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Review

# Moving in the right direction: How eukaryotic cells migrate along chemical gradients

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ABSTRACT

Many cells have the ability to grow or migrate towards chemical cues. Oriented growth and movement require detection of the external chemical gradient, transduction of signals, and reorganization of the cytoskeleton. Recent studies in *Dictyostelium discoideum* and mammalian neutrophils have revealed a complex signaling network that enables cells to migrate in chemical gradients.

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**Contents**



## 1. Introduction

Many types of cells have an internal "compass" that enables them to sense chemicals in the environment and direct their movements (Fig. 1). This process referred to as chemotaxis or directed cell migration plays a critical role in a variety of biological processes. *Dictyostelium* amoebae rely on chemotaxis to find bacterial prey. When food is scarce, chemotactic responses towards secreted cyclic AMP (cAMP) allow the cells to aggregate into multicellular structures and produce spores that are resistant to harsh environmental conditions. During embryogenesis in metazoans, specific chemoattractants guide primordial germ cells to proper locations, mediate the formation of organs, and control the wiring of the nervous system. In the adult, chemotaxis is involved in immune cell trafficking, wound healing, tissue regeneration, and stem cell homing. Conversely, improper chemotaxis is the origin of many pathological conditions [1–3]. For example, during metastasis, chemotaxis is required for cancerous cells to escape the primary tumor, enter the circulation, and emigrate to specific tissues.

Chemotaxis initiates with the binding of chemoattractants (or chemorepellents) to specific receptors on the cell surface, which triggers a wide range of biochemical responses. These events influence the organization of the cytoskeleton in distinct ways at the front and the rear of the cell to bias migration. Chemotaxis can be conceptually divided into three distinctive yet interconnected processes: motility, directional sensing, and polarity (Fig. 2). Motility, in both *Dictyostelium* and mammalian neutrophils, involves periodic extension and retraction of pseudopodia, which coordinate

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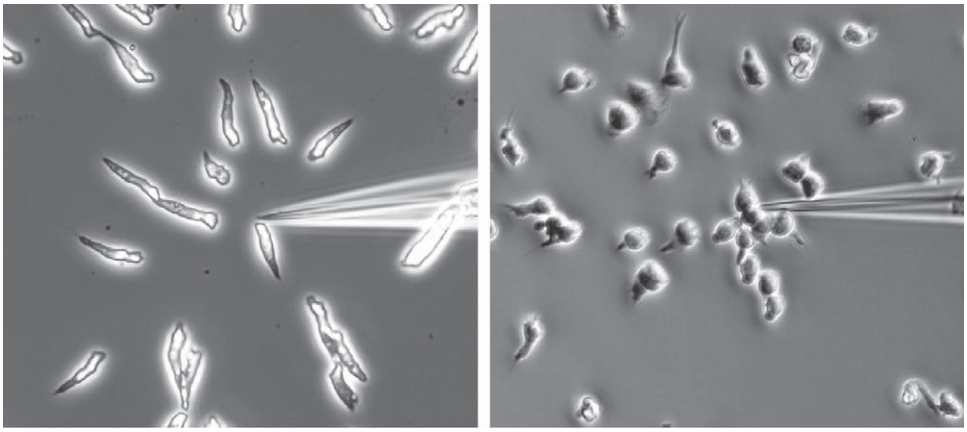

**Fig. 1.** *Dictyostelium* and neutrophils migrate along chemoattractant gradients. Left panel: *Dictyostelium* cells were placed on a chambered cover glass and exposed to a cAMP gradient generated by a micropipette. Right panel: Neutrophil-like HL-60 cells were placed on fibronectin-coated surface and exposed to an fMLP gradient.

with adhesion to propel cellular movement in random directions [4,5]. Actin polymerization drives pseudopod formation at the leading edge whereas actomyosin filaments generate contractile forces at the sides and the rear. Directional sensing refers to the ability of chemotactic cells to determine the direction and proximity of external cues and bias the movement accordingly. This is generally achieved by converting shallow extracellular gradients of chemoattractant into steeper gradients of intracellular signals [6,7]. Polarity refers to an elongated cell morphology, which is accompanied by the redistribution of cytoskeletal components and signaling molecules to either the anterior or the posterior of the cell. In polarized amoebae and neutrophils, the anterior becomes more sensitive to chemoattractants. When the direction of the gradient is changed, these cells maintain the original front and turn towards the new highest concentration rather than extend a new front [8]. It should be noted that neither motility nor polarity is required for a cell to sense direction. For example, cells treated with compounds that depolymerize the cytoskeleton, such as latrunculin A, are round and immobile, yet functional gradients of intracellular signaling molecules can still be established in response to external gradients [9,10].

Observations of chemotaxing cells have led to a series of intriguing questions. How do cells convert chemoattractant gradients into intracellular signals? Which portions of the signaling network are involved in gradient detection and which control cell movements? What is the basis of the rhythmic production of pseudopodia? How are the temporal responses of cells to global chemotactic stimuli related to directed movement? Studies of chemotaxis in the two model cell types *Dictyostelium* amoebae and neutrophils have greatly facilitated our understanding of this process. Despite differences in the migratory behaviors and variations in the molecular details of the signaling pathways, many guidance mechanisms have turned out to be highly conserved among eukaryotic cells.

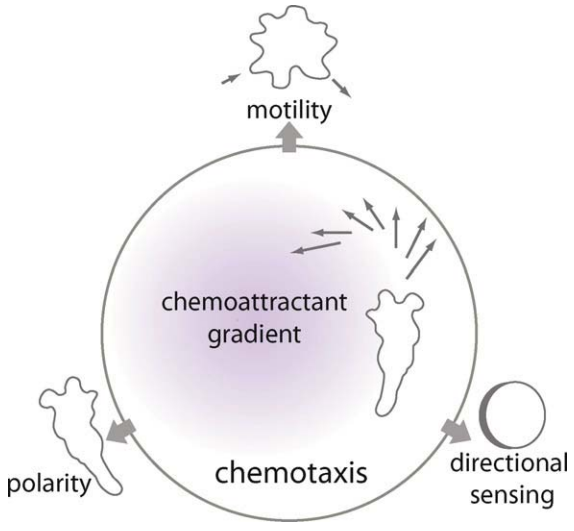


**Fig. 2.** Chemotaxis is composed of motility, directional sensing, and polarity. Motility involves the periodic extension and retraction of pseudopodia that drive cellular movement in the absence of chemoattractant gradients. Directional sensing is demonstrated by the gradient-mediated relocalization of proteins in cells that have been immobilized by treatment with inhibitors of actin polymerization. Polarity is evident in an elongate cell shape as well as the asymmetric distribution of the cytoskeletal components and signaling molecules. These processes have overlapping, but distinct, properties as described in the text.

## 2. G-protein coupled receptors (GPCRs) detect chemoattractant gradient

Chemoattractants are detected by the seven-transmembrane-spanning serpentine receptors. In *Dictyostelium*, four cAMP receptors (cAR1–4) have been identified, which have different affinities for cAMP and are expressed at different stages of differentiation [11,12]. The cARs signal through the heterotrimeric G-protein consisting of α2-, β-, and γ-subunits (there are only single copies of the Gβ and Gγ genes in *Dictyostelium*) [13]. Current evidence suggests that the β- and γ-subunits form a complex that mediates downstream signaling, whereas the α2-subunit is required for interaction with the receptor [14–16]. As shown by FRET analysis, binding of cAMP to the receptor leads to a rapid dissociation of the βγ-complex from the α2-subunit, which activates multiple downstream effectors that ultimately give rise to polarization and cellular movement [15,16]. In neutrophils, chemoattractant receptors for ligands such as fMLP, LTB4, and C5a are predominantly linked to pertussis-sensitive Gαi proteins [17]. Signals from the Gαi-coupled receptor also seem to be mediated by the Gβγ subunits, but some studies have suggested that $G\alpha_{12/13}$ plays a direct role in chemotaxis [18].

Studies of both *Dictyostelium* and neutrophils have shown that gradient sensing does not require redistribution of the receptor or G-protein. In *Dictyostelium*, cAR1-GFP and Gβ-GFP were found to distribute fairly evenly along the plasma membrane during random or chemotactic movements (Fig. 3) [19,20]. Furthermore, FRET analysis revealed that the dissociation of the Gα- and βγ-subunits mirrors receptor occupancy and parallels the shallow external gra-

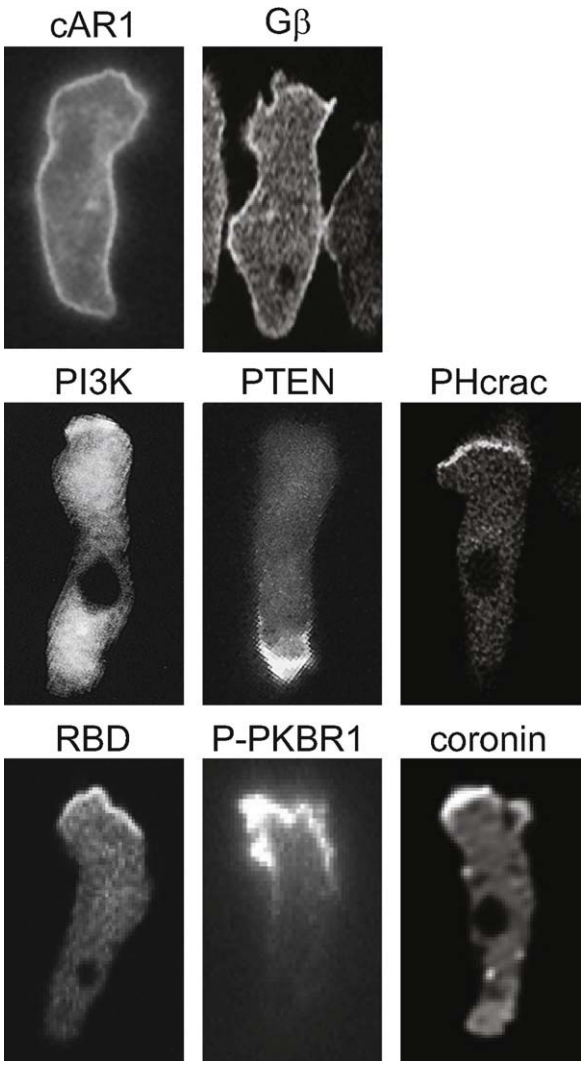


**Fig. 3.** Fluorescent images depicting the localization of signaling components. Top panel: In migrating *Dictyostelium* cells, the cAMP receptor cAR1 and Gβ subunit distribute evenly along the plasma membrane. Middle panel: PI3K is recruited to the leading edge whereas PTEN localizes to the lateral sides and the back. The reciprocal distribution of the two enzymes creates an internal PIP3 gradient as indicated by the localized accumulation of the PH domain from CRAC. Bottom panel: In some cases, although the proteins have a uniform distribution on the plasma membrane or in the cytosol, their activities are spatially restricted. Ras proteins and PKBR1 are activated at the cell's leading edge, as reflected by the localization of RBD and the phosphorylated form of PKBR1. Actin polymerization also enriches at the leading edge as indicated by the localization of the actin binding protein coronin.

dient [15]. Similarly, in neutrophils, the C5a receptor fused to GFP displays a uniform distribution on the plasma membrane in cells undergoing chemotaxis [21]. It is believed that evenly distributed receptors and G proteins enable a cell to constantly measure receptor occupancy across the entire perimeter so that it can respond swiftly to rapid changes in environmental conditions.

## 3. Signaling pathways steer chemotaxis

Despite the uniform distribution of the GPCRs, a number of downstream signaling events are restricted to either the high (the side of the cell experiencing higher concentrations of chemoattractants) or the low side of the cell. In this way, the initial small difference in receptor occupancy and G-protein activation is greatly amplified by conversion into sharply localized internal responses (Fig. 3). Important pathways (Fig. 4) responsible for signal amplification and guidance of cell migration will be discussed in detail in the following sections.

### *3.1. Phosphatidylinositol-3,4,5-triphosphate (PIP3) signaling in chemotaxis*

The observation that proteins containing the pleckstrin homology (PH) domain specifically localize to the leading edge of chemotaxing amoebae and neutrophils provided the first clue into how signals become amplified [9,22]. Translocation of these proteins to the plasma membrane reflects the local accumulation of PIP3. In *Dictyostelium*, the level of PIP3 is controlled by two classes of enzymes: PI3K (phosphatidylinositol 3-kinase), which produces PIP3 by phosphorylating PI(4,5)P2, and PTEN (phosphatase and tensin homologue deleted on chromosome 10), which catalyzes the reverse reaction [23–26]. When cells are migrating, PI3K is recruited to the front of the cell, conversely, PTEN falls off the membrane at the front and localizes to the lateral sides and the back. As a result of this reciprocal distribution, PIP3 accumulates specifically at the leading edge (Fig. 3). The mechanisms that control the cellular localization and activity of PI3K and PTEN are therefore central to our understanding of signal amplification and directional sensing.

The *Dictyostelium* genome encodes five class I PI3Ks. PI3K1 and PI3K2 are responsible for most of the PIP3 production in response to cAMP [23,25,27]. PI3K activation requires localization to the plasma membrane. Membrane-targeted PI3Ks are active even in the absence of chemoattractants [23,25]. All five PI3Ks contain a Ras-binding domain (RBD). PI3K1 and PI3K2 carrying point mutations in the RBD fail to rescue the defects in PIP3 production in the *pi3k1*$^{-}$/*pi3k2*$^{-}$ cell, suggesting that PI3K receives additional signal from the Ras family small GTPases [25]. Studies of a collection of knockout mutants implicated that RasG is an upstream regulator of PI3K in *Dictyostelium* [10,28], but it is unlikely to be the membrane recruiter. The N-terminal domains of PI3K1 and PI3K2, which lack the RBD, are sufficient for recruitment of GFP to the plasma membrane in response to stimulus [25]. In addition to signals from the receptor, a positive feedback loop involving PI3K and F-actin has also been proposed to regulate the localization of PI3K [28]. It should be noted that F-actin is not absolutely required for PI3K activation since PIP3 gradient remains largely intact in cells treated with inhibitors of actin polymerization [9]. The interaction of *Dictyostelium* PTEN with the plasma membrane requires a N-terminal PI(4,5)P2 binding motif [29]. Mutant version of PTEN lacking the PIP2 binding motif does not bind to the plasma membrane nor does it rescue the phenotypes of *pten*$^{-}$ cells. Some studies suggested that PLC, through its ability to degrade PI(4,5)P2, regulates PTEN distribution by preferentially removing PTEN binding sites at the leading edge [30].

In neutrophils, PIP3 accumulation dependents on the activities of PI3Kγ, a class IB PI3K, the 5′-phosphatase SHIP-1, and the 3′-phosphatase PTEN [31–34]. PI3Kγ appears to be regulated by the synergistic action of Gβγ-subunits and Ras proteins [35]. In addition, a large body of work indicated that Rac proteins and components of the F-actin networks form part of a positive-feedback loop to stimulate PIP3 production at the leading edge [36–38]. The localization of PTEN in moving neutrophils is currently unresolved. Although some studies localized PTEN to the posterior of the cell, others found it to be evenly distributed in the cytosol [18,32,39].

Accumulating evidence suggests that the localized accumulation of PIP3 provides an important signal for directed cell migration. First, the internal PIP3 gradient is a highly conserved signature of chemotactic signaling observed in many cell types. Second, PIP3 is a strong candidate for a directional sensing cue since cells treated with inhibitors of F-actin are still able to localize PIP3 when exposed to a gradient. Third, chemotaxis is impaired in cells with genetic loss

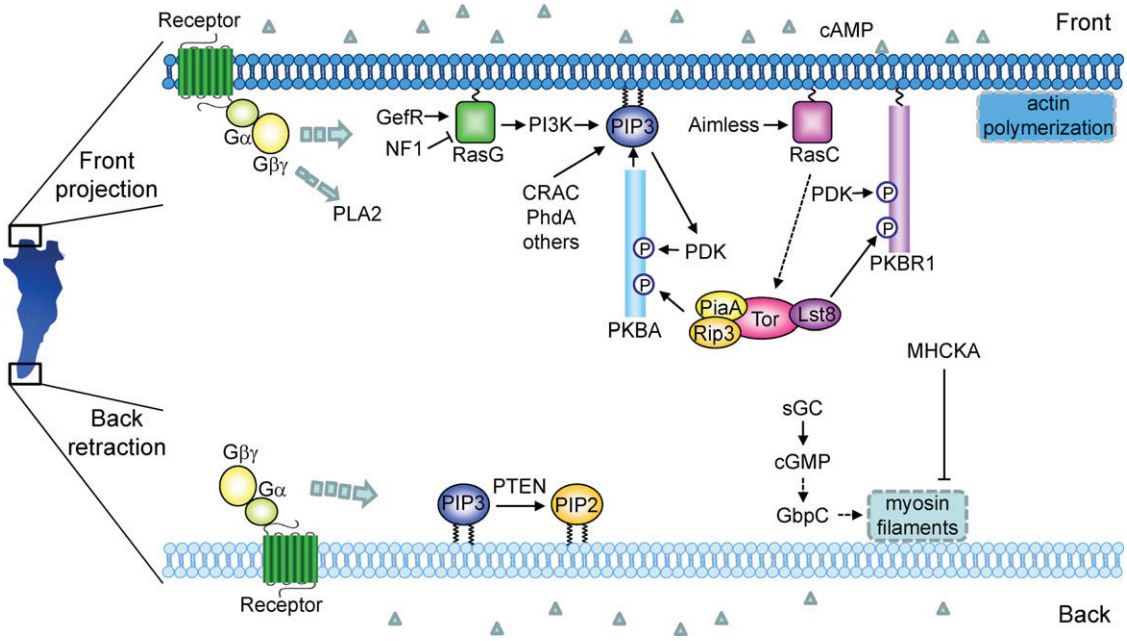


**Fig. 4.** Signaling networks control chemotaxis. Depicted is a network of signaling events triggered by cAMP to control front projection and back contraction in *Dictyostelium*. At the front, binding of cAMP to GPCR leads to the activation of RasG and RasC, which in turn stimulate the activities of PI3K and TORC2, respectively. PI3K produces PIP3, which recruits PH-domain containing proteins including PKBA, CRAC and PhdA. PDK [86] and TORC2, composed of PiaA, Rip3, Lst8, and the Tor kinase, mediate the phosphorylation and activation of PKBA and PKBR1. PLA2 acts in parallel with the PIP3 pathway to regulate actin polymerization. Front signals also inhibit myosin II activity through the activation of the myosin heavy chain kinase (MHCKA) [87,88]. At the back, PTEN is responsible for the degradation of PIP3. Myosin II is assembled into contractile filaments that suppress pseudopod formation and promote back retraction. The cGMP binding protein GbpC promotes the assembly and activity of myosin II. Positive links between components are indicated by (→) or dotted arrows (less defined steps) and inhibitory links are indicted by (⊥).

of PI3Ks or treated with PI3K inhibitors [17,25,26,40]. Finally, a collection of data has linked PIP3 production to actin polymerization and pseudopod formation. In *Dictyostelium* cells lacking PTEN and mouse neutrophils lacking SHIP-1, excess PIP3 diffuses away from the leading edge, resulting in unrestricted pseudopodia extensions, a severe loss of cell polarity and defects in chemotaxis [24,34]. Furthermore, delivery of PIP3 to or activation of PI3K in neutrophils is sufficient to trigger actin polymerization, induce polarity and initiate migration [36,41].

PIP3 signaling is believed to be transduced by effector proteins that bind selectively to its head group. As noted before such proteins often contain PH domains and are recruited to the cell's leading edge. In *Dictyostelium*, CRAC (cytosolic regulator of adenylyl cyclase), PhdA (PH domain-containing protein A), and PKBA (protein kinase B A) are three well-characterized PIP3 binding proteins and a recent study identified three additional ones, designated PhdB, PhdG, and PhdI [9,26,42,43]. CRAC is required for the process of signal relay, in which extracellular cAMP stimulates further production and release of cAMP [44,45]. Cells lacking PhdA, PhdB, or PhdG all show reduced chemotaxis [26,42]. PKBA, also known as Akt, is a serine/threonine protein kinase. cAMP stimulates the activity of PKBA, which in turn mediates the phosphorylation of a series of signaling and cytoskeletal proteins [43,46,47]. Deletion of PKBA in *pten*$^-$ cell rescues the chemotaxis defects seen in *pten*$^-$ cell. Even though the level of PIP3 remains elevated in the double knockout cell, pseudopod projection is confined to the leading edge, suggesting that PKBA is a critical downstream effector of PIP3 in regulating the cytoskeletal activity [47]. Recently, a recursive-learning algorithm was developed to predict PIP3 responsive PH-domain containing proteins [48]. This algorithm predicted as many as twenty potential PIP3 binding proteins in *Dictyostelium*. Characterization of these new proteins is currently in progress. A number of PIP3-binding proteins have also been identified in neutrophils, many of which are regulators for small GTPases of the Rac, Rho, and Cdc42 family. PIP3 may signal through these proteins to modulate the cytoskeleton [40,49]

### 3.2. PIP3-independent pathways in chemotaxis

Although PIP3 clearly plays an important role in cell polarity and migration, inhibiting the pathway does not always block chemotaxis. PI3K inhibitors such as LY294002 and wortmannin produce only partial defects, and in some cases, cells can recover from the treatment and resume efficient chemotaxis [50]. *Dictyostelium* cells lacking all five PI3Ks are still able to carry out chemotaxis [51]. Studies in neutrophils have revealed a similar pattern of results. In one study it was shown that the dependence on PI3Kγ activity is context dependent, with respect to the state of priming of the neutrophils and the type of surface on which they are migrating [31]. These observations led to the search for signaling pathways that act independently or in parallel with the PIP3 pathway.

#### 3.2.1. TORC2

Target of rapamycin complex 2 (TORC2) is a conserved serine/threonine kinase complex, which has been shown to regulate cytoskeleton-based events in many cell types [52–55]. Pianissimo (PiaA, the mRictor homolog), an essential component of the *Dictyostelium* TORC2, was identified fourteen years ago in a genetic screen for mutants that failed to aggregate upon starvation [56]. It was demonstrated only recently that TORC2 regulates *Dictyostelium* chemotaxis and the cell-to-cell relay of cAMP signal through the phosphorylation of two Akt/PKB kinases, PKBA and PKBR1 [46,57–59]. In response to chemoattractant stimulation, both PKBs are transiently phosphorylated within their hydrophobic motifs by TORC2 [46]. In migrating cells, these phosphorylation events are restricted to the leading edge, suggesting that they may

play a role in regulating the actin cytoskeleton. Indeed, PKB activity was found to mediate the phosphorylation of the regulators of the Rac and Rho family small GTPases [46,47]. The activation of PKBA, as noted in Section 3.1, requires an additional interaction with PIP3. In contrast, PIP3 is not required for the phosphorylation and activation of PKBR1 [46,57,60]. Furthermore, cells lacking PiaA or PKBR1 exhibit more severe defects in chemotaxis compared to cells lacking PKBA, implying that the PIP3-independent TORC2-PKBR1 pathway provides the major PKB activity required for migration [46]. TORC2 has also been found to regulate chemotaxis in neutrophils. A recent study showed that, as in *Dictyostelium*, TORC2 signaling promotes chemoattractant-induced production of cAMP in neutrophils. cAMP accumulation in the cell body modulates the RhoA-ROCK-myosin II pathway, which in turn controls back retraction [61]. Neutrophils lacking TORC2 activity display a loss of polarity and a strong defect in chemotaxis. Interestingly, TORC2 stimulates cAMP production through PKC but not the PIP3-responsive substrate Akt, suggesting that this may also be a PIP3-independent process [61].

How do cells transduce chemoattractant stimuli to TORC2 activation? In *Dictyostelium*, three recent studies provided strong evidence that a Ras family small GTPase, RasC, exerts both spatial and temporal control of TORC2 during chemotaxis [46,57,58]. Cells deleted of RasC exhibit attenuated TORC2 signaling. The expression of persistently activated forms of RasC prolong the time course of TORC2 mediated activation of PKBR1 and the phosphorylation of PKB substrates. Paralleling these changes pseudopodial activity is increased and mislocalized and chemotaxis is impaired [57]. Furthermore, the effects of activated RasC can be suppressed by deletion of PiaA and addition of immunopurified TORC2 to membranes containing activated RasC and PKBR1 reconstitute the phosphorylation event in the absence of chemoattractant stimuli [57]. In neutrophils, it remains a mystery how chemoattractants are relayed within the cell to activate TORC2. Chemoattractant-stimulated Ras activation has been observed in human neutrophils [62,63] and it will be interesting to learn whether Ras plays a similar role in neutrophil chemotaxis to activate TORC2.

#### 3.2.2. Other PIP3-independent pathways

An unbiased genetic screen in *Dictyostelium* for mutants that were selectively impaired in chemotaxis when PI3K was inhibited led to the identification of a gene with homology to patatin-like phospholipase A2 (PLA2) [64]. The role of this gene in chemotaxis was further supported by work using PLA2 inhibitors [65]. The simultaneous loss of PI3K and PLA2 activities causes a stronger chemotactic defect and a further reduction in receptor-mediated actin polymerization than does the loss of either activity alone, suggesting that PLA2 and PI3K may act in parallel to mediate chemotaxis [64,65]. PLA2 specifically cleaves the second acyl chain of phospholipids to produce fatty acids, predominantly arachidonic acid (AA) and lysophospholipids. It was shown that chemoattractants stimulation leads to a rapid and transient production of AA derivatives in *Dictyostelium* [64]. The pathway leading to PLA2 activation and the exact function of its lipid products have yet to be determined.

In *Dictyostelium* cells that are allowed to progress further in their developmental program, the lack of PI3K and PLA2 activities can be compensated by a pathway involving the soluble guanylyl cyclase (sGC), which is responsible for the synthesis of the second messenger cyclic GMP (cGMP) [66]. cGMP promotes the formation of myosin filaments at the back and the lateral sides of the cell through the cGMP-binding protein GbpC [67,68]. Cells expressing catalytically deficient sGC mutants migrate slowly and have more lateral pseudopods compared to wild-type cells.

## 4. A mechanistic understanding of chemotaxis

Over the years, investigations of chemotaxis in amoeboid cells have revealed some salient, consistent features of the process. First, a large portion of the signaling network downstream of the receptor and G-protein (Fig. 4) appears to act in a coordinated fashion not only when stimulated but also during spontaneous extension of pseudopod. For example, chemoattractants trigger activation of Ras proteins and PI3Ks and loss of PTEN and myosin II from the cell surface; the same events occur at the tips of pseudopodia formed spontaneously in the absence of stimuli [69,70]. Moreover, recent studies have shown that the cortical cytoskeleton and parts of the signaling network behave as an excitable medium. Observations by total internal reflection fluorescence microscopy (TIRFM) reveal propagating waves of recruitment of actin binding proteins to the cell cortex, local Ras activation, and $PIP_3$ accumulation [71–73]. We speculate that this excitable biochemical network underlies the random motility of cells. Second, directional sensing is linked to a general characteristic of chemotactic systems referred to as adaptation. Cells respond to increases in receptor occupancy, adapt when occupancy is held constant, and respond again when occupancy is increased further or when the stimulus is removed and reapplied after a period of deadaptation [74–76]. Most cellular responses adapt, including activation of Ras proteins, production of cAMP, cGMP, and $PIP_3$, phosphorylation of PKB, polymerization of actin, and changes of cell shape. Third, chemoattractant-mediated responses are actually biphasic in both *Dictyostelium* and neutrophils (Fig. 5A) [77–79]. That is, there is an initial peak that declines sharply, followed by broader, weaker responses which occur over the next several minutes. When visualized with a $PIP_3$ biosensor, the PH domain from CRAC (Fig. 5A), the first response is seen to occur uniformly around the perimeter and then disappear during a "cringe". The second response consists of a series of intermittent patches at the tips of projections on the spreading cells.

Several general classes of models have been put forth to explain these observations [80]. The Local Excitation-Global Inhibition (LEGI) model (Fig. 5B) was proposed to explain the temporal and spatial responses of immobilized cells to chemoattractants [81]. The LEGI model accounts for perfect adaptation displayed by cells exposed to uniform step increases in chemoattractants and also explains the persistent, directional responses displayed by immobilized cells towards spatial gradients. However, it does not amplify the external gradient nor does it account for the dynamic behavior of migrating cells or the development of stable polarity. A variety of schemes have attempted to model the spontaneous acquisition of polarity and the excitability of signaling events in cells [36,41,71,72,82–85].

In the LEGI-Biased Excitable Network hypothesis (LEGI-BEN) [71], upstream signaling components including chemoattractant receptor and G-protein, and an inhibitor, read the stimulus and produce a Response Regulator (RR) as described by the LEGI module. RR serves as an input that biases a downstream excitable biochemical network that spontaneously generates pseudopodia (Fig. 5C). The time scale of the RR is a few minutes, corresponding to the rate of adaptation, whereas the time constant of the excitable network is much faster. Fig. 5D shows one simulation produced by implementation of this hypothesis. Assuming that the patches of biochemical activity are correlated with cellular projections, the LEGI-BEN model can account for most of the observed behaviors of chemotaxing cells. First, the simulated cells display spontaneous activity in the absence of stimulation. The excitable network serves as a "clock" that regulates the extension of projections. Second, the simulated cells respond and adapt to uniform stimuli and display persistent directional sensing in gradients. Third, since the RR and the excitable network have different time constants, the responses

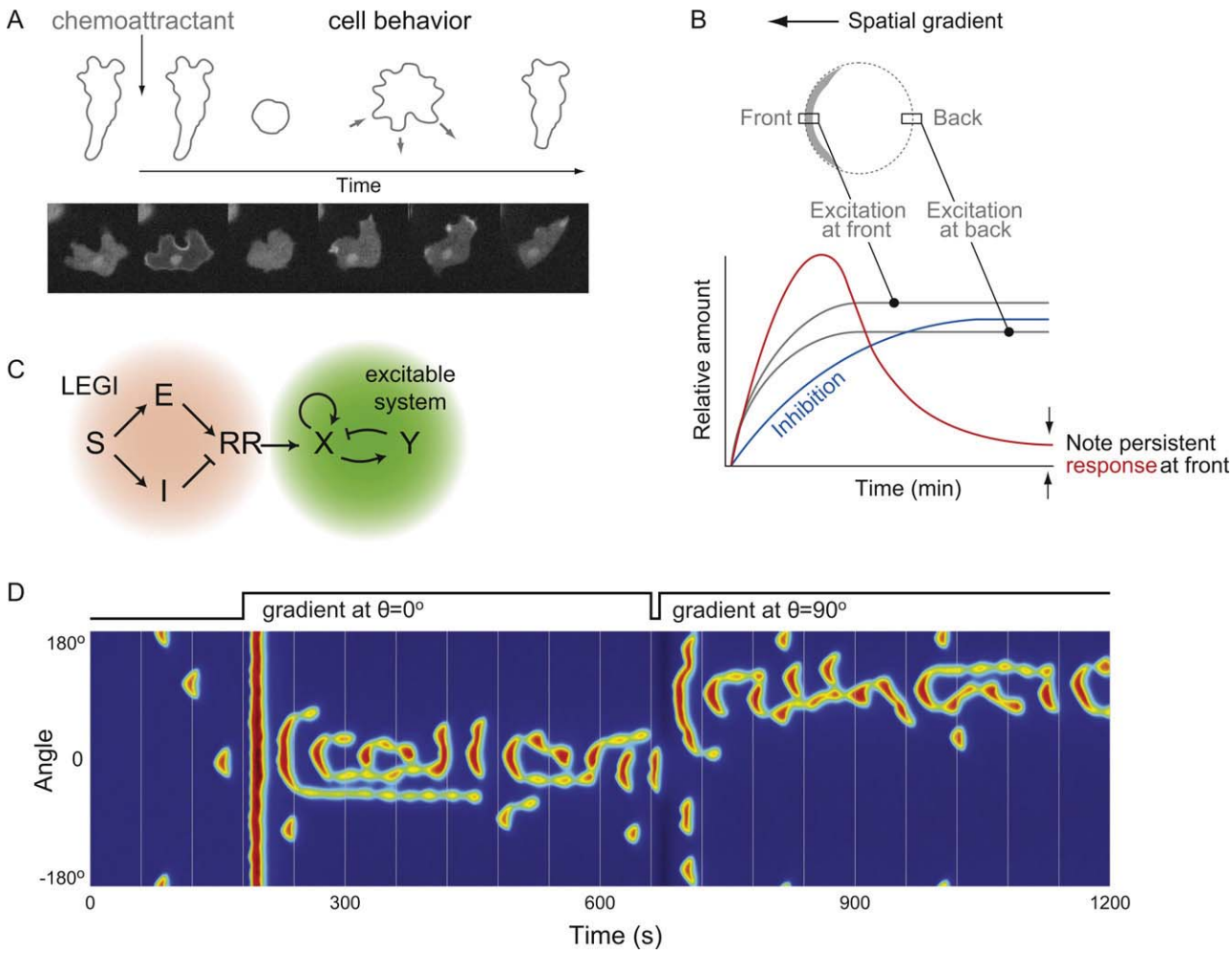


**Fig. 5.** Chemotactic responses and the LEGI-BEN model. (A) Top: Schematic of a biphasic response triggered by a global stimulation. Bottom: A biphasic response of PIP3 production in cells expressing GFP-labeled $PH_{CRAC}$. (B) In the LEGI model, chemotactic stimuli generate a local excitor (LE), which rises faster than the global inhibitor (GI). The excitor reflects the local receptor occupancy, whereas the inhibitor depends on the averaged receptor occupancy. When a gradient is applied, there is an initial response (red line). However, when it reaches a steady state, chemotactic response persists at the front of the cell (red line pointed by the two arrows) because excitation (grey lines) exceeds inhibition (blue line) at the front while it is lower at the back. (C) In the LEGI-BEN model, the LEGI module controls a response regulator, RR, which is a positive driver of an excitable network triggered by stochastic noise. (D) Kymograph of a one-dimensional simulation produced by implementation of the LEGI-BEN hypothesis. The kymograph is a plot of *Y*. Before stimulation (from 0 s to 180 s), patches of activity are at random sites around the cell perimeter. When a gradient stimulus is applied at ~180 s, activities appear all along the perimeter producing an initial uniform response that quickly "shuts-off" and becomes localized towards the high side of the gradient. When the gradient is repositioned at ~660 s, a second uniform response is produced followed by a persistent directional response in the new direction. It should be noted that the excitable network is triggered by stochastic noise. If noise crosses threshold, it will trigger a response. Therefore signals sometimes appear at different points on the cell perimeter at the same time. For the same reason, even when most responses are biased to occur on the side of the cell facing the gradient, some events can take place by chance away from the gradient. These simulations are consistent with observations of real cells.

to stimuli are biphasic. Interestingly, the extraordinary sensitivity of chemotaxing cells is a natural consequence of the model since the excitable network amplifies small differences in the level of RR. Forth, alterations of the feedback loops within the excitable network can give rise to alternate behaviors. For example, enhancing the positive feedback causes spontaneous and stimulated activities to be greatly prolonged and exaggerated, a behavior that has been described for *pten*$^-$ cells and cells expressing constitutively activated RasC and RasG [10,24,57].

## 5. Conclusion

Eukaryotic cells have developed a robust and sophisticated machinery to guide their movements in complicated environmental conditions. Multiple signaling pathways act in parallel to convert extracellular chemical gradients into localized intracellular signals. A large portion of the signaling network appears to be excitable, and this excitability underlies the random motility of cells and is biased by chemoattractants in directed migration. Notwithstanding the significant progress we have made in the study of chemotaxis, many new questions have been raised. What is the molecular mechanism of adaptation? From which point does the receptor and G-protein enter the excitable signaling network? What are the components that make up the positive and negative feedback loops? What is the mechanism of wave generation and propagation and what is the relationship between the waves and cell motility? To what extent do different signaling pathways regulate chemotaxis? It is no doubt that answers to these questions will provide further insights into this fascinating area of cell biology.

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