on the plasma membrane, where they initiate random pseudopod formation (5, 35). In a gradient, however, PI3K is stably recruited from the cytosol to a defined region of plasma membrane that labels the front of the cell along with PH domain proteins (FIGURE 2). Conversely, PTEN falls off this region of membrane but remains persistently associated with membranes at the side and back of the cell. This distri-

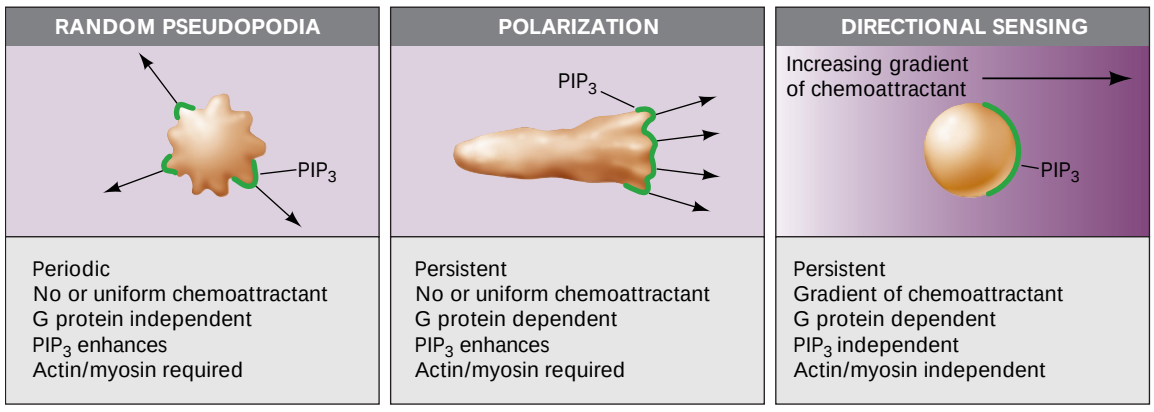

FIGURE 1. **Chemotactic migration**
Chemotactic migration can be divided conceptually into 3 separate processes: random pseudopod extensions, polarization, and directional sensing. In a chemoattractant gradient, directional sensing amplifies the spatial information of the gradient into sharp internal asymmetries by localizing proteins to either the front or the back of cells. This is thought to bias periodic pseudopod formation that otherwise occurs randomly in unstimulated cells. Directed movements then lead to polarization, an elongation of the cell shape, and persistent differences between the anterior and posterior of the cell that further restrict pseudopod formation to the front. Indicated in this diagram are the dependence of these 3 processes on chemoattractants, heterotrimeric G proteins, phosphatidylinositol-3,4,5-trisphosphate (PIP_3), actin, and myosin.

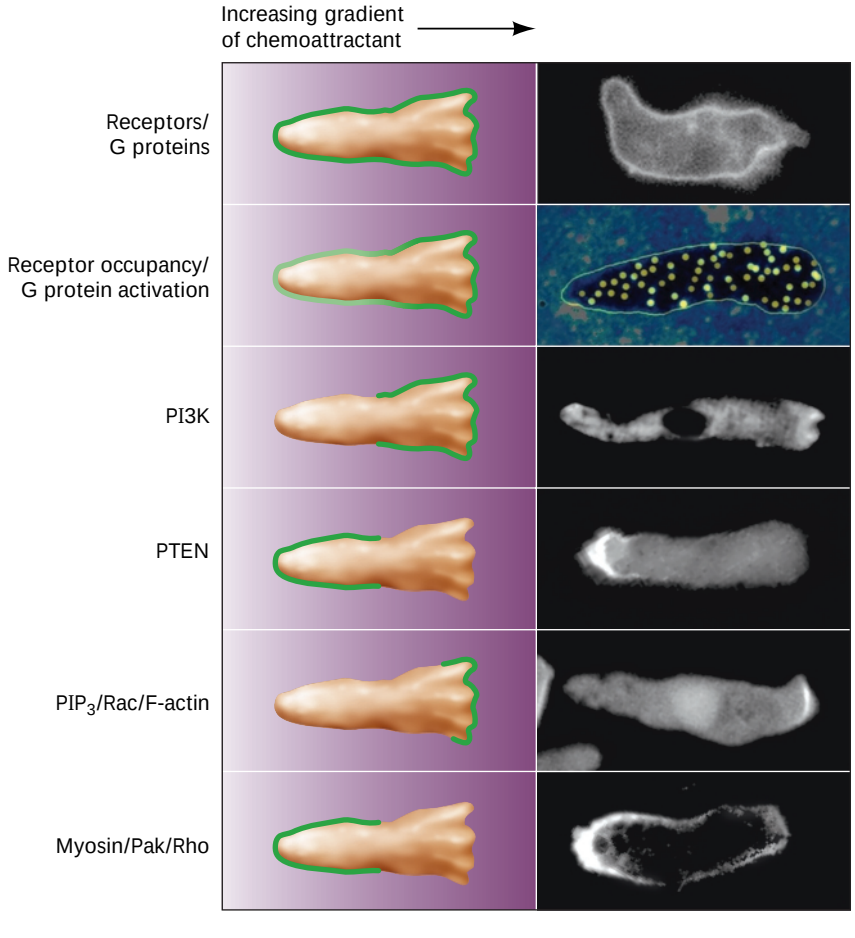

FIGURE 2. **Localization of key signaling molecules during chemotaxis**
Left: localization of the indicated signaling molecules in Dictyostelium cells migrating toward a chemoattractant source. *Right*: examples of (from *top* to *bottom*) cAMP receptor 1-green fluorescent protein (GFP), single-molecule imaging of Cy3-labeled cAMP bound to receptors at the cell surface, phosphoinositol 3-kinase (PI3K) 2-GFP, phosphate and tensin homolog (PTEN)-GFP, cytosolic regulator of adenylyl cyclase-GFP, and myosin II-GFP.

bution creates a situation in which PI3K generates PIP_3 at the front and PTEN focuses the distribution of this lipid by degrading it as it diffuses outside of this region (FIGURE 2).

In contrast to the localization of PI3K and PTEN, upstream components of this signaling pathway, such as the receptors and heterotrimeric G proteins, remain evenly distributed or in highly polarized cells become marginally enriched at the front of the cell (21, 23). Thus the gradient-induced effects on PI3K and PTEN localization are currently the earliest indication of symmetry being broken within the cell. Consequently, we and others (8, 18) have proposed that the ability to regulate the localization of these components forms the core of the directional sensing system and serves to orient PIP_3 accumulation. The strongest evidence for this model comes from PTEN loss-of-function mutations, which in both *Dictyostelium* and mammalian cells promote the frequency and duration of membrane extensions (17, 28). This effect is particularly striking in the *Dictyostelium* deletion mutant. In these cells, PIP_3 levels remain elevated for a markedly prolonged time after stimulation and are also higher in the absence of chemoattractant. This results in increased actin polymerization that at the cellular level translates into excessive membrane protrusions, often in directions other than that of the gradient, and a severe chemotaxis defect. Also supporting this model is the recent observation that diphosphoinositol pentakisphosphate (IP_7) acts as a negative regulator of chemotaxis in vivo by competing with PIP_3 for binding to PH domains (29). Production of this second messenger is stimulated by cAMP in *Dictyostelium*, and, interestingly, cells unable to produce IP7 aggregate faster in response to starvation than wild-type cells.

Other data, however, suggest that the story may not be that simple. Disruption of the two most active PI3K isoforms in *Dictyostelium* has only a partial effect on chemotaxis (5, 11). The cells move slower and deviate more frequently from the straight-line path to the chemoattractant source but nevertheless move toward chemoattractants and are able to aggregate when plated on nonnutrient agar. Arguing against the possibility of redundancy and compensating activity from other PI3K isoforms, treating the amoebae with the PI3K inhibitors also results in similarly mild defects (5). More pronounced effects have been reported for neutrophils lacking the G protein-regulated PI3Kγ gene, but even in this system there does not appear to be an absolute requirement for PIP_3 because studies using PI3K inhibitors have only partial effects (14, 40, 50). In summary, it is currently

unclear whether the confusion over the requirement for PI3K in chemotaxis can be explained by residual amounts of PIP_3 synthesis or whether phosphoinositide-independent pathways may operate in parallel.

The Role of Rho-GTPases in Directional Sensing

Recent studies in neutrophils have also suggested that members of the Rho family of small G proteins, which are established regulators of cell polarity and cytoskeletal dynamics in other systems, may play a role in directional sensing. Active RhoA accumulates in the rear of migrating cells and appears to direct uropod retraction by regulating the assembly of myosin II filaments and their interactions with actin at the cell cortex (55). In neutrophils, this proceeds through a cascade including ROCK and myosin light-chain kinase. The importance of the actinomyosin network in the rear and lateral regions of the cell has been demonstrated in neutrophils through the use of myosin light-chain kinase inhibitors and in *Dictyostelium* by knocking out the myosin II heavy-chain gene (10, 42, 53). These manipulations lead to reductions in the speed of migration due the failure of these cells to retract their uropods as they move forward and an increased production of lateral pseudopods

Intriguingly, myosin filament formation and activity may be regulated by a different pathway in *Dictyostelium*. Firstly, the genome does not appear to contain a specific Rho homolog, although it does encode several members of the Rac family. Instead, p21-activated kinase (PAK) localizes to the back of chemotaxing amoebae and is required for the assembly of myosin II filaments in the region, a function that is dependent on the presence of PKB (7). Secondly, cGMP signaling has been implicated in myosin filament localization. Mutations in the guanylyl cyclases disrupt this process and strongly impair chemotaxis, as does loss of cGMP-binding proteins that have been identified in *Dictyostelium* (3). Finally, the binding of myosin heavy-chain kinase to filamentous actin at the front of the cell antagonizes myosin assembly at this location, thus restricting myosin activity to the sides and rear of the cell (39).

Recent studies have also implicated Rac and Cdc42 in directional sensing. In neutrophils, activated Rac1 and Cdc42 both concentrate at the leading edge during chemotaxis, and inhibiting their activities by dominant negative proteins significantly impairs chemotaxis (45). Dominant negative Rac1 largely abolishes chemoattractant-induced actin polymerization and polarization, whereas dominant negative Cdc42 interferes with the persistence and direction of pseudopods. An increasing body of data has begun to reveal interactions between the PIP_3 pathway, actin polymerization, and Rac and Cdc42 activation. Firstly, providing neutrophils with exogenous PIP_3 is able to induce polarization and migration in the absence of chemoattractants (51). The simplest interpretation of this result is that the initial dose of phospholipid is reinforced by a positive feedback loop that stimulates actin polymerization and further PIP_3 synthesis. This is supported by the findings that persistent migration is dependent on PI3K activity and actin polymerization (50). Although a mechanism for the contribution of actin polymerization to this positive feedback remains unclear, the ability of several Rac GEFs to be recruited and activated by PIP_3 is likely to play a role in this process (52). Although this mechanism is essential for polarization, both *Dictyostelium* cells and neutrophils in which this loop has been disrupted retain the ability to sense direction. This is clear from the ability of cells immobilized by pharmacological inhibitors of actin polymerization to localize PH domain proteins to the front when placed in a gradient (33, 50). A recent study has also shown that the localization of PIP_3 to the leading edge confines the activity of the Cdc42 exchange factor PAK-interacting exchange factor-α to this region (27). In this study, the loss of PI3Kγ or treating cells with LY-294002 prevented the localized activation of Cdc42.

A negative-feedback pathway has been observed between Rac activity at the front and RhoA activity at the back of the cell, which may also contribute to directional sensing by spatially separating the activity of the two G proteins. This is based on the findings that overexpression of constitutively active RhoA inhibits Rac activity and pseudopod formation at the leading edge, whereas the inhibition of actin polymerization by treating cells with latrunculin B or LY-294002 increased the levels of RhoA-GTP (55).

Models of Directional Sensing

Directional sensing is remarkable for its sensitivity, dynamic range, and responsiveness to changes in direction. Sensitivity is reflected in the ability to sense both shallow gradients and low concentrations of chemoattractants. Cells are also able to rapidly adjust to a change in the direction of chemoattractant, either by extending another pseudopod in the new direction or by turning around to reorientate the leading edge, depending on the extent of the cell's polarization. The studies described above detail the progress that has been made in understanding how directional movement is regulated and reveal how small asymmetries

specified by chemoattractant gradients are refined and amplified. This raises the key question of how this asymmetry is initially generated, and a number of models that try to explain how this is achieved have been proposed. A detailed evaluation of the various models is outside the scope of this article, and readers are directed to Refs. 8, 16, 36, and 37 for further discussion. Attention in this review will be focused on the local excitation-global inhibition model, which to date has proved to be the most successful in accounting for the behavior of chemotactic cells. The foundation of this model is the observation that cells respond to uniform chemoattractant stimulation by transiently activating downstream signaling pathways. This is the case for PIP_3 synthesis, as discussed previously, and is also true for all of the known signaling responses. This suggests that chemoattractants trigger two signaling pathways, a rapid excitation response and a slower inhibitory pathway that allows cells to adapt to constant stimulation. Consistent with this hypothesis, in cells exposed to a uniform stimulus, PI3K is recruited to the entire cell membrane (rapid excitation response) and then falls off once the slower inhibition takes effect (FIGURE 3). Conversely, under the same conditions, PTEN transiently dissociates and then returns to the plasma membrane. At the cellular level, this results in an initial "cringing" response as actin polymerization is initiated throughout the cell, followed by slower, randomly localized pseudopodia and then a return to the prestimulation morphology as the cell adapts (35).

If the excitation response is a local, spatially restricted phenomenon and inhibition is mediated by a freely diffusible, globally acting factor, a simple mechanism for gradient amplification emerges (FIGURE 3). The level of excitation at the front of a cell in a gradient will be marginally greater than the back, corresponding to the differences in receptor occupancy. In contrast, the level of inhibition, because of its diffusible nature, will be equal throughout the cell and depend on total receptor occupancy. As the activity of the inhibitory response slowly reaches a steady state, the level of excitation only exceeds global inhibition at the front of the cell. Thus when a gradient is first applied, there will be an initial cringing response that is followed by persistent migration in the direction of the concentration gradient. A mathematical model incorporating these principles accounts for many of the observed features of chemotaxis: its sensitivity, its adaptation to constant levels of stimulation, its response to changes in the direction of gradient, the range of concentrations that can direct chemotaxis, and the ability to form two leading edges if cells are exposed to two sources of chemoattractant (22, 26).

Polarization and Directional Sensing

This model fails, however, to explain the polarization of chemotaxing cells and the response of highly polarized cells to changes in the direction of the chemoattractant gradient. The local excitation-global inhibition model predicts that cells should change direction by retracting the original pseudopod and generating a new leading edge when the direction of the gradient is changed. However, neutrophils and highly developed *Dictyostelium* cells become increasingly elongated and polarized as they migrate and will generally respond to changes in gradient direction by turning around and redirecting the existing leading edge. These features can be accounted for if some form of positive feedback is incorporated in to the local excitation-global inhibition framework.

Although directional movement necessarily demands some degree of polarization, several lines of evidence indicate that acquisition of the high level of polarity referred to above is a distinct process that is guided by directional sensing but is not an intrinsic characteristic. *Dictyostelium* cells starved for <5 h or vegetative cells sensing folate gradients do not become particularly elongated during chemotaxis and remain uniformly sensitive around their perimeter. Conversely, extensive polarization can be achieved in the absence of directional sensing. For example, highly developed amoebae and neutrophils migrate randomly in the absence of a gradient and are highly polarized. This may reflect strong positive feedback at the leading edge of these cells that is not present in less-developed or vegetative amoebae. This mechanism probably serves to enhance the efficiency of chemotaxis, and, consistent with this hypothesis, the extent of polarization correlates with the speed and persistence of migration.

Regulation of PI3K and PTEN Localization

The dynamic association of PI3K and PTEN with the plasma membrane and the spatial distribution of these proteins in vivo strongly suggest that their localization is regulated through the creation and destruction of membrane binding sites. Determining the identity of these sites and how they are regulated are key goals for future research, because the answer to these questions is likely to provide insight into how chemoattractant gradients establish the initial intracellular differences that become amplified in the process of directional sensing. Currently these issues are very poorly understood, but some recent studies have begun to investigate this question, and these data are discussed below.

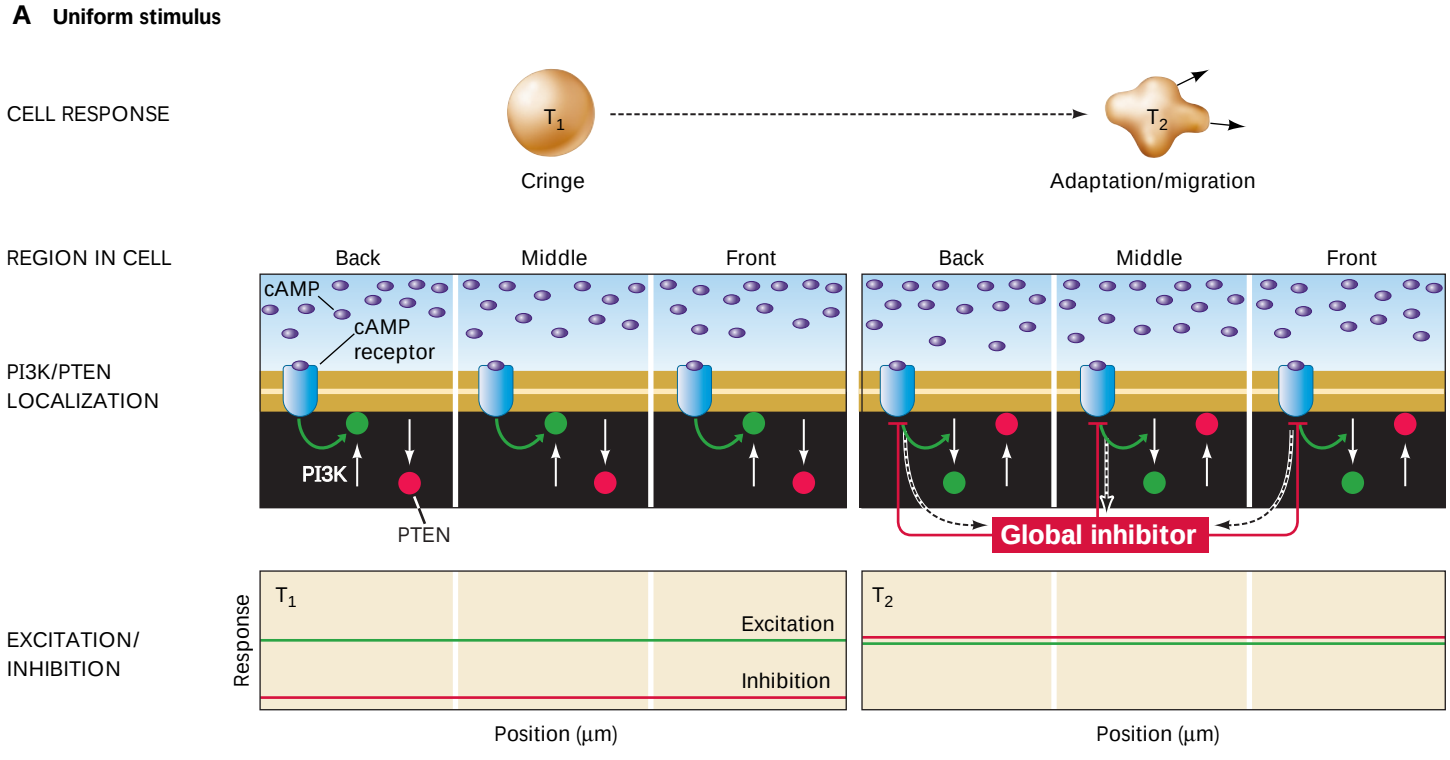

FIGURE 3. **Local excitation-global Inhibition model of directional sensing**
The outcomes predicted by this model in response to either a uniform stimulus (*A*) or a gradient stimulus (*B*) immediately after stimulation (T_1) and after the cells adapt (T_2) are shown. *Top*: changes in cell shape. *Middle*: receptor signaling to excitatory (green arrows) and inhibitory (red arrows) pathways along the back, middle, and front of the cell. *Bottom*: relative activities of excitation and inhibition along the length of the cell.

The three PI3K genes that have been reported in *Dictyostelium* are most closely related to the class I isoforms of mammalian PI3K (57). They share a conserved Ras-binding domain, a C2-like domain, and catalytic domains but have an extended amino terminus that is not present in the mammalian homolog. The interaction of Ras with the Ras-binding domain is required for activation but does not appear to mediate membrane association (11). Instead, in the case of *Dictyostelium* PI3K, it is the amino terminus that plays a key role in this process. Deletion of this region abolishes binding, whereas fusing this domain to green fluorescent protein (GFP) results in cAMP-dependent membrane translocation. This region does not, however, share significant homology with any other known domains, and interestingly, the amino terminals of the three *Dictyostelium* isoforms diverge considerably from one another, even though the localization of all three proteins is thought to be similarly regulated. In vertebrates, a regulatory subunit named p101 has been implicated in directing the membrane association of PI3Kγ, the G protein-regulated isoform. No obvious homologs of p101 exist in the completed *Dictyostelium* genome, but it remains an open question whether or not a functionally similar protein plays a role. The regulatory subunit interacts with both the catalytic subunit of PI3Kγ and the Gβγ complex, and this interaction has been suggested to mediate membrane association in mammalian cells. In favor of this model, a recent study reported that p101 and Gβγ cooperate in promoting the membrane localization of PI3Kγ (4). However, other reports have suggested that PI3Kγ constitutively binds lipids and that the role of G proteins and p101 is to regulate kinase activity (24, 47). Yet another report attributes PI3Kγ localization to its affinity for lipid rafts (13). Determining the localization of endogenous PI3Kγ in response to signals will be critical in resolving these conflicts. A final point is that although the Gβγ complex does interact with PI3K, free Gβγ dimers cannot be the sole determinant of PI3K membrane binding because their localization and activation kinetics do not correspond with those of PI3K.

The potential for PIP_3 itself to promote PI3K binding and thus act as a mechanism for positive feedback at the leading edge has also been a subject of speculation. This has largely been based on the result that adding exogenous PIP_3 induces motility and polarization in unstimulated neutrophils. Arguing against this hypothesis, however, in *Dictyostelium* at least, is the finding that the membrane translocation of a kinase-dead PI3K-GFP fusion protein in response to cAMP is not significantly inhibited in either *pi3k1/2*-null cells or wild-type cells treated with PI3K inhibitors (15). Thus PIP_3 probably induces positive feedback by mechanisms other than increasing PI3K membrane association.

The binding of PTEN to the plasma membrane requires a small region with homology to a PIP_2-binding motif found in other proteins that is located in the amino terminus of PTEN (19). This region, however, is not sufficient for membrane binding and cannot direct the translocation of heterologous proteins, indicating that other domains play a role in this process. Unlike *Dictyostelium*, PTEN has not been observed to constitutively associate with the plasma membrane of mammalian cells. The reason for this difference is not clear, and conflicting results have been described for PTEN localization during chemotaxis. Using antibodies against endogenous PTEN, Wu and colleagues (27) have been able to detect the asymmetric localization of PTEN to the rear of neutrophils performing chemotaxis. Interestingly, in this study PTEN was distributed generally throughout the rear of these cells, rather than being localized predominantly to the plasma membrane. How this localization pattern is achieved is unclear, but the authors have suggested a role for Cdc42 and the MAPK pathway (54). Other studies, however, have not been able to detect this redistribution of PTEN. Another recent study has implicated the membrane protein neutral endopeptidase in binding to PTEN and recruiting it to the plasma membrane (48). However, no regulation of this interaction was observed, indicating that this is unlikely to be the critical binding site in regulating the dynamic localization of PTEN during chemotaxis.

Conclusions and Future Directions

The research discussed above summarizes our current understanding of how cells sense and interpret chemoattractant gradients to generate directed cell movement by concentrating actin polymerization at the leading edge of the cell and myosin activity at the rear. The localization and activities of PI3K and PTEN as well as Rac, Cdc42, and Rho amplify small internal asymmetries that are induced by the external concentration gradient to define the region sensing the highest level of chemoattractant as the front and the regions sensing the lowest levels as the back. This system biases the generation of random pseudopods in unstimulated cells and consequently promotes migration in the direction of the gradient. Positive feedback loops may then reinforce this asymmetry and lead to increased polarization of the cell, which in turn enhances the efficiency of chemotaxis and the sensitivity of the leading edge to chemoattractant signals.

Several fundamental questions, however, remain

unanswered. Although the ability of cells to adapt to constant or uniform stimulation clearly plays an important role in directional sensing, the mechanisms underlying this process are not known. In other G protein-coupled receptor systems, such as the photoreceptors of the eye or the yeast mating factor receptors, desensitization is thought to be largely achieved via regulator of G protein signaling (RGS) proteins and agonist-induced receptor phosphorylation. RGS proteins stimulate the GTPase activity of the Gα subunits and thus promote reassociation of the G protein heterotrimers, whereas receptor phosphorylation leads to the binding of arrestin proteins that prevent further receptor-G protein interactions. In *Dictyostelium*, however, the Gα and Gβγ subunits remain disassociated as long as receptors are occupied, even after they become phosphorylated (21). A further question is how does the directional sensing apparatus regulate actin polymerization? Previous models suggested that WAVE/SCAR proteins act downstream of PIP_3 to regulate actin polymerization through the Arp2/3 complex. However, recent findings indicate that the SCAR complex is not required for chemoattractant-induced actin polymerization, suggesting an important role for other proteins (2). Finally, we may also have a very limited understanding of the complexity of the signaling pathways involved in chemotaxis. Studies in *Dictyostelium* and other organisms have identified a number of other genes required for chemotaxis that are either of unknown function or do not appear to play a role in the mechanisms discussed in this review (6, 20, 44, 56). Research into these areas will undoubtedly stretch our current models of chemotaxis and challenge investigators for many years to come. ■

We acknowledge the National Institutes of Health for funding and thank the members of the Devreotes lab for helpful discussions and comments. We also thank Chris Janetopoulos for the image of myosin II-GFP and T. Yanagida and M. Ueda for the image of receptor occupancy in Figure 2.

References

1. Aubry L and Firtel R. Integration of signaling networks that regulate *Dictyostelium* differentiation. *Annu Rev Cell Dev Biol* 15: 469-517, 1999.
2. Blagg SL, Stewart M, Sambles C, and Insall RH. PIR121 regulates pseudopod dynamics and SCAR activity in *Dictyostelium*. *Curr Biol* 13: 1480-1487, 2003.
3. Bosgraaf L, Russcher H, Smith JL, Wessels D, Soll DR, and Van Haastert PJ. A novel cGMP signaling pathway mediating myosin phosphorylation and chemotaxis in *Dictyostelium*. *EMBO J* 21: 4560-4570, 2002.
4. Brock C, Schaefer M, Reusch HP, Czupalla C, Michalke M, Spicher K, Schultz G, and Nurnberg B. Roles of Gβγ in membrane recruitment and activation of p110 γ/p101 phosphoinositide 3-kinase γ. *J Cell Biol* 160: 89-99, 2003.
5. Chen L, Janetopoulos C, Huang YE, Iijima M, Borleis J, and Devreotes PN. Two phases of actin polymerization display different dependencies on PI(3,4,5)P3 accumulation and have unique roles during chemotaxis. *Mol Biol Cell* 14: 5028-5037, 2003.
6. Chen MY, Long Y, and Devreotes PN. A novel cytosolic regulator, Pianissimo, is required for chemoattractant receptor and G protein-mediated activation of the 12 transmembrane domain adenylyl cyclase in *Dictyostelium*. *Genes Dev* 11: 3218-3231, 1997.
7. Chung CY and Firtel RA. PAKα, a putative PAK family member, is required for cytokinesis and the regulation of the cytoskeleton in *Dictyostelium discoideum* cells during chemotaxis. *J Cell Biol* 147: 559-576, 1999.
8. Devreotes P and Janetopoulos C. Eukaryotic chemotaxis: distinctions between directional sensing and polarization. *J Biol Chem* 278: 20445-20448, 2003.
9. Dormann D and Weijer CJ. Chemotactic cell movement during development. *Curr Opin Genet Dev* 13: 358-364, 2003.
10. Eddy RJ, Pierini LM, Matsumura F, and Maxfield FR. Ca^{2+}-dependent myosin II activation is required for uropod retraction during neutrophil migration. *J Cell Sci* 113: 1287-1298, 2000.
11. Funamoto S, Meili R, Lee S, Parry L, and Firtel RA. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* 109: 611-623, 2002.
12. Funamoto S, Milan K, Meili R, and Firtel RA. Role of phosphatidylinositol 3′ kinase and a downstream pleckstrin homology domain-containing protein in controlling chemotaxis in dictyostelium. *J Cell Biol* 153: 795-810, 2001.
13. Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez AC, and Manes S. Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *J Cell Biol* 164: 759-768, 2004.
14. Hannigan M, Zhan L, Li Z, Ai Y, Wu D, and Huang CK. Neutrophils lacking phosphoinositide 3-kinase gamma show loss of directionality during *N*-formyl-Met-Leu-Phe-induced chemotaxis. *Proc Natl Acad Sci USA* 99: 3603-3608, 2002.
15. Huang YE, Iijima M, Parent CA, Funamoto S, Firtel RA, and Devreotes P. Receptor-mediated regulation of PI3Ks confines PI(3,4,5)P3 to the leading edge of chemotaxing cells. *Mol Biol Cell* 14: 1913-1922, 2003.
16. Iglesias PA and Levchenko A. Modeling the cell's guidance system. *Sci STKE* 2002: RE12, 2002.
17. Iijima M and Devreotes P. Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell* 109: 599-610, 2002.
18. Iijima M, Huang YE, and Devreotes P. Temporal and spatial regulation of chemotaxis. *Dev Cell* 3: 469-478, 2002.
19. Iijima M, Huang YE, Luo HR, Vazquez F, and Devreotes PN. Novel mechanism of PTEN regulation by its PIP2 binding motif is critical for chemotaxis. *J Biol Chem*, 2004.
20. Insall RH, Borleis J, and Devreotes PN. The aimless RasGEF is required for processing of chemotactic signals through G-protein-coupled receptors in *Dictyostelium*. *Curr Biol* 6: 719-729, 1996.
21. Janetopoulos C, Jin T, and Devreotes P. Receptor-mediated activation of heterotrimeric G-proteins in living cells. *Science* 291: 2408-2411, 2001.
22. Janetopoulos C, Ma L, Devreotes PN, and Iglesias PA. Chemoattractant-induced phosphatidylinositol 3,4,5-trisphosphate accumulation is spatially amplified and adapts, independent of the actin cytoskeleton. *Proc Natl Acad Sci USA*, 2004.
23. Jin T, Zhang N, Long Y, Parent CA, and Devreotes PN. Localization of the G protein βγ complex in living cells during chemotaxis. *Science* 287: 1034-1036, 2000.
24. Krugmann S, Cooper MA, Williams DH, Hawkins PT, and Stephens LR. Mechanism of the regulation of type IB phosphoinositide 3OH-kinase byG-protein βγ subunits. *Biochem J* 362: 725-731, 2002.
25. Kunkel SL and Godessart N. Chemokines in autoimmunity: from pathology to therapeutics. *Autoimmun Rev* 1: 313-320, 2002.
26. Kutscher B, Devreotes P, and Iglesias PA. Local excitation, global inhibition mechanism for gradient sensing: an interactive applet. *Sci STKE* 2004: pl3, 2004.
27. Li Z, Hannigan M, Mo Z, Liu B, Lu W, Wu Y, Smrcka AV, Wu G, Li L, Liu M, Huang CK, and Wu D. Directional sensing requires Gβγ-mediated PAK1 and PIX α-dependent activation of Cdc42. *Cell* 114: 215-227, 2003.

28. Liliental J, Moon SY, Lesche R, Mamillapalli R, Li D, Zheng Y, Sun H, and Wu H. Genetic deletion of the Pten tumor suppressor gene promotes cell motility by activation of Rac1 and Cdc42 GTPases. *Curr Biol* 10: 401-404, 2000.

29. Luo HR, Huang YE, Chen JC, Saiardi A, Iijima M, Ye K, Huang Y, Nagata E, Devreotes P, and Snyder SH. Inositol pyrophosphates mediate chemotaxis in *Dictyostelium* via pleckstrin homology domain-PtdIns(3,4,5)P3 interactions. *Cell* 114: 559-572, 2003.

30. Meili R, Ellsworth C, Lee S, Reddy TB, Ma H, and Firtel RA. 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.

31. Murphy P, Ahmed N, and Hassan HT. Increased serum levels of vascular endothelial growth factor correlate with splenomegaly in polycythemia vera. *Leuk Res* 26: 1007-1010, 2002.

32. Parent CA. Making all the right moves: chemotaxis in neutrophils and *Dictyostelium*. *Curr Opin Cell Biol* 16: 4-13, 2004.

33. Parent CA, Blacklock BJ, Froehlich WM, Murphy DB, and Devreotes PN. G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* 95: 81-91, 1998.

34. Pollard TD and Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112: 453-465, 2003.

35. Postma M, Roelofs J, Goedhart J, Gadella TW, Visser AJ, and Van Haastert PJ. Uniform cAMP stimulation of *Dictyostelium* cells induces localized patches of signal transduction and pseudopodia. *Mol Biol Cell* 14: 5019-5027, 2003.

36. Postma M and Van Haastert PJ. A diffusion-translocation model for gradient sensing by chemotactic cells. *Biophys J* 81: 1314-1323, 2001.

37. Rickert P, Weiner OD, Wang F, Bourne HR, and Servant G. Leukocytes navigate by compass: roles of PI3Kγ and its lipid products. *Trends Cell Biol* 10: 466-473, 2000.

38. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, and Horwitz AR. Cell migration: integrating signals from front to back. *Science* 302: 1704-1709, 2003.

39. Rubin H and Ravid S. Polarization of myosin II heavy chain-protein kinase C in chemotaxing dictyostelium cells. *J Biol Chem* 277: 36005-36008, 2002.

40. Sadhu C, Masinovsky B, Dick K, Sowell CG, and Staunton DE. Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. *J Immunol* 170: 2647-2654, 2003.

41. Saga Y and Yanagisawa K. Macrocyst development in Dictyostelium discoideum. I. Induction of synchronous development by giant cells and biochemical analysis. *J Cell Sci* 55: 341-352, 1982.

42. Saito H, Minamiya Y, Kitamura M, Saito S, Enomoto K, Terada K, and Ogawa J. Endothelial myosin light-chain kinase regulates neutrophil migration across human umbilical vein endothelial cell monolayer. *J Immunol* 161: 1533-1540, 1998.

43. Servant G, Weiner OD, Herzmark P, Balla T, Sedat JW, and Bourne HR. Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. *Science* 287: 1037-1040, 2000.

44. Sobko A, Ma H, and Firtel RA. Regulated SUMOylation and ubiquitination of DdMEK1 is required for proper chemotaxis. *Dev Cell* 2: 745-756, 2002.

45. Srinivasan S, Wang F, Glavas S, Ott A, Hofmann F, Aktories K, Kalman D, and Bourne HR. Rac and Cdc42 play distinct roles in regulating PI(3,4,5)P3 and polarity during neutrophil chemotaxis. *J Cell Biol* 160: 375-385, 2003.

46. Stites J, Wessels D, Uhl A, Egelhoff T, Shutt D, and Soll DR. Phosphorylation of the *Dictyostelium* myosin II heavy chain is necessary for maintaining cellular polarity and suppressing turning during chemotaxis. *Cell Motil Cytoskeleton* 39: 31-51, 1998.

47. Suire S, Hawkins P, and Stephens L. Activation of phosphoinositide 3-kinase gamma by Ras. *Curr Biol* 12: 1068-1075, 2002.

48. Sumitomo M, Iwase A, Zheng R, Navarro D, Kaminetzky D, Shen R, Georgescu MM, and Nanus DM. Synergy in tumor suppression by direct interaction of neutral endopeptidase with PTEN. *Cancer Cell* 5: 67-78, 2004.

49. Uchida KS, Kitanishi-Yumura T, and Yumura S. Myosin II contributes to the posterior contraction and the anterior extension during the retraction phase in migrating *Dictyostelium* cells. *J Cell Sci* 116: 51-60, 2003.

50. Wang F, Herzmark P, Weiner OD, Srinivasan S, Servant G, and Bourne HR. Lipid products of PI(3)Ks maintain persistent cell polarity and directed motility in neutrophils. *Nat Cell Biol* 4: 513-518, 2002.

51. Weiner OD, Neilsen PO, Prestwich GD, Kirschner MW, Cantley LC, and Bourne HR. A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity. *Nat Cell Biol* 4: 509-513, 2002.

52. Welch HC, Coadwell WJ, Ellson CD, Ferguson GJ, Andrews SR, Erdjument-Bromage H, Tempst P, Hawkins PT, and Stephens LR. P-Rex1, a PtdIns(3,4,5)P3- and Gβγ-regulated guanine-nucleotide exchange factor for Rac. *Cell* 108: 809-821, 2002.

53. Wessels D, Soll DR, Knecht D, Loomis WF, De Lozanne A, and Spudich J. Cell motility and chemotaxis in *Dictyostelium* amebae lacking myosin heavy chain. *Dev Biol* 128: 164-177, 1988.

54. Wu Y, Hannigan MO, Kotlyarov A, Gaestel M, Wu D, and Huang CK. A requirement of MAPKAPK2 in the uropod localization of PTEN during FMLP-induced neutrophil chemotaxis. *Biochem Biophys Res Commun* 316: 666-672, 2004.

55. Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, Kelly K, Takuwa Y, Sugimoto N, Mitchison T, and Bourne HR. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* 114: 201-214, 2003.

56. Zhan Q, Bamburg JR, and Badwey JA. Products of phosphoinositide specific phospholipase C can trigger dephosphorylation of cofilin in chemoattractant stimulated neutrophils. *Cell Motil Cytoskeleton* 54: 1-15, 2003.

57. Zhou K, Takegawa K, Emr SD, and Firtel RA. 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.