Eukaryotic Chemotaxis at a glance

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Introduction

Chemotaxis is a fundamental process in which cells migrate directionally when they are exposed to external chemical gradients. It is exhibited by a wide variety of cell types and involves distinct strategies that depend on the environmental conditions. For example, the migration of fibroblasts or kératinocytes within tissues is relatively slow as it relies on strong adhesion forces to the extracellular matrix. Conversely, fast moving and highly deformable leukocytes navigate to sites of inflammation using dynamic actin remodeling and minimal adhesion forces. In this Cell Science at a Glance article, we describe the signals that control directed migration for two cellular systems that are commonly accepted as paradigms for fast movement: the genetically tractable eukaryotic unicellular organism *Dictyostelium discoideum* and mammalian neutrophils (see supplementary material Movies 1 and 2). These two systems are exceptionally tuned to dynamically sense and respond to very shallow external chemoattractant gradients. Although evolutionarily separated by millions of years, their chemotactic machinery is controlled by highly conserved signal transduction pathways, which – in many instances – were first identified in *Dictyostelium.* The spatial activation of these pathways ultimately leads to the redistribution of polymerized actin at the front of the cell for propulsion and the assembly of myosin II at the back for retraction.

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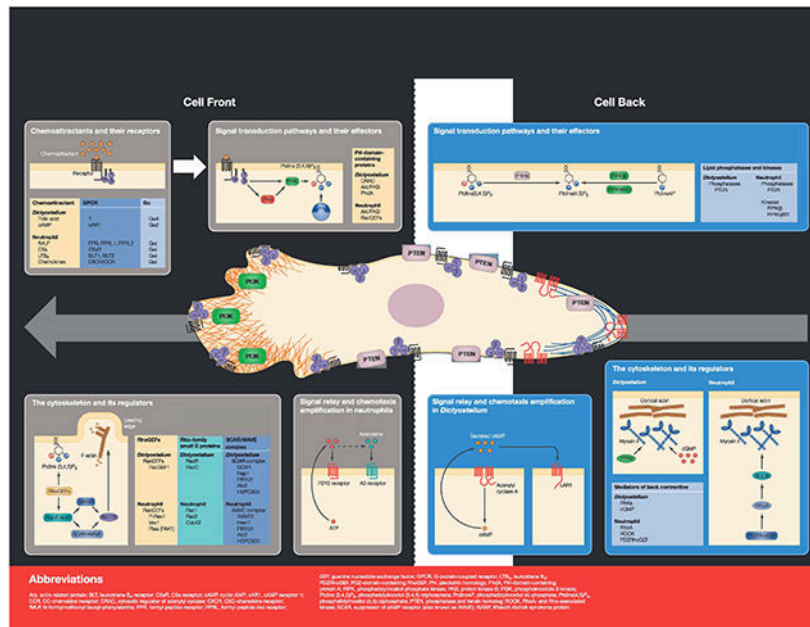
Chemoattractants and their receptors

The first event that occurs during chemotaxis is the binding of a chemoattractant molecule to its specific receptor. In *Dictyostelium*, two main chemoattractant molecules promote chemotaxis: folic acid and cAMP (Mahadeo and Parent, 2006). Folic acid, a bacterial by-product, acts as a directional cue to attract *Dictyostelium* to their food source during growth, as these organisms live as single cells that feed on bacteria. Under conditions of starvation, *Dictyostelium* enter a developmental program and lose their ability to sense folic acid. Within 4–5 hours, they then acquire the chemotactic machinery that is responsible for the detection, synthesis and degradation of cAMP, the most potent chemoattractant for *Dictyostelium* (Manahan et al., 2004; Saran et al., 2002). Concurrently, starved cells become highly chemotactic and migrate directionally to form aggregates that later differentiate into a multicellular structure that can resist harsh environmental conditions (Chisholm and Firtel, 2004).

Receptors for folic acid and cAMP belong to the G-protein-coupled receptor (GPCR) family, each member of which comprises an extracellular N-terminal domain followed by seven transmembrane helices and a C-terminal tail (Milligan and Kostenis, 2006). These receptors mediate most of their effects through heterotrimeric G proteins, although a subset of responses occur independently of G proteins (Brzostowski and Kimmel, 2001). Four distinct cAMP receptors (cARl to cAR4), which are expressed at different times during *Dictyostelium* development, have been cloned. cARl is expressed during early development, when the cells are highly chemotactic, and is linked to the G protein $G\alpha_2\beta\gamma$ (Parent and Devreotes, 1996). Both cARl and G proteins are uniformly distributed around the cell periphery and remain this way during chemotaxis (Parent and Devreotes, 1999). The folic-acid receptor, which is linked to $G\alpha_4\beta\gamma$, has yet to be cloned.

Leukocyte chemotaxis is induced by several chemoattractants and chemokines that signal through pertussis-toxin-sensitive G_i-coupled as well as G_{12}- and G_{13}-coupled GPCRs (Baggiolini, 2001; Niggli, 2003; Stephens et al., 2008). Classical chemoattractants include formylated peptides that are secreted by bacteria [such as N-formylmethionyl-leucyl-phenylalanine (fMLP)], products of the complement cascade (such as C5a) and phospholipid metabolites [such as leukotriene B_4 (LTB_4)], whereas the family of chemokines includes chemotactic mediators that are derived from a variety of cells. As first observed for cARl in *Dictyostelium,* C5a receptors remain uniformly distributed around the plasma membrane in chemotaxing neutrophils (Niggli, 2003; Parent, 2004). Although an even distribution of receptors around the cell periphery provides optimal sensitivity to highly dynamic chemoattractant gradients, there are cases in which chemokine receptors redistribute to the front of migrating cells; such behavior characterizes CXC-chemokine receptor 4 (CXCR4) in hematopoietic progenitor cells and CC-chemokine receptor 5 (CCR5) in Jurkat cells (Manes et al., 2005).

Signal transduction pathways and their effectors

Binding of the chemoattractant to its receptor triggers the dissociation of the heterotrimeric G protein into α- and βγ-subunits. In both *Dictyostelium* and mammalian neutrophils, the βγ dimer acts as the main transducer of chemotactic signals and activates several downstream effectors, including adenylyl cyclase, guanylyl cyclase and phospholipase C (PLC) (Bagorda et al., 2006; Stephens et al., 2008). The Gβγ stimulation is locally transduced via the activation of phosphoinositide 3-kinase (PI3K) at the front of cells in a Ras-dependent manner, although Gβγ can directly regulate PI3K in neutrophils (Kolsch et al., 2008; Stephens et al., 2008). PI3K converts phosphatidylinositol (4,5)-bisphosphate (Ptdins(4,5)P_2) into phosphatidylinositol (3,4,5)-trisphosphate (Ptdlns(3,4,5)P_3), thereby promoting a localized Ptdins(3,4,5)P_3 production. In *Dictyostelium,* the spatial restriction of the Ptdins(3,4,5)P_3 signal to the front is ensured by the presence of PTEN (the 3′-phosphatase) at the sides and back of cells (Parent, 2004). The Ptdlns(3,4,5)P_3 that is locally generated and retained at the front of cells provides a binding site for a subset of PH-domain-containing proteins, which eventually translocate to the leading edge and act as nucleation factors to activate key effectors of migration (Iijima et al., 2002).

The *Dictyostelium* cytosolic regulator of adenylyl cyclase (CRAC) protein was the first PH-domain-containing protein that was shown to translocate specifically to the front of chemotaxing cells. CRAC, originally identified as regulator for adenylyl-cyclase activity, also regulates chemotaxis. Another PH-domain-containing protein, Akt/PKB (protein kinase B), also translocates to the cell front and controls chemotaxis and cell polarity (Bagorda et al., 2006). Interestingly, PI3K, although necessary for the chemotaxis of *Dictyostelium* in shallow chemical gradients, appears to become dispensable in steeper gradient conditions. Indeed, *Dictyostelium* cells that lack the five class I PI3Ks as well as PTEN move surprisingly well in steep gradients, suggesting that other pathways are required to control chemotaxis. Recently, phospholipase A_2 (PLA_2) and guanylyl cyclase were proposed to represent candidate pathways to regulate chemotaxis in parallel with PI3K signaling (Insall and Andrew, 2007; Kolsch et al., 2008). In addition, components of the target of rapamycin

complex 2 (TORC2) have been shown to control *Dictyostelium* chemotaxis (Sasaki and Firtel, 2006), although the cellular distribution of this complex remains to be determined.

The use of Akt/PKB-GFP fusions revealed that Ptdlns(3,4,5)P_3 is also euriched at the leading edge of chemotaxing neutrophils (Iijima et al., 2002; Rickert et al., 2000). In addition, neutrophils that are harvested from mice that lack PI3Kγ, the principal PI3K isoform that is activated following chemoattractant stimulation, show reduced chemotaxis, although this trait appears to be environment- and chemokine- dependent (Ferguson et al., 2007; Franca-Koh et al., 2007; Liu et al., 2007; Stephens et al., 2008). Interestingly, in contrast to *Dictyostelium,* neutrophils that have been isolated from mice that lack PTEN do not show significant chemotaxis defects. Instead, in these cells, the $5'$-PtdIns(3,4,5)P_3 phosphatase SHIPI regulates chemotaxis – *ship1$^{-/-}$* neutrophils behave much like PTEN-deficient *Dictyostelium* cells (Nishio et al., 2007). However, the cellular distribution of SHIP1 during neutrophil chemotaxis has yet to be determined. These findings provide evidence that Ptdins(3,4,5)P_3 pathways are important regulators during mouse neutrophil chemotaxis.

Studies that were performed using HL-60 cells, a pluripotent human hematopoietic cell line that can be differentiated into neutrophil-like cells, showed that the PI3Kγ-dependent PtdIns(3,4,5)P_3 enrichment at the cell front is balanced by the local phosphatidylinositol phosphate kinase Iβ (PIPKIβ)- and PIPKI-γ661-dependent PtdIns(4,5)P_2 production at the back of chemotaxing neutrophils (Lacalle et al., 2007; Lokuta et al., 2007). Such a balance appears to be crucial for human neutrophil chemotaxis, as abrogation of either kinase impairs neutrophil polarization and directional migration. Furthermore, the signal transduction pathway that is activated during human neutrophil chemotaxis is dependent on the type of chemoattractants. Primary chemoattractants trigger a response that depends on p38 MAPK, whereas secondary chemoattractants act via PI3K (Heit et al., 2002). In addition, and similar to *Dictyostelium,* other phospholipid signaling pathways that are mediated by PLD and PLC have been shown to impact neutrophil chemotaxis, although the exact mechanism by which this occurs remains to be determined (Kolsch et al., 2008).

The cytoskeleton and its regulators

To migrate directionally in a chemoattractant gradient, major cytoskeleton rearrangements that promote F-actin polymerization at the front and actomyosin assembly at the back of cells must occur in a coordinated fashion. Such redistributions, which are mediated by members of the Rho-family small G proteins, ultimately promote leading-edge formation at the cell front and retraction at the cell back. Actin polymerization occurs through the generation of newly formed barbed ends by the Arp2/3 complex, the activity of which is controlled by adaptor proteins of the WASP (Wikott-Aldrich synodrome protein) and SCAR/WAVE (suppressor of cAMP recuptor; also known as WAVE) families (Vartiainen and Machesky, 2004). In both *Dictyostelium* and neutrophils, SCAR/WAVE complexes are localized at the front of cells and have been shown to locally bind Rae proteins, which go on to stimulate F-actin assembly during pseudopod extension (Ibarra et al., 2005; Weiner et al., 2006). In neutrophils, Rae proteins are activated by the PH-domain-containing Rac guanine-nucleotide-exchange factors (GEFs) (P-Rexl and Vavl) as well as DOCK2 (a DOCK180

homologue that is predominantly expressed in hematopoetic cells), the activities of which are regulated by PI3K signaling (Andrews et al., 2007; Stephens et al., 2008). In *Dictyostelium,* a single RacGEF, RacGEFl, has been identified (Park et al., 2004), although DOCK180 homologues are present in the genome. Although actin reorganization at the front of both *Dictyostelium* and neutrophils has been suggested to enhance PI3K activity, generating positive-feedback loops to reinforce polarity and directional sensing, the mechanisms by which this takes place, remain elusive (Kolsch et al., 2008; van Haastert and Devreotes, 2004).

The retraction that occurs on the trailing edge of chemotaxing neutrophils is mediated by RhoA, which is activated by $G\alpha_{12/13}$ – another G protein that has been shown to mediate chemotactic processes, and by PDZRhoGEF (PDZ-domain-containing RhoGEF), a RhoAGEF. RhoA, through its effector ROCK (RhoA- and Rho-associated kinase), regulates myosin-II-mediated contractility (Meili and Firtel, 2003; Wong et al., 2007). The RhoA-dependent actomyosin contraction is in regulated by Rae (Pestonjamasp et al., 2006) and Cdc42, which is activated at the front of cells by the Cdc42 GEF PIXα, and PAKl (Li et al., 2003). In this manner Rae and Cdc42 control polarity by acting locally at the front to promote actin polymerization, and distally at the back (via RhoA) to mediate retraction (Van Keymeulen et al., 2006). In *Dictyostelium,* in which no Rho or Cdc42 homologues have been found, cell retraction is controlled by the nucleotide cGMP, which is produced by soluble guanylyl cyclase (sGC) (van Haastert and Devreotes, 2004). High cGMP levels give rise to increased myosin II phosphorylation and assembly. Myosin II assembly is also controlled by PAKa, which localizes at the back of cells. PAKa, however, is phosphorylated by Akt/PKB in a PI3K-dependent fashion (Kolsch et al., 2008). These findings highlight the importance of front-to-back crosstalk during chemotactic signaling in both *Dictyostelium* and neutrophils (Ridley et al., 2003) – a process that is also evident for the activation of adenylyl cyclase in *Dictyostelium* (see below).

Signal relay and chemotaxis amplification

During chemotaxis, *Dictyostelium* and neutrophils relay the chemoattractant signal to neighboring cells to amplify the chemotactic response. In *Dictyostelium,* CRAC – in conjuncture with TORC2 – stimulates the adenylyl cyclase ACA, which leads to the production of cAMP that is rapidly secreted to stimulate neighboring cells. During chemotaxis, ACA is highly enriched at the back of cells. This distribution is essential for the ability of *Dictyostelium* cells to align in a head-to-tail manner and migrate in the form of chains during chemotaxis (Bagorda et al., 2006). It is proposed that the ACA that is positioned at the back of cells provides a compartment from which cAMP is locally released to attract neighboring cells to migrate in chains.

Interestingly, in neutrophils, the release of purines (such as ATP) has been shown to occur from the leading edge of the cell (Chen et al., 2006). As a neutrophil moves towards a source of chemoattractant, ATP is released and, together with its derived adenosine, provides an autocrine feedback loop by activating transmembrane purinergic receptors, thereby amplifying chemotaxis. Mammalian neutrophils also release a variety of chemokines during migration towards sites of inflammation. For example, chemoattractants such as fMLP and

C5a stimulate the production and secretion of LTB$_4$, as well as interleukin-8. Although not much is known about the mechanisms that guide the secretion of chemokines, there is increasing evidence that signal relay is responsible for the amplification of chemotaxis and is a key regulator of inflammatory responses.

Perspectives

In the past 10 years, incredible progress has been made in understanding how cells sense and respond to chemotactic gradients. The use of GFP technology has revolutionized the field and allowed scientists to get a glimpse of the intracellular working of live migrating cells (Van Haastert and Devreotes, 2004). Although the PI3K pathway is highly polarized in migrating cells and has a role in regulating chemotaxis, other parallel pathways are clearly involved. The challenge now resides in identifying additional pathways and, most importantly, in assessing how they work in concert to control polarity and chemotaxis. In this context, powerful *Dictyostelium* genetic screens will be invaluable and continue to provide key insight. However, as neutrophils are exposed to a complex array of chemotactic agents, more elaborate signaling cascades are surely operational in higher eukaryotes. Continued studies involving gene manipulation in mice as well as specific pharmacological inhibitors will certainly bring significant insight.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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