Minireview

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Eukaryotic Chemotaxis: Distinctions between Directional Sensing and Polarization*

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Directional sensing and polarization are fundamental cellular responses that play a central role in health and disease. In this review we define each process and evaluate a series of models previously proposed to explain these phenomena. New findings show that directional sensing by G protein-coupled receptors is localized at a discrete step in the signaling pathway downstream of G protein activation but upstream of the accumulation of PIP_3. Local levels of PIP_3, whether triggered by chemoattractants, particle binding, or spontaneous events, determine the sites of new actin-filled projections. Robust control of the temporal and spatial levels of PIP_3 is achieved by reciprocal regulation of PI3K and PTEN. These observations suggest that a local excitation-global inhibition model can account for the localization of PI3K and PTEN and thereby explain directional sensing. However, elements of other models, including positive feedback and the reaction of the cytoskeleton, must be invoked to account for polarization.

Directional Sensing Is a Fundamental Cellular Process

Many types of cells are able to sense extracellular directional cues and respond with asymmetric changes in cell morphology and motility. For example, during chemotaxis a chemical gradient serves as a directional signal that organizes cell movement. This intriguing process plays a central role in development, immunity, and tissue homeostasis (1–4). During embryogenesis, movements of cells in response to chemotactic stimuli bring form and organization to tissues and organs and steer axons in the formation of the nervous system (5–7). In the immune system, an elaborate network of chemoattractants directs leukocytes to their correct locations and facilitates cell-cell interactions. Chemotaxis is also central to wound healing and has been implicated in disease states such as metastasis and atherosclerosis (8–12). This review will focus on mechanisms of directional sensing with emphasis on chemotactic systems.

By investigating model chemotactic systems such as *Dictyostelium discoideum*, researchers are uncovering the general principles by which cells sense asymmetric environmental stimuli (13–15). Mechanisms of chemotaxis in mammalian cells are remarkably similar to those in this genetically tractable organism (16–18). During growth, *D. discoideum* amoebae use chemotaxis to track down and phagocytose bacteria. When starved, the cells differentiate, polarize, and migrate directionally toward secreted 3′,5′-cyclic adenosine monophosphate (cAMP). The cAMP is detected by four serpentine receptors, designated cAR1–cAR4, coupled to a single heterotrimeric G protein (19). A similar situation is found in mammalian leukocytes where 20 types of chemoattractant, or chemokine, receptors couple to the same G protein, G_i (20, 21). Other similarities with mammalian systems include chemoattractant-elicited transient increases in phosphoinositides (PIs),[1] cAMP, cGMP, inositol trisphosphate, and Ca^{2+} and rearrangements in the cytoskeleton (16, 22). PIP_3 has emerged as an important intermediate in chemotactic signaling in *D. discoideum* amoebae and mammalian leukocytes (23–34).

The terms directional sensing, polarity, and chemotaxis are often used interchangeably. We offer these definitions to more clearly distinguish these phenomena. Directional sensing refers to the ability of a cell to detect an asymmetric extracellular cue and generate an internal amplified response (15). In cells exposed to shallow gradients in chemoattractant concentration, signaling molecules accumulate at the membrane adjacent to the higher concentration and initiate downstream responses locally. This localized activation can be visualized, for example, with proteins containing a PH domain fused to green fluorescent protein (GFP) (Fig. 1). The directional sensing response does not require the cell to be polarized. Unpolarized, immobilized cells can also detect gradients with a similar degree of signal amplification (Fig. 1, *left*). The sensitivity to chemoattractant is uniform around the perimeter, and when the gradient is shifted, the PH domain proteins rapidly redistribute according to the new direction (Fig. 1, *left*).

Polarization defines the propensity of the cell to assume an asymmetric shape with a defined anterior and posterior. Molecules associated with the "leading edge" include actin and actin-binding proteins Scar, WASP, filopodin, cofilin, and coronin, whereas molecules associated with the trailing edge include myosin II and cortexillin (35–39). In polarized cells the anterior surface is more sensitive to chemoattractants than other regions. When the direction of chemoattractant gradient is changed, a polarized cell generally turns toward the new highest concentration and maintains its original anterior instead of redistributing PH domains (Fig. 1, *right*). A very steep gradient in an opposing direction can sometimes override this asymmetry and generate a new axis in the new direction (not shown). The localized sensitivity afforded by polarization focuses the activity of the actin cytoskeleton at the leading edge, resulting in faster movement toward a chemoattractant source. However, the sensing must occur within a small zone at the front rather than across the entire cell diameter. In contrast, the symmetrical sensitivity of the unpolarized cell means the area involved in gradient detection is larger (Fig. 1, *left*).

Cells display various degrees of polarization that may also change with conditions. In general, neutrophils are immobile until exposed to chemoattractant. They then polarize, acquire a distinct leading edge and uropod, and begin to move (16, 40–42). Growth stage *D. discoideum* amoebae are unpolarized and move randomly without exogenous chemoattractant. These cells can still sense direction and carry out chemotaxis. As they differentiate, they become elongated, motile, and highly chemotactic (43). Polarization can also be enhanced by a period of directed movement in a gradient. Unlike directional sensing, polarization depends critically on the actin cytoskeleton, and inhibitors of actin polymerization convert a polarized cell to an unpolarized one. This treatment eliminates both polarized morphology and sensitivity, suggesting that an interaction of key signaling molecules with the cytoskeleton stabilizes the polarized state (44, 45). Here we focus on the mechanisms of directional sensing and speculate on emerging models for polarization and chemotaxis.

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[1] The abbreviations used are: PI, phosphoinositide; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; GFP, green fluorescent protein; PH, pleckstrin homology; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphatidylinositol 3-kinase; PTEN, PI 3-phosphatase.

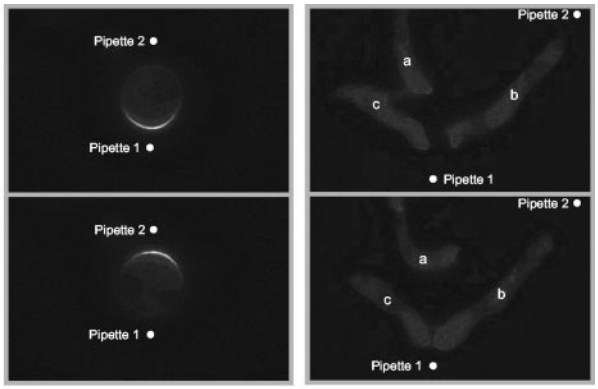

FIG. 1. **Unpolarized *D. discoideum* cells are equally responsive at all points on their perimeters whereas polarized amoebae have restricted sensitivity.** Cells expressing PH_{Crac}-GFP sense a gradient of cAMP released from a micropipette. A latrunculin-treated cell (*top panel*, *left*) displays PH_{Crac}-GFP binding to the membrane on the side of the cell exposed to gradient emanating from pipette 1 (*dot*), and then rapidly (within 60 s) translocates to the other side when pipette 2 (*dot*) is turned on. Polarized cells initially chemotax toward pipette 1 (*top panel*, *right*). When a competing gradient from pipette 2 (*dot*) is turned on, they either turn or continue forward. (The rear of cell *b* is actually closer to pipette 2.) Time between frames in *right panels* is 30 s.

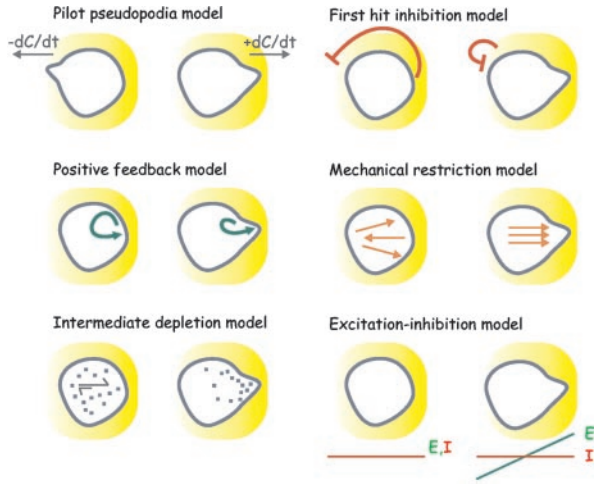

FIG. 2. **Salient models proposed to explain chemotaxis and polarity.** Each *panel* indicates the same cell at an initial and an advanced stage of gradient sensing. In the gradient represented by the *yellow shading*, the highest concentration is on the *right*. In the "pilot pseudopodia" model, pseudopodia are reinforced only when they detect an increasing concentration ($+dC/dt$). In the "first hit inhibition" model, an inhibitory molecule (*red line*) diffuses rapidly through the cell or along the membrane and blocks the back of the cell from responding further. "Positive feedback loops" of internal signaling components (*green arrows*) have also been proposed to amplify the shallow gradient across the cell. "Mechanism restriction" models invoke the cytoskeleton (*red arrows*) to couple an extension at the front of the cell to a retraction in the back. The "intermediate depletion" model proposes that binding of a limited internal signaling component (*gray dots*) is highly cooperative. The excitation-inhibition model proposes that the response depends on a balance between rapid excitation and slower inhibition processes. Excitation (*E*, *green*) reflects local receptor occupancy whereas an inhibition (*I*, *red*) reflects average receptor occupancy across the cell.

Series of Models Proposed to Account for Directional Sensing and Polarization

Fig. 2 illustrates some of the ideas that have been put forward to explain direction sensing and polarization. An early proposal, based on the temporal mechanism of chemotaxis in *Escherichia coli*, holds that a eukaryotic cell extends "pilot pseudopodia" in random directions (16, 46, 47). Those extended up the gradient experience a positive change in chemoattractant concentration and are reinforced whereas those projected down the gradient receive a negative signal and are extinguished. The random walk of pseudopodia tends to move the cell steadily toward the attractant. A second proposal reasons that a gradient applied to a cell must first contact the cell on one side (48). This "first hit" triggers a rapid inhibitory response that spreads across the cell and prevents the posterior from responding. When the gradient is repositioned, there is again an initial contact and the direction of the response is reset.

A third class of models is based on powerful internal "positive feedback" loops. Signaling molecules are selectively amplified at the anterior of the cell and thereby localize the response (49, 50). Several models link a positive action at the front of the cell to an opposing action at the back. In the "mechanical restriction" model an extension at the front of the cell is physically coupled to a retraction at the back. In the "intermediate depletion" model highly cooperative binding at the front limits the availability of free signaling molecules at the back of the cell (51). Finally, the "local excitation-global inhibition" model proposes that directional sensing depends on a balance between a rapid, local "excitation" and a slower global "inhibition" process (15, 16, 52–54). Receptor occupancy controls the steady-state levels of each process, and the difference between the two regulates the response. Because inhibition depends on average receptor occupancy, its steady-state level is less than that of local excitation at the front of the cell. At the back, the situation is reversed.

Many of these concepts are useful to our understanding of directional sensing and polarization, but none can account for observed responses under all experimental paradigms. The "pilot pseudopodia" model cannot explain how a completely immobile cell that is unable to extend projections is still able to amplify a stable external gradient (Fig. 1, *left*). The "first hit" inhibition model cannot account for the ability of a cell to sense a gradient formed by lowering the concentration from an initially high uniform level.[2] The "positive feedback" models provide large amplification, but once initiated the response becomes relatively independent of the external signal. This property is useful for polarization but is inconsistent with the ability of an unpolarized cell to respond to rapid shifts in directional input. The mechanical restriction model is incompatible with the capacity of a paralyzed cell to sense the external gradient (Fig. 1, *left*). The "intermediate depletion" model requires strong cooperative binding and cannot account for the ability of the cell to respond over a wide range of stimulus concentrations.

The "local excitation-global inhibition" model is consistent with many features of the chemotactic responses. Cells respond to *changes* in receptor occupancy and *adapt* when occupancy is held constant. The model accounts for transient responses, the directional responses to spatial gradients, and for observed responses to combinations of temporal and spatial stimuli. It is also consistent with the ability of the cell to respond to gradients with a wide range of midpoint concentrations. However, the model lacks the large amplification afforded by positive feedback and does not explain the slow reactions of polarized cells to shifts in the external gradients (see Fig. 1, *right*). A comprehensive, predictive scheme for directional sensing *and* polarization will likely bring together elements from a number of these models.

Directional sensing of chemoattractants occurs within the signaling pathway after G protein activation and before the accumulation of PIP_3. During directional sensing and polarization there is surprisingly little redistribution of the upstream components and biochemical reactions in the signaling pathway. In unpolarized cells, the chemoattractant receptors and G proteins are distributed uniformly along the cell membrane, whereas receptor occupancy closely mirrors the shallow concentration gradient of chemoattractant (Fig. 3) (55–57).[2] Cell polarization leads to only subtle changes in these parameters; the G protein subunits acquire a slightly asymmetric distribution toward the front of the cell, and the on and off rates of cAMP binding are faster at the anterior end (55, 58). G protein activation has not been directly imaged, but its kinetics suggests that it is not sharply confined to the front of cells whether or not they are polarized. During chemoattractant stimulation, the G protein α- and $\beta\gamma$-subunits remain dissociated as long as receptors are occupied (59). It is difficult to envision then how the G proteins would be inactivated at the back of the cell where receptor occupancy is only slightly lower than at the front. Rather, it seems likely that a global inhibitory process offsets G protein activation at the back of the cell and thereby localizes responses to the front.

The accumulation of PIP_3 at the cell anterior is an early point where strong asymmetric activation of the signaling pathway is observed (Fig. 1). This was first shown in *D. discoideum* by visualization of these PIs with a variety of GFP-tagged PH domain-

[2] P. Devreotes and C. Janetopoulos, unpublished observations.

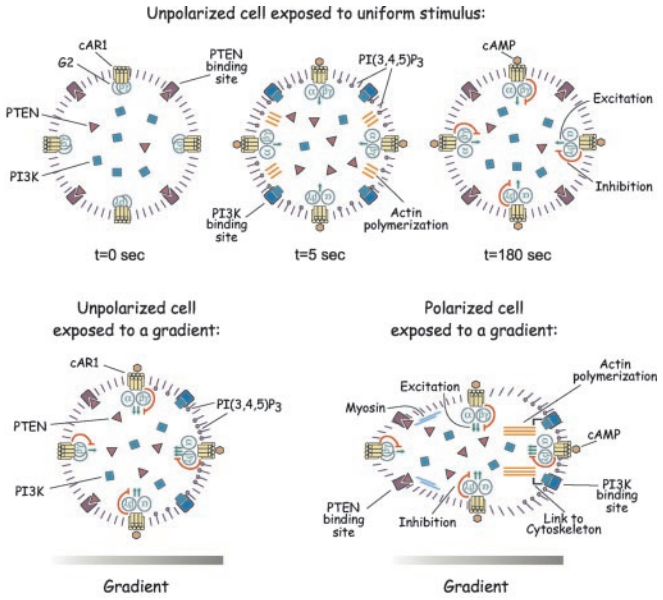

FIG. 3. **Cartoon depicting the distribution and activity of signaling molecules in unpolarized and polarized cells during temporal and spatial stimulation.** Chemoattractant receptors (cAR1), receptor occupancy, associated and dissociated (activated) G protein $\alpha\beta\gamma$-subunits, excitation, inhibition, PI3Ks, PTEN, PI3K and PTEN binding sites, PIP_3, F-actin, and myosin are indicated. *Upper diagram* illustrates the response to a temporal stimulus. In resting cells, PTEN is bound to the membrane and PI3Ks are in the cytosol (t = 0 s). An increase in receptor occupancy by chemoattractant (*orange hexagons*) triggers, through the heterotrimeric G proteins, a rapid increase in excitation (*green arrow*), which leads to binding of PI3Ks (*light blue squares*) to their binding sites (*dark blue squares*) at the membrane and causes PTEN (*maroon triangles*) to dissociate from binding sites (*purple squares*) at the membrane (t = 5 s). The combined effect causes a large increase in PIP_3 (*green lollipops*). At longer times, inhibition (*red line*) increases and eventually balances excitation. PI3Ks return to the cytosol, PTEN returns to the membrane, and PIP_3 returns to prestimulus levels (t = 180 s). *Lower diagram* shows the response of a cell treated with latrunculin A (*left*) and of a polarized cell (*right*) in a spatial gradient. The appearance of the polarized cell would be similar in a uniform concentration of attractant (see text). The "global" inhibition (*red line*) is equal at both ends of the cell. "Local" excitation is slightly higher at the front causing the binding of PI3K to and the loss of PTEN from the membrane at the front. This leads to a large steady-state accumulation of PIP_3 selectively at the front and, in untreated cells, actin polymerization and directed motility.

containing proteins (44, 60). Similar asymmetric localizations of PH domains occur in leukocytes exposed to gradients of chemoattractants (33). PI accumulations are transient in cells exposed to uniform chemoattractant, whereas the G protein subunit dissociation is not (Fig. 3, *top*). In cells lacking functional G proteins, chemoattractants do not elevate PIP_3 levels (45, 61), whereas inhibitors of the cytoskeleton, such as latrunculin A, do not interfere with the response (see Fig. 1, *left*). Taken together, these observations and others suggest that gradient detection becomes sharply localized at a step downstream of G protein activation and upstream of the generation of the PIs (14, 59).

Further evidence for a key role of PIP_3 in directional sensing has come from studies of the subcellular distribution of PI3Ks and PTEN in *D. discoideum* (62, 63). These enzymes are reciprocally regulated in response to chemotactic stimulation (Fig. 3). In resting cells, the PI3Ks are cytosolic whereas a fraction of PTEN is bound to the plasma membrane. Uniform addition of chemoattractant results in the translocation of PI3Ks to the membrane while PTEN rapidly dissociates. Then, PI3Ks return to the cytosol and PTEN reassociates with the membrane. The PTEN reassociation results in a higher than prestimulus level of membrane-bound PTEN. In a gradient, the PI3Ks are recruited to the front of the cell, and PTEN associates with the membrane at the back (Fig. 3, *bottom*). Interestingly, this spatial asymmetry in the distributions of the two enzymes is greater in polarized *versus* unpolarized cells.

The movements of PI3K and PTEN and the changes in PIP_3 levels suggest that the enzyme activities are reciprocally regulated during the response. Indeed, there is an extremely rapid increase in PI3K activity following an increase in chemoattractant (64). Cell lysates prepared within 5 s of addition of a stimulus incorporate ^{32}P-labeled γ-ATP into [^{32}P]PIP_3 about 6-fold higher than lysates

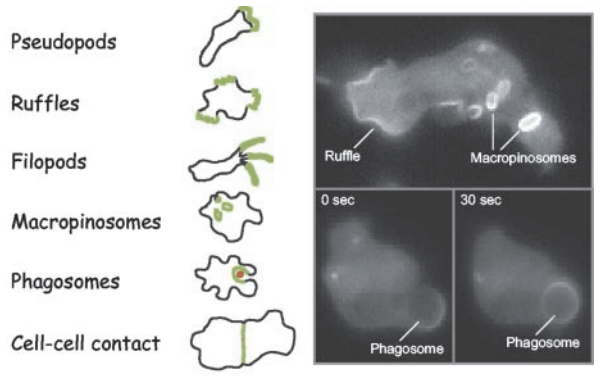

FIG. 4. **Cells expressing PH_{Crac}-GFP show localization of this marker for PIP_3 on a variety of membrane structures.** The *cartoons* on the left illustrate PIP_3 (*green* labeling) in a number of events such as pseudopodia extension, membrane ruffling, filopod extension, macropinocytosis, phagocytosis, and cell-cell contact that require cytoskeletal remodeling. Examples on the right include PH_{Crac}-GFP localization on ruffles, which mediate random movements of *D. discoideum* cells, and on macropinosomes (*top panel*). *Bottom two frames* show a phagocytic cup during phagocytosis of a yeast cell by growing cells. PH_{Crac}-GFP signal at the membrane appears with the initial encounter of the yeast cell and usually disappears from the phagosome after engulfment.

from unstimulated cells. This activation is transient; PI3K activity in lysates of cells pretreated for 30 s or more returns to a plateau level that is slightly elevated. Thus, receptor-mediated activation of PI3K contributes to the transient increases in PIP_3. It is expected that the rapid loss of PTEN from the membrane enhances the accumulation of the PIs, and the return of the enzyme to the membrane helps terminate the response. Cells lacking PTEN display changes in PI3K activity essentially identical to those in wild-type cells, yet increases in PIP_3 are higher and prolonged (64). The parallel regulation of PI synthesis and degradation provides a robust system that is resistant to perturbation (14). Since changes in both enzymes contribute to the accumulation of the PIs, partial inhibition of either is unlikely to completely impair the response.

These observations focus attention on the membrane binding sites and activators of PI3Ks and PTEN. These regulatory events create the initial asymmetry in signaling that leads to directional sensing. The movements and regulation of PI3K and PTEN can be explained by the excitation-inhibition model described above (see Figs. 2 and 3). We propose that the balance between an excitation and an inhibition process controls the membrane binding and activity of each enzyme. For PI3K, excitation reflecting local levels of receptor occupancy leads to recruitment and activation of the enzyme whereas global inhibition, determined by the average receptor occupancy, counteracts these effects. For PTEN, local excitation decreases its association with the membrane whereas global inhibition restores binding. Recent structural information has shed some light on the membrane binding and activation of these enzymes. For the PI3Ks, the N-terminal hydrophilic regions can target GFP to the membrane whereas a Ras binding domain is not required for enzyme recruitment but might be important for activation (62). PTEN contains an N-terminal $PI(4,5)P_2$ binding motif, and its deletion completely redistributes the enzyme to the cytosol. This mutated PTEN, when expressed in *pten*$^-$ cells, is unable to rescue their chemotactic defects, suggesting that membrane association is crucial for function (63).

Local PIP_3 Increases Lead to Directional Actin Polymerization Responses

Evidence suggests that PIP_3 plays a central role in directing where and when sites of actin-filled projections form in a variety of cellular responses. First, the stimulus-induced accumulation of PIP_3 as assessed by binding of specific PH domains to the membrane co-localizes with sites of new actin filament formation (14, 30, 62). In *D. discoideum* the PH domains label the surface membranes of pseudopodia, ruffles, filopods, macropinosomes, phagosomes, and sites of cell-to-cell contact (Fig. 4) (65–69). Interestingly, many of these events occur spontaneously in the absence of functional G proteins, implying G protein-independent activators can also lead to local accumulations of PIP_3. Second, interference with PI3K alters actin polymerization and inhibits many of these actin-based events. In macrophages the later stages of phagocytosis

are blocked by PI3K inhibitors (66). *D. discoideum* cells lacking PI3Ks or treated with PI3K inhibitors display profound defects in ruffling, macropinocytosis, and phagocytosis.[2] Third, elevation of PIP_3 by disruption of PTEN induces excess actin polymerization (63). In wild-type cells, chemoattractant stimulation typically triggers a biphasic actin polymerization response. In *pten*$^-$ cells, the second phase of actin polymerization is 6-fold that in wild-type cells. Attempts to reduce PIP_3 and thereby block actin polymerization triggered by chemoattractant led to a surprising observation. Inhibitors of PI3K and gene disruptions, which reduce increases in PIP_3 by over 90%, completely block the second phase of actin polymerization but do not affect the initial rapid phase. The first phase of the elicited actin polymerization response may be independent of or require only very slight increases in these PIs. It is not clear whether the effects of PIP_3 on actin polymerization require Akt-mediated phosphorylation events. Alternatively, the PIs may activate an exchange factor for a Rac family protein that is recruited to the membrane. There is a recent report of synergistic activation of exchange factor by G protein $\beta\gamma$-subunits and PIP_3 (70).

Positive Feedback and Actin Cytoskeleton May Stabilize Directional Sensing and Establish Polarity

Recent investigations have advanced our understanding of directional sensing, and these findings allow us to speculate on mechanisms of polarity. Although the local excitation global inhibition scheme is sufficient to explain directional sensing by an unpolarized cell, we believe that polarity must involve many of the concepts outlined in earlier models such as mechanical restriction and positive feedback loops. Although directional sensing does not require actin polymerization, polarity depends critically on a signal input as well as a reorganization of the cytoskeleton. We therefore propose that establishment of polarization involves a dynamic, coordinated interaction of directional sensing events with the activities of the cytoskeleton (see Fig. 3, *bottom*). How might the components involved in directional sensing, together with the cytoskeleton, bring about gradient-induced or even "spontaneous" polarization? We suggest that an essential role of the actin cytoskeleton is to stabilize the asymmetric distribution of key components of the directional response apparatus. Possibly, in a polarized cell, the associations of PI3K and PTEN with the membrane at the front and back of the cell, respectively, are reinforced by interactions with elements of the cytoskeleton localized to these regions. Because PIP_3 promotes actin polymerization, were components of the anterior cytoskeleton to stabilize the interaction of PI3K with the membrane, a positive feedback loop would result and reinforce the initial asymmetry. Similarly, a connection of PTEN to components of the cytoskeleton such as myosin II or Pak A, which are known to be modulated by chemoattractant at the back, might create a second feedback loop at the rear. With sensitive feedback loops, small perturbations would be expected to trigger the cell to acquire polarized morphology and sensitivity even in the presence of a uniform concentration of chemoattractant. This would lead to persistent generation of PIP_3 at the leading edge, which, in turn, would maintain the asymmetry in the cytoskeleton in the absence of a gradient.

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