Chemotaxis: moving forward and holding on to the past

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Summary
The ability of cells to sense external chemical cues and respond by directionally migrating towards them is a fundamental process called chemotaxis. This phenomenon is essential for many biological responses in the human body, including the invasion of neutrophils to sites of inflammation. Remarkably, many of the molecular mechanisms involved in controlling neutrophils chemotaxis arose millions of years ago in the simple eukaryotic organism Dictyostelium discoideum. Both neutrophils and Dictyostelium use G protein-coupled signaling cascades to mediate chemotactic responses, which are responsible for transducing external cues into highly organized cytoskeletal rearrangements that ultimately lead to directed migration. By using the genetically and biochemically tractable organism Dictyostelium as a model system, it has been possible to decipher many of the signal transduction events that are involved in chemotaxis.

Keywords
Dictyostelium, neutrophils, chemotactic signaling

Dictyostelium discoideum: A powerful model for the study of chemotaxis

The capacity of a cell to sense gradients of extracellular chemical signals (chemoattractants) and respond by migrating directionally towards their source is a fundamental process called chemotaxis. This process is remarkably conserved between mammalian leukocytes and the simple eukaryotic amoeboid organism Dictyostelium discoideum (1). The strong similarities that these two systems share during chemotaxis and the ease by which Dictyostelium cells can be manipulated in the laboratory have led scientists to use this system to investigate the molecular mechanisms that regulate chemotaxis. Dictyostelium, is a versatile model for genetic, biochemical and cell biological analyses. Its small (34 Mbp) haploid genome has been completely sequenced (2) and harbors many genes homologous to those found in higher eukaryotes (3). Endogenous genes can be easily disrupted by homologous recombination, novel genes can be identified using insertional mutagenesis, and structure-function analyses can be performed by random mutagenesis approaches. Moreover, strains that can grow in liquid culture are available, making it easy to obtain up to 10¹² clonal cells in just a few days. The amoeba are easy to lyse and process for a multitude of biochemical assays and for protein purification as well as for subcellular fractionations. Furthermore, these cells can be easily transfected and the use of fluorescent probes has allowed in-depth cell biological analyses. For all these reasons, Dictyostelium is perfectly poised for studies of fundamental cellular processes such as cytokinesis, motility, chemotaxis, signal transduction and development.

A life cycle driven by chemotaxis

The amoeba Dictyostelium was first isolated from the soil of a North Carolina forest by Kenneth Raper during a camping trip in 1933 (4). Since then, this system has been used extensively as an experimental model to study the developmental biology of higher organisms. In fact, the amoeba shares many of the properties of more complex creatures, including an extraordinary ability to escape a solitary life in order to initiate a multicellular developmental program (5–7).

Chemotaxis is essential during the entire life cycle of Dictyostelium, which consists of two distinct phases: growth and development (Fig. 1). The natural habitat of the organism is the soil where, during the growth (vegetative) phase, Dictyostelium exist as single, free-living amoeboid cells that feed on bacteria and divide by binary fission (5). During this phase the cells can hunt bacteria due to their ability to chemotax towards folic acid, a by-product of bacterial metabolism (4). When challenged with ad-
verse conditions, such as starvation, Dictyostelium cells stop dividing and enter a differentiation program that leads the organism into the developmental phase of its life cycle where a newly expressed set of proteins shifts the chemotactic sensitivity of the cells from folic acid to cAMP (8–10). Although cAMP acts as a ubiquitous intracellular second messenger, in Dictyostelium it also functions extracellularly as a chemotactant. Indeed, chemotaxis towards cAMP is required for the aggregation of ~100,000 single cells that later differentiate into a true multicellular organism. The end goal of this process is the formation of a fruiting body that contains resistant spores that allow the organism to survive the harsh conditions. Upon reappearance of nutrients these spores germinate to produce single celled amoebae and the cycle is re-initiated (Fig. 1).

During early development, within 4 hours of starvation, cells spontaneously begin to produce and release molecules of cAMP. Cyclic AMP binds to specific surface receptors and activates a plethora of signal transduction pathways that lead to chemotaxis, the synthesis and secretion of additional cAMP, and gene expression (11). Through an adaptation mechanism, the adenylyl cyclase A (ACA), that generates cAMP, shuts down and a secreted form of phosphodiesterase rapidly degrades the extracellular cAMP. This process results in oscillations of cAMP every 6 min. This exquisitely regulated series of events generates propagating waves of cAMP that attract neighboring cells. Intriguingly, during their movement toward the aggregation center, the cells align themselves in a head to tail fashion and form characteristic streams (12, 13). After 10 hours, as many as $10^5$ cells aggregate into a mound, where the cells differentiate into either presstalk or prespore cells (14). During this later stage of development, the aggregate behaves as a multicellular organism: the mound gradually elongates into a slug structure that migrates along heat or light gradients in search of the perfect environment for further development. The prestalk cells move forward to the front of the slug, while the prespore cells stay behind in the back. Once in an optimal environment, the slug ceases migration and rounds up: the prestalk cells push their way down from the front of the stalk, giving the appearance of a Mexican hat (Fig. 1). Then the stalk grows, by successive addition of prestalk cells, and the prespore cells are gradually elevated to the top of the stalk. During this process, known as culmination, prespore and prestalk cells terminally differentiate into either viable spores, or dead stalk cells (15). During slug migration and fruiting body formation cAMP is continuously secreted in a pulsatile fashion – a process that is essential to coordinate cell movement and shape changes necessary to build the multicellular fruiting body (16). The final act of development consists of encapsulation of the spores: they lose water and build a thick wall, producing a barrier for molecules the size of proteins, but not for water or other small molecules (15). The amoebae contained in this coat are not dormant; they are sensitive to food availability and osmolarity where high concentrations of osmoletes keep them as spores and prevent germination. Although little is known about spore germination, it is clear that spores continuously sample their en-

Figure 1: A schematic representation of the life cycle of Dictyostelium. Morphological changes occurring during the growth and developmental phases are depicted. Next to each stage of development, the corresponding time (in hours) after the beginning of starvation is shown. The end goal of the differentiation process is the formation of a multicellular fruiting body containing resistant spores. Under favorable conditions, the spores germinate to produce single celled amoebae and re-initiate the cycle (see text for details).
Bagorda et al.: Chemotactic signaling in Dictyostelium and neutrophils

A life cycle driven by conserved signals

Dictyostelium and neutrophil chemotaxis is controlled by signal transduction pathways mediated through the activation of G-proteins coupled receptors (GPCRs). GPCRs constitute a large family of seven transmembrane receptor proteins that mediate signals by activating bound intracellular heterotrimeric G proteins. GPCR-mediated signaling is highly conserved (18) and represents a primary mechanism by which cells sense changes in the external environment and convey this information to the intracellular compartment.

The initial step of chemotactic signaling in Dictyostelium cells is binding of the chemoattractant, folic acid or cAMP, to its environment through cAMP-dependent signals (17), until they find the appropriate conditions to re-initiate their life cycle as independent single cells.

Figure 2: A schematic representation of chemotactic signaling in Dictyostelium and mammalian neutrophils. The middle panel shows a cell chemotaxing towards a source of chemoattractant. The chemotaxing cell has two morphologically and biochemically distinct compartments, the front and the back, regulated by distinct signaling events. Localized polymerization of F-actin at the side of the cell facing the highest concentration of chemoattractant leads to the extension of a leading pseudopod. F-actin polymerization is suppressed at the sides and the back of the cell, ensuring efficient migration towards the source of the signal. The back of the cell is enriched in actomyosin that contracts for retraction. The upper and lower panels depict in greater detail the major signaling events that take place at the front and at the back of chemotaxing Dictyostelium (2A, B) and neutrophils (2C, D). The dashed stimulatory and inhibitory arrows between the “front” and “back” boxes represent the dynamic signaling interactions between the front and the back of chemotaxing cells (e.g. CRAC – ACA, Akt – PAK in Dictyostelium; Cdc42 – RhoA in neutrophils) (see text for details).
cognate GPCR (Fig. 2B). This activates intracellular signaling pathways that mediate, among other responses, the directional migration toward the source of the chemoattractant. A similar system operates in mammalian neutrophils, where the formylated tripeptide, fMLP (N-formyl-L-methionyl-L-leucyl-L-phenylalanine), which is released by bacteria, the complement factor C5a, the platelet-activating-factor (PAF), and chemokines bind GPCRs and activate highly conserved downstream intracellular pathways (1) (Fig. 2D). The *Dictyostelium* GPCRs that bind cAMP are known as cARs (cAMP receptors). Four different members of this family have been cloned: cAR1, cAR2, cAR3, cAR4 (19–21). They are differentially expressed during development and display distinct binding affinities for cAMP, with cAR1 and cAR3 possessing the highest affinity binding sites (22). The first cAR to be expressed during development is cAR1; its function is partially overlapped by cAR3, which is expressed slightly later. cAR1 deficient mutants arrest in early development because of their inability to carry out chemotaxis. Cells lacking both cAR1 and cAR3 are completely insensitive to cAMP (23). Cells that lack either cAR2 (the third expressed) or cAR4 (the fourth cAR to be expressed), despite their ability to aggregate, arrest during the multicellular stage of development (24, 25).

GPCRs are coupled to heterotrimeric G proteins composed of α, β and γ subunits. In response to stimulation, the GPCRs activate the exchange of GDP for GTP on the α subunit leading to the dissociation of the GTP-bound α-subunit from the βγ dimer, which can both modulate downstream effectors. In mammalian cells, there are 4 major families of heterotrimeric G proteins distinguishable by their a subunit isoforms: Gα1, Gαq, Gqα11, G12/13 (26). Gαi activates all mammalian isoforms of adenyl cyclase (AC) resulting in increased levels of cAMP, while Gαq is also linked to AC, inhibiting a subset of AC isoforms. Gαq activates phospholipase C (PLC) β isoform, which catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate to diacylglycerol (DAG) and inositol trisphosphate (IP3). The most recently identified class of heterotrimeric G proteins includes the G12/13 family, which activates the small GTPases Ras and Rho. *Dictyostelium* cells differentially express 14 distinct Gα subunits, all closely related to the mammalian Gαi family (27). Genetic studies (28) and FRET analyses (29) have shown that the main isoform linked to cAR1 is Gαi. The function of the other Gα subunits is not clear, except for Gαq and Gαs. It has been shown that Gαs is linked to the folic acid receptor (30) and that Gαq acts as a regulator of chemotactic response, attenuating the activities of multiple pathways downstream of the chemotacti-

The mammalian GPCRs for chemoattractants and chemokines are mostly coupled to G1, although neutrophils also exhibit G12/13-mediated chemotactic processes (26, 32). With the exception of G12/13, which directly mediates signaling, G1-regulated chemotactic responses are mainly controlled through the Gβγ subunits, released following receptor activation (33). Mammalian cells have 5 different β subunits and 14 distinct γ subunits, which do not pair indiscriminately, but combine selectively (34). The agonist-induced activation of mammalian chemokine receptors promotes the release of Gβγ, which is responsible for the activation of PLCβ2 and phosphatidylinositol-3 kinase (PI3K) γ. Gene deletion studies in mice revealed that PI3Kγ plays a crucial role in the activation of downstream phagocyte function, including chemotaxis and oxygen radical formation (35, 36). PLCβ2, on the other hand, is important in chemoattractant-mediated superoxide production but does not appear to be required to regulate chemotaxis (37, 38). However, Ca++ signals may contribute to chemotaxis as cyclic ADP-ribose-induced Ca++ mobilizations have been reported to positively regulate neutrophil chemotaxis toward a discreet subset of chemokines (39, 40).

In *Dictyostelium*, the major effectors controlling chemotaxis are also predominantly regulated by Gβγ. In the early stage of *Dictyostelium* development, cAMP interacts with cAR1 (the key receptor involved in chemotaxis at this stage) and stimulates the dissociation of Gαi from Gβγ, where both β and γ isofoms are present in the genome. While PI3K activation, plays a critical role in the chemotactic response (41–43), the involvement of PLC activity appears to be dispensable (44). Intriguingly, *Dictyostelium* cells use alternative pathways to generate IP3 and the chemotactic behavior of cells lacking the putative IP3 receptor – and the role of Ca++ influx – remains to be clarified (45–48).

Although heterotrimeric G proteins are essential for chemotaxis, the binding of cAMP to cAR1 induces responses that are independent of G proteins (49, 50). It has been shown that cAMP-mediated calcium influx (51), in *Dictyostelium*, still occurs in the absence of functional G proteins. In addition, several other cAR1-mediated responses, including Gαi phosphorylation (52), loss of ligand-binding (53), ERK2 activation (54) and various gene expression events take place in a G protein-independent manner. It has been shown that differentiated HL-60 cells (a neutrophil-like cell line) also exhibit Gαi-independent responses to chemoattractant stimulation. Under pertussis toxin (PTX) treatment, which specifically ADP ribosylates the Gαi subunit thereby preventing the dissociation of the Gβγ complex, these cells lose several chemokine responses, such as actin polymerization and PKB phosphorylation (32) but still display a well-defined uropod-like structure at their back, which is a fundamental component of cell polarity and chemotaxis (see below).

**Chemotaxis requires signaling events to be spatially confined**

**Seeing is believing**

*Dictyostelium* and neutrophils possess the extraordinary ability to precisely detect and respond to very shallow chemoattractant gradients (as low as 2% difference across their length) (55, 56). They do so by amplifying very small receptor occupancy differences into highly polarized intracellular events that give rise to a dramatic redistribution of cytoskeletal components. F-actin is locally polymerized at the front and actomyosin is localized to the back of the cells (Fig. 2) (57). This asymmetric enrichment of cytoskeletal components leads to cellular polarity, a prerequisite for migration. The exposure of *Dictyostelium* cells and neutrophils to gradients of chemoattractants induces a rapid change in polarity through the extension of anterior pseudopods. Pseudopod extension occurs through increased F-actin polymerization and is mediated by the Arp 2/3 complex, a seven subunit complex
that binds the sides of pre-existing actin filaments and induces the formation of branched polymers (58). As cells require a single pseudopod for optimal migration, an equally important component of chemotaxis is the suppression of lateral pseudopods. This event is promoted by myosin II filaments, which are concentrated at the rear of migrating cell and provide the power to retract the back. When considering this chemotactant-mediated acquisition of cellular polarity and migration, a fundamental question arises: how do cells transduce shallow external chemical gradients into an highly polarized cytoskeletal reorganization?2

Deciphering the signaling and cytoskeletal dynamics that occur during chemotaxis has been possible with the introduction of an imaging technique based on a natural fluorescent protein, the green fluorescent protein (GFP), produced by the bioluminescent jellyfish *Aequorea Victoria*. The cloning of GFP in 1992 (59) marked a new era, as it quickly became a convenient and versatile reporter for use in cell imaging. GFP can be used as a reporter by itself or, most interestingly, can be fused in-frame to a gene of interest, therefore permitting visualization of the dynamic localization of proteins in live migrating cells. GFP was the only representative of the family of ‘GFP-like fluorescent proteins’ until 1998, when blue, cyan, yellow (60) and, very recently, red emissions (61), have been successfully generated from the *Aequorea* GFP to significantly expand the range of colors available for cell biological applications. The GFP mutants allow studies with living cells to be extended to more than one intracellular protein, in multi-color labeling experiments. This gave rise to more sophisticated imaging techniques, such as Fluorescence Resonance Energy Transfer (FRET) (62) to study the dynamics of protein-protein interactions. Moreover, newly developed techniques, such as Fluorescence Recovery After Photobleaching (FRAP), provide tools for studying the trafficking and diffusion kinetics of proteins (63).

**Signals are amplified downstream of receptors and G proteins**

The binding of chemoattractants to their surface receptors represents the first event in a series of steps that eventually give rise to a polarized morphology and directed migration. The fact that cells become highly polarized when exposed to shallow gradients of chemoattractants, implies that the external signals must, at some point, be amplified to allow for distinct front and back behaviors. As receptors represent the link between external signals and the internal signaling machinery, it had been proposed that this amplification could arise from the asymmetric distribution of the receptors themselves. However, studies in both *Dictyostelium* and neutrophils, have established that chemoattractant receptors are uniformly distributed on the surface of chemotaxing cells. Indeed, using a cAR1-GFP fusion construct, it was shown that, in a chemoattractant gradient, cAMP receptors remain uniformly distributed around the cell periphery (64). Similarly, Bourne et al. (65) later demonstrated that C5a receptors fused to GFP are uniformly distributed showing no apparent increase anywhere on the plasma membrane of polarized differentiated PLB-985 cells, a neutrophil-like leukemia cell line. Although this uniform distribution, which allows cells to quickly respond to highly dynamic chemoattractant gradients is conserved, it does not appear to apply to all GPCR-mediated chemotactic events. In some cases the receptors redistribute to the leading edge, as has been reported for CXCR4, in hematopoietic progenitor cells (66), and CCR5, in motile Jurkat cells (67). These results suggest that the mechanisms regulating chemotactic responsiveness may vary among different cell types and different receptors.

A similar line of investigation established that the amplification of the signal occurs downstream of heterotrimeric G proteins. By studying the cellular distribution of Gβ-GFP in *Dictyostelium*, Jin and collaborators (68) showed that Gβγ subunits, like chemoattractant receptors, remain mostly uniformly distributed on the plasma membrane during chemotaxis. Considering that actin-binding proteins and F-actin accumulate in extending pseudopods at the leading edge of *Dictyostelium* and neutrophils, the uniform distribution of both receptors and G proteins suggest that the signals that lead to actin polymerization must occur by the selective activation of signaling pathways downstream of receptors and G proteins and upstream of actin (57, 69).

**PI3K/PTEN: Key components that spatially confine signals in chemotaxing cells**

The involvement of PI3K/PTEN as regulators of chemotaxis came from studies of two PH domain-containing proteins: CRAC and PKB.

In *Dictyostelium* binding of cAMP to cAR1 leads to the production of cAMP via the activation of the adenylyl cyclase ACA, a 12 transmembrane protein related to the mammalian G protein-coupled adenyl cyclases. While part of the synthesized cAMP remains inside the cell and activates PKA to regulate gene expression, the majority of the produced cAMP is secreted and a signal relay loop is initiated. The activation of ACA requires, in addition to the Gβγ subunits, a regulator called CRAC (cytosolic regulator of adenyl cyclase), which contains a pleckstrin homology (PH) domain at its N-terminus (70) – cells lacking CRAC show no receptor-mediated ACA activation. Under unstimulated conditions CRAC is found in the cytoplasm. However, following a uniform chemoattractant addition, CRAC rapidly and transiently associates with the plasma membrane. Remarkably, when CRAC-GFP expressing cells are placed in a gradient of chemoattractant, CRAC is specifically and persistently recruited to the leading edge (71). This redistribution is mediated by the binding of the PH domain of CRAC to PtdIns (3, 4) P2 and PtdIns (3–5) P3 (3-PI) (41, 72). In addition to its role in ACA activation, it was recently determined that CRAC is involved in regulating chemotaxis (72). Cells lacking CRAC move very slowly in gradients of chemoattractants and display broad fronts. However, as they are still capable of polymerizing cortical actin in response to chemoattractant stimulation, it was determined that the chemotaxis defect is not due to alterations in the motility machinery but to defects in translating the external chemoattractant gradient into directed pseudopod extension. Interestingly, using mutagenesis analyses, it was established that CRAC regulates ACA activation and chemotaxis independently. Mutants harboring C-terminal deletions, although defective in their ability to activate adenylyl cyclase, are still able to chemo-
tax, similarly to cells lacking ACA (72). Conversely, a CRAC point mutant that can no longer interact with 3-PI shows defects in both ACA activation and chemotaxis. These observations underline a key role for the spatial localization of CRAC in two distinct, yet related, responses: chemotaxis and ACA activation. Searches of the mammalian genome databases have not led to the identification of CRAC homologues.

Another PH-domain containing protein, Akt/PKB, associates with the leading edge of both Dictyostelium (73) and neutrophils (74), again underlining the evolutionary conservation of the chemotactic signaling cascade. Studies performed with Dictyostelium have established that Akt/PKB is involved in maintaining the leading edge polar structure as well as proper chemotaxis. Cells lacking Akt/PKB function less well and show significant defects in their ability to localize PAKs (a homologue of mammalian p21-activated kinases) and myosin II to their posterior (75). As active PAKs are required for myosin II assembly to the rear of moving cells, it was proposed that the role of Akt/PKB, once translocated and activated at the leading edge, is to phosphorylate PAKs, which moves to the posterior of chemotaxing cells where it co-localizes and promotes myosin II assembly (Fig. 2A). The role of Akt/PKB in neutrophil chemotaxis has yet to be determined.

PI3K and PTEN regulate chemotaxis

The chemoattractant-mediated stimulation of PI3K, together with its enzymatic activity, produces phosphatidylinositol 3-phosphate PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome 10), regulating the production of 3-PI. While PI3K phosphatase phosphorylates the 3′ phosphate, PTEN removes the 3′ phosphate thereby counteracting the action of PI3K. Pharmacological inhibition of PI3Ks blocks chemotaxis and migration to various degrees in Dictyostelium, as well as in a variety of mammalian cells (reviewed in [69]). Moreover, Dictyostelium cells lacking PI3K1 and PI3K2, two of the three class I PI3Ks expressed in this organism, and neutrophils harvested from mice lacking PI3Kγ, which is the principal isoform responsible for 3-PI production in these cells, have defects in polarity and show reduced efficiency of chemotaxis (35–37, 76). As expected, Dictyostelium cells lacking PI3K activity have strongly diminished levels of 3-PI following chemoattractant addition, as measured by a dramatically reduced recruitment of CRAC to the plasma membrane (76). Conversely, cells lacking PTEN show a persistent association of CRAC to the plasma membrane following chemoattractant stimulation and have strong polarity defects. These cells polymerize F-actin robustly in response to chemoattractant stimulation and, when placed in a chemoattractant gradient, move slowly and with less direction, compared to wild type cells (77). Taken together, these studies suggest an involvement of 3-PI in directional migration.

The cellular distribution of PI3K and PTEN in chemotaxing cells is mutually exclusive. Studies in chemotaxing Dictyostelium cells have shown that GFP-tagged PI3K1 and PI3K2 are specifically recruited to the leading edge (42). Simultaneously, PTEN-GFP is selectively removed from the leading edge, and remains associated with the plasma membrane at the sides and back of cells (42, 77). These responses seem to be conserved in mammalian leukocytes, as Li et al. have shown that PTEN is restricted to the rear of chemotactic neutrophils (78). This targeted cellular distribution of PI3K and PTEN therefore promotes the restricted production of 3-PI at the leading edge of chemotaxing cells and serves to amplify external chemical gradients into sharply polarized internal signals (Fig. 2).

The polarizing signal responsible for the recruitment of PI3K and PTEN remains to be fully understood, although recent studies have shown that the actin cytoskeleton might be involved (79, 80). Work performed with Dictyostelium has shown that the activation of PI3K requires an input from a small Ras GTPase and it was recently demonstrated that RasG, one of five Ras GTPases expressed in Dictyostelium, is activated exclusively at the leading edge of chemotaxing cells (42, 79, 81). It was pro-

Figure 3: Dictyostelium – a paradigm for signal relay. The image represents the current model for chemotactic signal propagation in Dictyostelium. Upon stimulation with chemotaxant (depicted as red dots), the PH-domain containing cytosolic protein CRAC is recruited to the leading edge (CRAC-GFP localization is shown on the far top right panel). By a mechanism that is still not elucidated CRAC activates ACA, which is enriched at the back (ACA-YFP localization is shown on the far bottom right panel). Produced cAMP is proposed to be released from the back to locally recruit neighboring cells (see text for details).
posed that Ras activation promotes leading edge formation by stimulating basal PI3K activity, which leads to F-actin polymerization, and subsequently, a feedback loop, mediated through localized F-actin, recruits more cytosolic PI3K to the leading edge to amplify the signal (79). Similarly, an actin-dependent feedback loop has previously been proposed to regulate PH-domain recruitment in neutrophils, where treatment with latrunculin, an inhibitor of actin polymerization, diminishes the recruitment of the PH-domain containing protein Akt/PKB to the plasma membrane, thereby decreasing further downstream activation (80).

Chemoattractant signaling to actin and myosin II

The cytoskeletal rearrangements induced by chemoattractants are mediated by Rho family GTPases, including Rac, Cdc42, and Rho. Thus, the chemoattractant-mediated spatiotemporal activation of Rho GTPases is a key regulatory event during chemotaxis. GTPases are GTP binding proteins that cycle between inactive GDP-bound and active GTP-bound forms. GTPase-activating proteins (GAPs) keep the enzymes in their inactive form by stimulating their low intrinsic GTP hydrolytic activity. Conversely, guanine nucleotide-exchange factors (GEFs), which catalyze the release of the bound GDP for GTP, are essential to activate the GTPases. It has been demonstrated that Rac is responsible for mediating leading edge formation, stimulating F-actin assembly at the front of both neutrophils and Dictyostelium (82–84). In neutrophils, Cdc42 is proposed to be required exclusively for directional migration (32) and for maintaining leading edge stability (83) in chemoattractant gradients. Interestingly, no Cdc42 homologues are found in Dictyostelium cells, thereby suggesting that alternative mechanisms should exist to stabilize the leading edge during directional migration.

F-actin polymerization at the front

Actin polymerization at the leading edge of chemotaxing cells is initiated through the generation of newly formed barbed ends by the Arp2/3 complex, whose activity is controlled by the adaptor proteins WASP (Wiskott-Aldrich Syndrome Protein) and SCAR (Suppressor of cAR). Through their CRIB domains (Cdc42/Rac-interacting binding domains), WASP/SCAR family members act as linkers between Rac/Cdc42 and actin filament formation (85) (Fig. 2B, D). GEFs for Rac and Cdc42 harbor PH domains and are therefore perfectly positioned to spatially control actin polymerization during chemotaxis (Fig. 2) (86).

In neutrophils, the RacGEFs P-Rex1 (PtdIns(3,4,5)P_3-dependent Rac exchanger), whose activity is directly activated by 3-PI and Gβγ (87), and Vav1, which is regulated by PtdIns(3,4,5)P_3, seem to be responsible for Rac activation during chemotaxis (88, 89). Another GEF, PIXa (PAK-interacting exchange factor), specific for Cdc42, has also been proposed to spatially control actin polymerization during chemotaxis. Li et al. (78) have described a pathway where Cdc42 is activated at the front of moving cells, through the formation of a complex with PAK1 and PIXa. They propose that chemoattractants induce the release of Gβγ subunits (from Gαi), which binds to PAK1-PIXa, thereby recruiting the ternary complex to the leading edge. PIXa then activates Cdc42, which in turns activates PAK1 to mediate Cdc42 activation in a feedback positive loop. Here again, 3-PI appear to have a role in controlling the spatial activation of Cdc42, albeit unclear at this point, as Yoshii et al. (90) have shown that PI3K products activate PIXa.

In Dictyostelium, among the 18 isoforms of RhoGTPases expressed, only the role of RacB has been specifically assessed during chemotaxis (84). It is required for proper chemotaxis and upon chemoattractant stimulation it shows two peaks of activation that correspond to the previously observed two peaks of chemoattractant mediated F-actin polymerization (91). The second peak of RacB activation, correlating to F-actin assembly during pseudopod extension, is dependent on the PI3K pathway, linking PI3K to F-actin polymerization at the leading edge. Furthermore, RacB binds with strong affinity to the CRIB domain of the Dictyostelium WASP. WASP has been recently shown to be localized to the leading edge of chemotaxing Dictyostelium cells via PI3K products – a mechanism which is also observed in mammalian fibroblasts (92, 93). Taken together these studies provide evidence that RacB is a possible candidate to stabilize the leading edge formation in Dictyostelium cells.

Myosin II assembly at the back

The signals that control the retraction of the back of a chemotaxis cell in Dictyostelium and neutrophils appear to be distinct. During neutrophil migration, Rho activity is required to regulate myosin-II-mediated contractility necessary for tail retraction (94), through a pathway that involves the action of its effector ROCK (Rho kinase) (95) (Fig. 2C). In differentiated HL-60 cells, stimulated with fMLP, RhoA is distributed predominantly to the sides and rear of stimulated cells, where it is thought to be activated by Goα12/13 (32, 96). Interestingly, the cytoskeleton also appears to regulate the back of migrating cells, as inhibitors of microtubule filament formation have recently been shown to enhance RhoA activity, leading to enhanced polarity and impaired chemotactic efficiency under chemoattractant stimulation (97, 98). Moreover, Li et al. presented evidence that activated Cdc42, at the front of chemotactic neutrophils, is required for the localization of activated RhoA at the back, where, through ROCK, it mediates the phosphorylation of PTEN. This pathway is then involved in controlling the localization and activation of PTEN to the back of chemotaxing cells (99).

In Dictyostelium Rho homologues are not present and two alternative pathways have been implicated to spatially control back retraction. One of these involves the phosphorylation of PAKα by Akt/PKB, which, as already mentioned, is required for myosin II assembly at the back of the cells (75). The other pathway involves cGMP, which has also been shown to be critical for myosin II assembly in Dictyostelium (Fig. 2A). The involvement of this soluble nucleotide in chemotaxis regulation emerged from studies using cell lines possessing either high or low cGMP levels. It was shown that high cGMP levels give rise to increased myosin II phosphorylation and cytoskeletal association as well as improved chemotaxis. On the other hand, low levels of cGMP generally produce the opposite effect (100, 101). Interestingly, the soluble guanylyl cyclase, the enzyme responsible for cGMP production following chemoattractant stimulation, has recently been shown to be enriched at the leading edge of cells (102), although its product mediates myosin filament formation at the back.
These results highlight an emerging theme during *Dictyostelium* and neutrophil chemotaxis where a requirement for dynamic signaling cross-talk between the front and back of cells is essential to properly orchestrate the complex cytoskeletal rearrangements that are required for migration.

**Signal relay**

As discussed above, the binding of chemoattractants to GPCRs triggers an exquisitely regulated series of signaling events that lead to the spatial activation of the cytoskeletal apparatus and directed migration. Chemotactic cells possess the ability to relay the chemotactic signal to surrounding cells through production and release of more attractants, which act in an autocrine and paracrine fashion to spread the chemotactic response. As with the signals that lead to cell polarity and motility, this signal relay response is shared by *Dictyostelium* and neutrophils.

Signal relay is essential for the development of *Dictyostelium*. Upon starvation, these cells spontaneously aggregate by producing, secreting, and detecting cAMP. The binding of cAMP to cAR1 leads to the synthesis of additional cAMP through the activation of ACA. As the cells are chemotaxing to the secreted cAMP signal, they recruit neighboring cells to the aggregate following each other and forming head-to-tail chains, called streams. As expected, cells lacking ACA do not form streams when subjected to an external cAMP gradient. Remarkably, it was shown that ACA-YFP is highly enriched at the back of chemotaxing cells (Fig. 3). This finding, coupled with other mutant analyses data, led Kriebel et al. (103) to propose that streaming depends not only on the presence of ACA, but also on its proper cellular distribution at the back of cells. This enrichment would provide a compartment from which cAMP is secreted to locally act as a chemoattractant. Interestingly, as mentioned, the essential regulator of ACA, CRAC, is recruited to the front of chemotaxing cells. Therefore, the activation of ACA, initiated at the front of cells, may reach a maximum at the back where it is perfectly localized to relay the chemotactic signal to neighboring cells.

The ability to relay signals is conserved in mammalian neutrophils. IL-8 (104, 105) and leukotriene B4 (LTB₄) (106) represent good examples of chemoattractants released by neutrophils following chemotactic activation. Several chemoattractants, including fMLP, C5a, PAF and LTB₄, stimulate neutrophil production and secretion of IL-8 (107). Although the secretory pathway of IL-8 remains to be established, there is evidence that this process could represent a mechanism used by neutrophils to attract a greater number of leukocytes to sites of inflammation (108) and to prime the superoxide production triggered by other chemokines (109). Furthermore, it has been demonstrated that the greater part of newly synthesized cytosolic IL-8 remains in the cells (110, 111), where it may constitute a source of IL-8 released when apoptotic neutrophils are digested by macrophages. The synthesis of LTB₄ in neutrophils involves a complex series of enzymatic reactions that are initiated at the plasma membrane (112). Under fMLP and C5a stimulation, a cytoplasmic phospholipase A₂, cPLA₂, translocates to the nuclear membrane of neutrophils, where it hydrolyzes membrane bound lipids to form arachidonic acid. Simultaneously, a 5-lipoxygenase (5-LO) is recruited to the nuclear membrane where it associates with the 5-LO activating protein (FLAP) and acts on arachidonic acid to generate LTA₄. LTA₄, by means of LTA₄ hydrolyase, is finally transformed into LTB₄, which is then secreted from the cells. The secreted LTB₄ binds to a specific class of Gi-coupled GPCRs and is part of an auto-amplification loop that mediates adhesion, migration and further accumulation of activated neutrophils to sites of inflammation (113, 114). Whether LTB₄ secretion is spatially localized during neutrophil chemotaxis remains to be determined.

**Perspectives**

*Dictyostelium* and mammalian neutrophils, although separated by millions of years of evolution, show remarkable similarities in the molecular mechanisms used to regulate the cytoskeletal rearrangements involved in directional sensing and migration. Indeed, *Dictyostelium* provides a simple model system where identical single cells respond to one major chemoattractant. Neutrophils, on the other hand, respond to a multitude of attractants that are generated from a wide variety of sources, including bacterially secreted formylated peptides, products of the complement cascade, and a plethora of chemokines derived from host endothelial, epithelial, and stromal cells. While signal cross-talk and integration is more complex in these cells, it is clear that the molecular mechanisms involved in generating asymmetric signals during neutrophil chemotaxis originated many years ago when *Dictyostelium* cells developed mechanisms to resist harsh environmental conditions. Further studies will continue to decipher how multiple signals are orchestrated into persistent and directed chemotaxis responses.

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