Review

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

Huaqing Cai, Peter N. Devreotes *

The Department of Cell Biology, Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA

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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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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 chemotactants 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...
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.

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

Chemotactants 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 Goi proteins [17]. Signals from the Goi-coupled receptor also seem to be mediated by the Gβγ subunits, but some studies have suggested that Goi12/13 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 Goi- and βγ-subunits mirrors receptor occupancy and parallels the shallow external gr-
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 activation 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 PI2 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...
of PI3Ks or treated with PI3K inhibitors [17,25,26,40]. Finally, a collection of data has linked PI3P production to actin polymerization and pseudopod formation. In Dictyostelium cells lacking PTEN and mouse neutrophils lacking SHIP-1, excess PI3P 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 PI3P to or activation of PI3K in neutrophils is sufficient to trigger actin polymerization, induce polarity and initiate migration [36,41].

PI3P 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 adenyl cyclase), PhdA (PH-domain-containing protein A), and PKBA (protein kinase B A) are three well-characterized PI3P binding proteins and a recent study identified three additional ones, designated PhdB, PhdG, and Phdl [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 PI3P remains elevated in the double knockout cell, pseudopod projection is confined to the leading edge, suggesting that PKBA is a critical downstream effector of PI3P in regulating the cytoskeletal activity [47]. Recently, a recursive-learning algorithm was developed to predict PI3P responsive PH-domain containing proteins [48]. This algorithm predicted as many as twenty potential PI3P binding proteins in Dictyostelium. Characterization of these new proteins is currently in progress. A number of PI3P-binding proteins have also been identified in neutrophils, many of which are regulators for small GTPases of the Rac, Rho, and Cdc42 family. PI3P may signal through these proteins to modulate the cytoskeleton [40,49].

3.2. PI3P-independent pathways in chemotaxis

Although PI3P 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 PI3Ky 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 PI3P 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]. Painsinopo (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 PKB, as noted in Section 3.1, requires an additional interaction with PIP3. In contrast, PIP3 is not required for the phosphorylation and activation of PKB [46,57,60]. Furthermore, cells lacking PiaA or PKBR1 exhibit more severe defects in chemotaxis compared to cells lacking PKB, 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. Parallelizing 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 P13K 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 P13K 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 P13K 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 stimulate 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 P13K 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 on 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 P13Ks 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 PIP3 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 deadadaptation [74–76]. Most cellular responses adapt, including activation of Ras proteins, production of cAMP, cGMP, and PIP3, 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 a broader, weaker responses which occur over the next several minutes. When visualized with a PIP3 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, once the RR and the excitable network have different time constants, the responses
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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