

Moving Forward: Mechanisms of Chemoattractant Gradient Sensing

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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 adenyl 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 (PIP₂) and phosphoinositide-3,4,5-triphosphate (PIP₃). 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 PIP₃ 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 PIP₃ from PIP₂, 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-

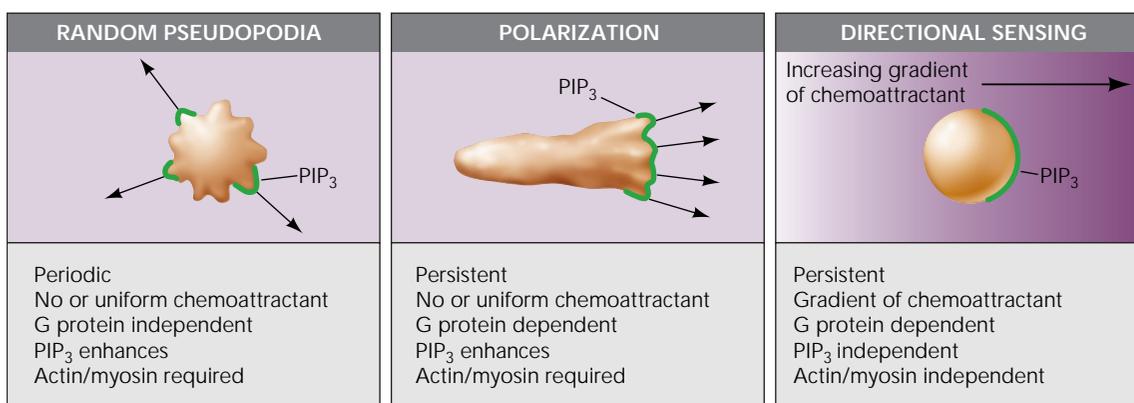


FIGURE 1. Chemotactic migration

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 (PIP₃), actin, and myosin.

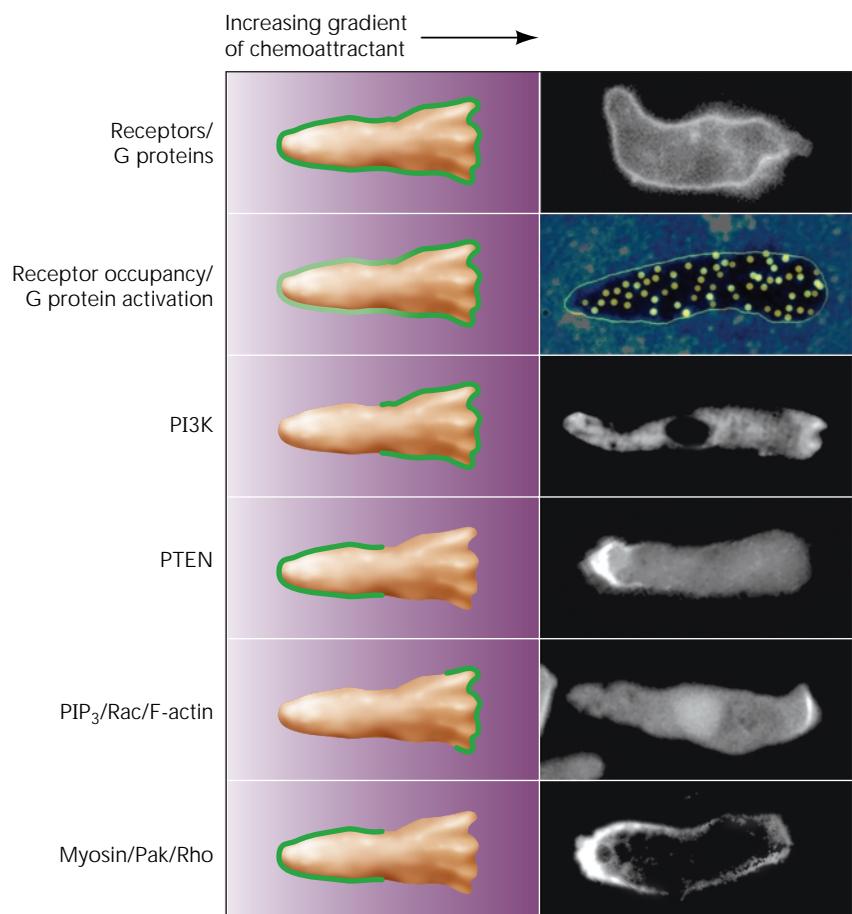


FIGURE 2. Localization of key signaling molecules during chemotaxis

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 IP_7 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 PI3K γ 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 PIP₃ 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 PIP₃ pathway, actin polymerization, and Rac and Cdc42 activation. Firstly, providing neutrophils with exogenous PIP₃ 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 PIP₃ 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 PIP₃ 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 PIP₃ 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 PI3K γ 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 pre-stimulation 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

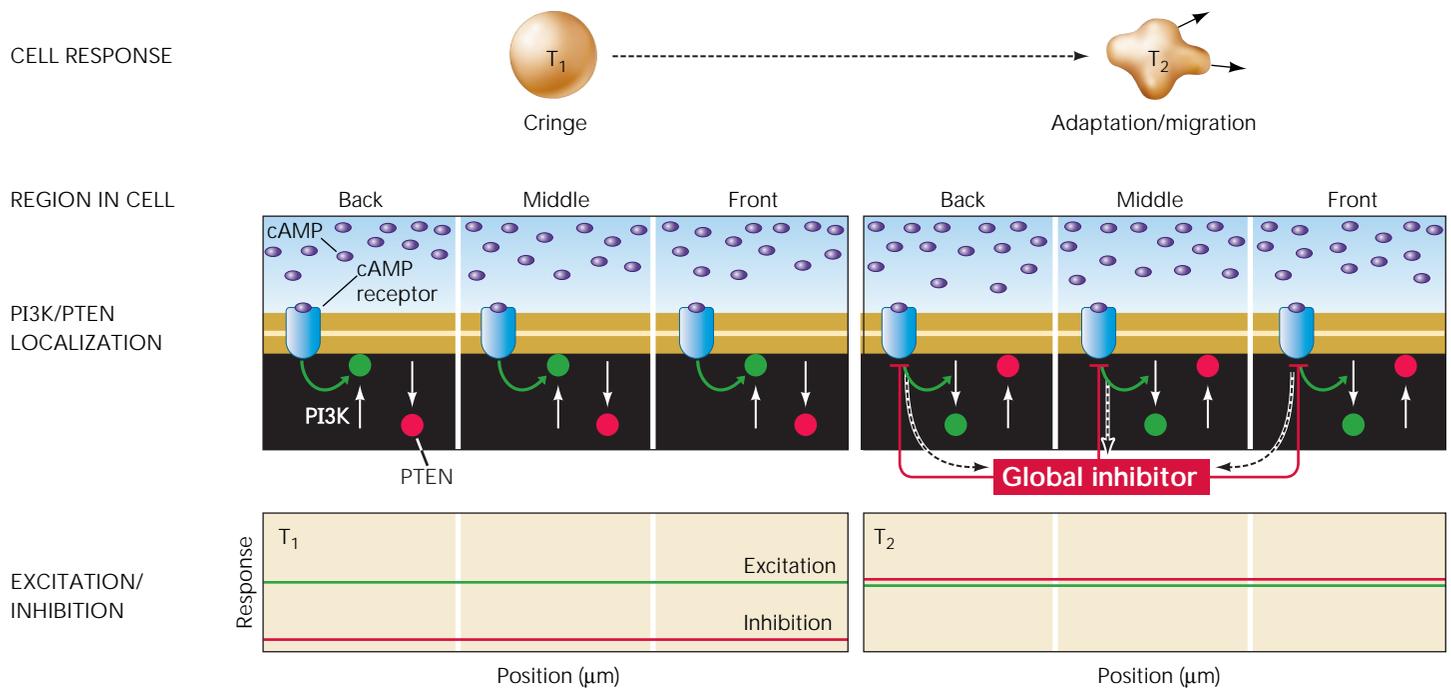
This model fails, however, to explain the polarization of chemotaxing cells and the response of highly polarized cells to changes in the direction of the chemoattractant gradient. The local excitation-global inhibition model predicts that cells should change direction by retracting the original pseudopod 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.

A Uniform stimulus



B Gradient stimulus

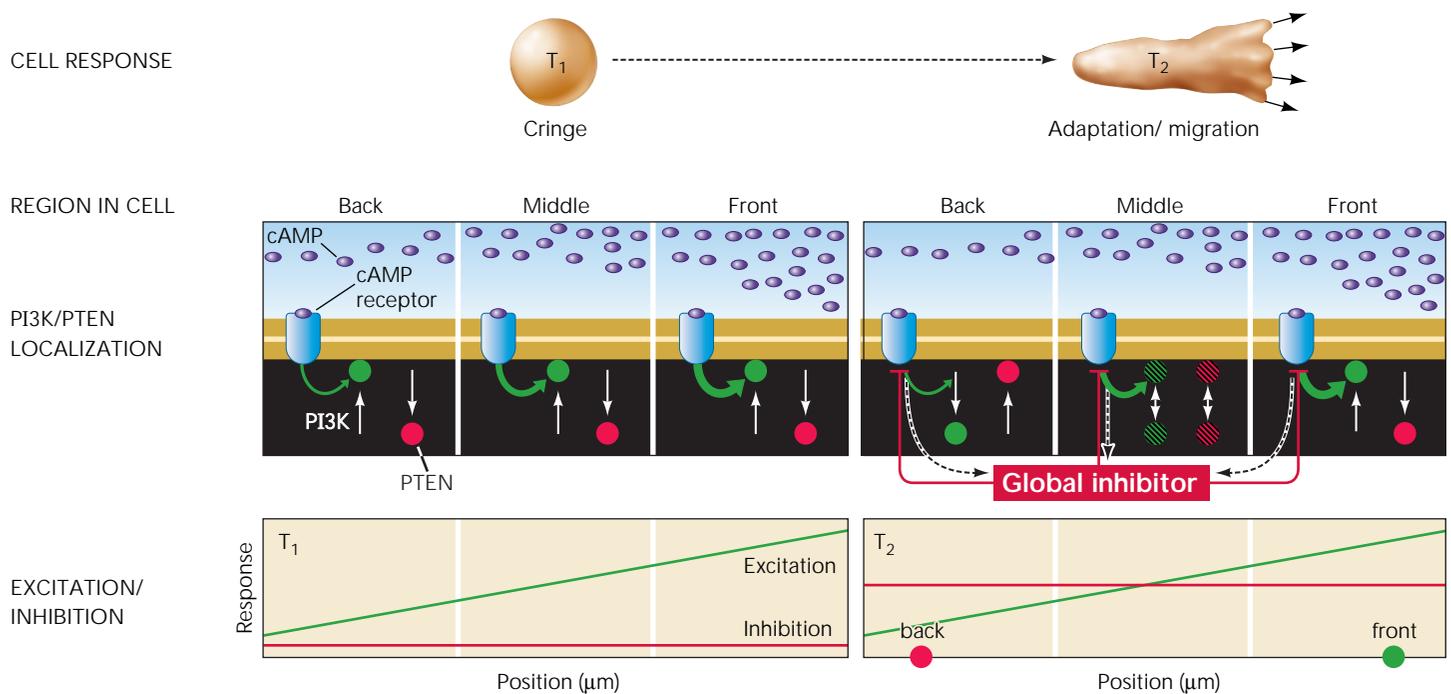


FIGURE 3. Local excitation-global Inhibition model of directional sensing

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 PI3K γ , 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 PI3K γ and the G $\beta\gamma$ 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 G $\beta\gamma$ cooperate in promoting the membrane localization of PI3K γ (4). However, other reports have suggested that PI3K γ constitutively binds lipids and that the role of G proteins and p101 is to regulate kinase activity (24, 47). Yet another report attributes PI3K γ localization to its affinity for lipid rafts (13). Determining the localization of endogenous PI3K γ in response to signals will be critical in resolving these conflicts. A final point is that although the G $\beta\gamma$ complex does interact with PI3K, free G $\beta\gamma$ 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 PIP₃ 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 PIP₃ 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 PIP₃ 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 PIP₂-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 $G\alpha$ 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 $G\alpha$ and $G\beta\gamma$ 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. ■

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