REVIEW ARTICLE

Big roles for small GTPases in the control of directed cell movement

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Small GTPases are involved in the control of diverse cellular behaviours, including cellular growth, differentiation and motility. In addition, recent studies have revealed new roles for small GTPases in the regulation of eukaryotic chemotaxis. Efficient chemotaxis results from co-ordinated chemoattractant gradient sensing, cell polarization and cellular motility, and accumulating data suggest that small GTPase signalling plays a central role in each of these processes as well as in signal relay. The present review summarizes these recent findings, which shed light on the molecular mechanisms by which small GTPases control directed cell migration.

Key words: cell polarity, cellular motility, directional sensing, eukaryotic chemotaxis, G-protein.

INTRODUCTION

The migration of neutrophils to sites of inflammation, the organization of embryonic cells during morphogenesis and the metastasis of tumour cells all result from the capacity of cells to detect and move towards the source of a signal (chemoattractant), i.e. to undergo chemotaxis. Chemoattractants are recognized by membrane receptors coupled to heterotrimeric G-proteins [GPCRs (G-protein-coupled receptors)], which translate the extracellular signal to intracellular signalling pathways. Through processes most likely involving multiple positive-feedback loops coupled to inhibitory signalling pathways, transduction of a slightly graded chemoattractant signal ultimately generates a highly polarized cellular response that allows the cells to move forward and up the chemoattractant gradient.

Our current understanding of eukaryotic chemotaxis results from studies mostly performed with neutrophils and the social amoeba *Dictyostelium discoideum*. *Dictyostelium* has proven to be an excellent model for the study of chemotaxis, not only because the key signalling pathways implicated in directional cell movement are conserved from the amoeba to higher eukaryotes, but also because *Dictyostelium* has the advantage of being a genetically and biochemically tractable organism [1]. In Nature, *Dictyostelium* grows as autonomous cells that chase bacteria, their food source, by performing amoeboid chemotaxis towards folate, which bacteria secrete. *Dictyostelium* cells also have the potential to perform chemotaxis towards cAMP, a chemoattractant they secrete when their bacterial food source has been depleted. Chemotaxis to cAMP allows *Dictyostelium* to survive starvation by promoting their aggregation and the subsequent formation of fruiting bodies containing spores [2].

Eukaryotic chemotaxis can be described as a modular process in which the directional sensing, cellular motility and cell polarity machineries work in a concerted fashion to generate efficient chemotactic migration [3]. In both neutrophils and *Dictyostelium*, while receptor-mediated heterotrimeric G-protein activation mirrors the extracellular gradient of chemoattractant [4,5], gradient sensing rapidly gives rise to the localized accumulation of PtdIns(3,4,5)P_3 on the side of the cell facing the highest chemoattractant concentration. This polarized PtdIns(3,4,5)P_3 response is generated through the differential local regulation of PI3K (phosphoinositide 3-kinase), which produces PtdIns(3,4,5)P_3 by phosphorylating PtdIns(4,5)P_2, and the PtdIns 3-phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome ten), which converts PtdIns(3,4,5)P_3 back into PtdIns(4,5)P_2. Upon gradient sensing, PI3K is recruited and activated at the plasma membrane, where the cell is exposed to a higher concentration of chemoattractant, leading to the localized activation and production of PtdIns(3,4,5)P_3. By contrast, PTEN is delocalized from the front, but stays associated with the membrane at the lateral sides and posterior of the cell, which would degrade any PtdIns(3,4,5)P_3 that might either diffuse laterally from the leading edge or be produced along the lateral sides of the cell, thus restricting the polarized accumulation of PtdIns(3,4,5)P_3. The importance of the regulation of PI3K and PTEN localization in the chemoattractant-sensing process is highlighted by the observation that mutations in either *Dictyostelium* PI3K or PTEN that impair their localization pattern lead to the production of adventitious pseudopodia and directionality defects [6,7].

It should be noted that, despite the recognized important role that PtdIns(3,4,5)P_3 signalling plays in directed cell movement, some evidence indicates that PI3K is not essential. *Dictyostelium*

Abbreviations used: ACA, adenylate cyclase for aggregation; APC, adenomatous polyposis coli; Arp2/3 complex, actin-related protein 2/3 complex; cAR, cAMP receptor; cdc42, cell-division cycle 42; CLIP170, cytoplasmic linker protein-170; CRAC, cytosolic regulator of adenylate cyclase; Epac1, exchange protein activated by cAMP-1; ERK2, extracellular-signal-regulated kinase 2; F-actin, filamentous actin; GAP, GTPase-activating protein; DGAP1, *Dictyostelium* GAP1; Gbp, cGMP-binding protein; GEF, guanine-nucleotide-exchange factor; GPCR, G-protein-coupled receptor; $G_{\beta\gamma}$, a subunit of heterotrimeric G-proteins; Hem-1, haemopoietic protein 1; IQGAP1, IQ-motif-containing GTPase-activating protein 1; MDia, formin protein mammalian homologue of Diaphanous; MHC, myosin heavy chain; MHCK, MHC kinase; MLC, myosin light chain; MLCK, MLC kinase; MTOC, microtubule-organizing centre; PAK, p21-activated kinase; PDGF, platelet-derived growth factor; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; Pia, Pianissimo; PTEN, phosphatase and tensin homologue deleted on chromosome ten; RBD, Ras-binding domain; RIP3, Ras-interacting protein 3; ROCK, p160-Rho-associated coil-containing protein kinase; SCAR, suppressor of cAMP receptor; TORC2, target of rapamycin complex 2; WASP, Wiskott–Aldrich syndrome protein; WAVE2, WASP family verprolin-homologous protein 2.

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cells that lack up to three of their five class I PI3Ks, as well as neutrophils lacking PI3Kγ, the principal isoform involved in PtdIns(3,4,5)P_3 production in leucocytes [8–10], exhibit reduced cell polarization and chemotaxis, but these cells are still able to move in the direction of the chemoattractant [9,11,12] (K. Takeda and R.A. Firtel, unpublished work). In addition, cells treated with the PI3K inhibitor LY294002 {[2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one]}, which exhibit severe chemotaxis defects, will eventually move up a steep chemoattractant gradient if given a strong enough gradient and sufficient time to polarize [13] (K. Takeda and R. A. Firtel, unpublished work). It was then postulated that the role of PI3K in gradient sensing is primarily to amplify the extracellular gradient of chemoattractant into a highly polarized response, allowing cells to sense and respond rapidly to very weak signals, which is characteristic of highly motile cells such as neutrophils and *Dictyostelium* [3,14], although other studies suggest that, in a weak chemoattractant gradient, PI3K is required for proper directional sensing (K. Takeda and R. A. Firtel, unpublished work). Along with the observation that PI3K localizes at the leading edge of chemotaxing cells, the above considerations suggest that gradient sensing occurs upstream of PI3K and raise the possibility that other chemoattractant-sensing pathways exist. The nature of these alternative pathways remains unknown.

Multiple findings from studies performed on different types of motile cells, including leucocytes, *Dictyostelium*, primary dendritic cells and fibroblasts, suggest that chemoattractant-induced localized PtsIns(3,4,5)P_3 signalling guides the local polymerization of F-actin (filamentous actin) [15]. Localized F-actin polymerization leads to pseudopod extension, which confers cellular motility. The persistent presence of PtdIns(3,4,5)P_3 at a particular site on the plasma membrane, as observed when cells are placed in a chemoattractant gradient, eventually results in major changes in global cell polarity. The cells acquire an elongated shape in which one dominant F-actin-enriched pseudopodium becomes the leading edge of the chemotactic cell, whereas suppression as well as retraction of pseudopodia at the back and sides of the cell are mediated by cortical myosin II [16]. In turn, cell elongation may also enhance the sensitivity of chemotactic cells to shallow chemoattractant gradients by increasing differences in receptor occupancy between the front and back of the cell. Polarization then contributes to directed cell migration by promoting persistent movement in a specific direction and producing two poles in which components of the respective signalling pathways are stabilized. Further, as will be discussed below, some anterior and posterior pathways function antagonistically: the enhancement of one often leads to an inhibition of the other. The co-ordinated regulation of directional sensing, cellular motility and polarity will therefore determine the efficiency of chemotaxis.

Another important component of the chemotactic response is signal relay, which allows chemoattractant-stimulated cells to transmit the signal to neighbouring cells. Chemotactic cells have the ability to synthesize and release chemoattractants, which then act in an autocrine and paracrine manner to spread the chemotactic signal. Such a signal relay plays an important role in the life cycle of *Dictyostelium*, driving its aggregation upon starvation [17]. cAMP induces its own production and secretion by binding and activating cAR1 (cAMP receptor 1) and cAR3, which then promote the activation of the cAMP-synthesizing enzyme adenylate cyclase. Several observations suggest that signal relay also occurs in chemotactic neutrophils, representing a potential mechanism which they use to attract a greater number of leucocytes to sites of inflammation [18–20].

Research over the past decade has started to uncover many key components of the chemotactic signal-transduction machinery downstream of heterotrimeric G-proteins. Of particular interest, studies revealed the importance of the involvement of small (20–35 kDa) GTPases at multiple levels of the chemotactic response. The present review will focus on the key roles that small GTPases play in the control of amoeboid chemotaxis, with particular emphasis on their newly appreciated functions.

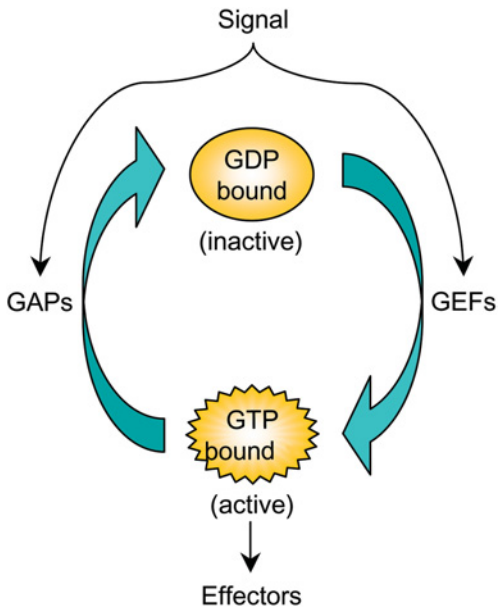

Figure 1 The GTPase cycle

Inactive GDP-bound small GTPases are activated by GEFs, which promote the release of GDP and allow its replacement by GTP. Active GTP-bound small GTPases can then promote the activation of different effectors until they return to their GDP-bound inactive state upon hydrolysis of the GTP into GDP, which is catalysed by GAPs. Extracellular or intracellular signals regulate the small GTPases by acting on either the GEFs or the GAPs.

SMALL GTPases

Although comparable with the α-subunit of heterotrimeric G-proteins, small GTPases function as monomeric entities. Every GTPase shares a common biochemical mechanism and function as a binary molecular switch, cycling between inactive GDP-bound and active GTP-bound forms (Figure 1). Two main classes of regulatory proteins control this cycle: GEFs (guanine-nucleotide-exchange factors) promote the exchange of GTP for GDP, and GAPs (GTPase-activating proteins) stimulate the otherwise-slow intrinsic GTPase activity, promoting the formation of the inactive GDP-bound configuration.

The superfamily of small GTPases, sometimes called the 'Ras superfamily' with reference to their founding member, can be divided into five major subfamilies according to their sequence and functional similarities: Ras, Rho, Rab, Ran and Arf [21]. In addition to the Ras proteins, the Ras subfamily includes Rap, Ral, and Rheb GTPases, whereas the Rho subfamily is further divided into three subgroups: Rho, Rac and Cdc42 (cell-division cycle 42). The superfamily of small GTPases is highly conserved throughout all eukaryotes. The *Dictyostelium* genome encodes multiple Rab, Arf and Ras proteins, including a Rap extremely similar to mammalian Rap1, as well as numerous Rac GTPases, but is distinct from many other cell types in that no homologues of Ral, Rho or Cdc42 have been identified [22].

Rab, Ran and Arf proteins mostly regulate vesicular or nucleocytoplasmic transport; the Ras and Rho subfamily proteins are important components of signalling networks implicated in the transduction of extracellular stimuli [21]. In particular, Ras proteins

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Table 1 Small GTPases involved in the control of *Dictyostelium* and leucocyte chemotaxis

Partial list of identified positive (+) and negative (−) regulators, as well as small GTPase effectors implicated in directed cell migration (see the text for references).

Small GTPase(s)	Regulator(s)	Effector(s)	Role
Dictyostelium			
RasC, RasG and other Ras	Aimless+ and RasGEFM+	PI3K, RIP3 (TORC2), ERK2, ACA and guanylate cyclase	Directional sensing, F-actin polymerization, ACA activation and adaptation
Rap1	GbpD+	Phg2	Cell polarity, motility, myosin regulation and adhesion
Rac1A, Rac1B, Rac1C, RacB and RacG	RacGEF1+	PAKa, PAKc, DGAP1 and SCAR/WAVE/WASP	F-actin polymerization
Leucocytes			
Rap1	Epac+	Riam, lamellipodin, Arap3 and RapL/β1, β2, β3 integrins	Cell motility, polarity and adhesion
Rac1 and Rac2	Tiam-1+, Vav+, P-REX-1+, DOCK2+, DOCK180+, PAK1+ and RhoA/FilGAP−	SCAR/WAVE and IQGAP1	Directional sensing, F-actin polymerization, microtubule regulation and myosin regulation
Cdc42	PIXα+ and PAK1+	PAK1, WASP and IQGAP1	Directional sensing, F-actin polymerization and microtubule regulation
RhoA	Lsc/p115 RhoGEF+, Rac1+, Cdc42+, Cdc42-Rac1/Tiam-1/p190RhoGAP− and Rac1/WAV2/Hem1−	ROCK and PTEN	Directional sensing and myosin assembly

are recognized for their involvement in signal-transduction cascades that regulate cell growth, proliferation, differentiation and survival, primarily through the modulation of gene expression [23]. Rho GTPases are key intermediates within signalling pathways that control cell-cycle progression, morphogenesis and motility, mostly by regulating the actin cytoskeleton, but also by affecting gene expression [24]. Moreover, both Ras and Rho GTPases regulate normal- as well as cancer-cell motility in fibroblasts and epithelial cells [25], but we are only beginning to identify the molecular mechanisms involved.

Consistent with their documented function as regulators of the cytoskeleton, Rho GTPases were found to control cellular motility and polarity in chemotaxing cells by regulating actin as well as myosin organization. Recent studies, mostly performed in *Dictyostelium*, also underscore the importance of Ras subfamily GTPases in eukaryotic chemotaxis, revealing their involvement in directional sensing, cytoskeleton regulation and signal relay (Table 1).

SMALL GTPases ARE IMPORTANT COMPONENTS OF THE DIRECTIONAL-SENSING MACHINERY

Upon exposure to a chemoattractant gradient, the restricted accumulation of PtdIns(3,4,5)P_3 at the edge of the cell closest to the chemoattractant source suggested that directional sensing occurs independently of global cell polarity. Indeed, chemoattractant-elicited localized PtdIns(3,4,5)P_3 production, albeit reduced, still occurs in neutrophils as well as *Dictyostelium* cells treated with the actin-polymerization inhibitor latrunculin, which generates round cells unable to form pseudopodia [26–28]. The decrease in PtdIns(3,4,5)P_3 production in latrunculin-treated cells can be explained by the observation that the recruitment of additional PI3K to the plasma membrane requires an F-actin cytoskeleton [28]. It is therefore postulated that amplification of the initial PtdIns(3,4,5)P_3 response is mediated through positive-feedback loops that involve F-actin, and that this is crucial for the subsequent formation of the leading edge as well as the maintenance of cellular asymmetry during chemotaxis [29].

It was only recently that several studies provided insights into the intermediate events that create the PtdIns(3,4,5)P_3 asymmetry in chemotactic cells. In particular, the molecular mechanisms regulating PI3K and PTEN activities were found to involve Ras and Rho GTPases respectively (Figure 2).

Ras signalling at the leading edge regulates PI3K

One of the first pieces of evidence of a role for Ras in chemotaxis came from a study performed with fibroblasts migrating towards PDGF (platelet-derived growth factor), which can induce both mitogenic and chemotactic responses through its binding to the PDGF receptor tyrosine kinase [30]. Inhibition of Ras signalling by the overexpression of either a dominant-negative mutant of Ras or a RasGAP was found to prevent PDGF-stimulated chemotaxis. In addition, the observation that cells producing excess constitutively active Ras or RasGEF also failed to migrate towards PDGF suggests that the Ras-dependent chemotactic response requires a tightly controlled regulation of Ras activity. The involvement of Ras in cellular motility is highlighted further by the findings that constitutively active K-Ras or H-Ras stimulate the motility of fibroblasts, and that a dominant-negative H-Ras mutant inhibits motility in many carcinoma cells [25].

The first clue that Ras could also play a role in GPCR-mediated directed cell movement was the finding that a *Dictyostelium* homologue of RasGEFs, Aimless (AleA) or RasGEFA, was required for aggregation [31]. It was found that disruption of the Aimless gene produced *aleA*-null (*aleA*$^-$) cells that are impaired with respect to both chemotaxis and adenylate cyclase activation. Since *aleA*$^-$ cells migrate, although with reduced speed and polarity, the chemotaxis defect seems to result from impaired gradient sensing. Moreover, inhibition of Ras signalling by the overexpression of a dominant-negative Ras protein in *Dictyostelium* in an *aleA*$^-$ background induces severe defects in directional movement, further suggesting that Ras plays a role in this process [28]. The *Dictyostelium* Ras proteins RasC and RasG are rapidly and transiently activated upon chemoattractant stimulation, which seems to require the heterotrimeric G-protein $G_{\alpha 2\beta\gamma}$ [28,32]. Among other things, disruption of RasC was reported to produce

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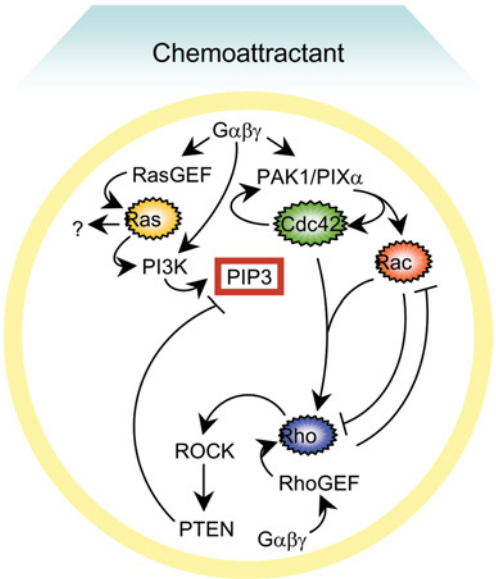

Figure 2 Directional-sensing model

Heterotrimeric G-protein signalling leads to Ras, Cdc42 and Rac activation at the plasma membrane facing the highest chemoattractant concentration, as well as to Rho activation. Ras activates PI3K, causing the local production of PtdIns(3,4,5)P_3 ('PIP3'). Rac and Cdc42 signalling excludes Rho from the leading edge, but also positively regulates Rho activity, which, in turn, promotes the ROCK-dependent activation of PTEN at the sides and posterior of the cell. Rho inhibits Rac and Cdc42 signalling at the back and sides, and PTEN degrades PtdIns(3,4,5)P_3, preventing it from diffusing along the plasma membrane, and therefore confining the PtdIns(3,4,5)P_3 signal to the front. Other, as-yet-unidentified, Ras effectors are likely to participate in the gradient-sensing process. This cartoon does not depict putative global inhibitors of leading-edge signalling that have been proposed to differentially inhibit the signalling at the cell's posterior over the anterior, thus promoting leading-edge formation [211].

cells that exhibit reduced chemotaxis to cAMP during early development [33], which could possibly result from a defect in gradient interpretation. In addition, a recent study shows that *rasG*-null cells display significant chemotaxis defects, and that *rasC*/*rasG* double null cells completely fail to perform chemotaxis towards cAMP [34]. Further studies indicate that Aimless most likely regulates both RasC and RasG since *aleA*$^-$ cells display reduced RasG activity and fail to induce RasC activation ([28]; H. Kae and G. Weeks, unpublished work cited in [34]; P. G. Charest and R. A. Firtel, unpublished work).

Whereas Ras protein is uniformly distributed along the plasma membrane in chemotactic cells, Ras activation primarily takes place at the leading edge [28]. Using the actin-polymerization inhibitor latrunculin, Sasaki et al. [28] observed that chemoattractant-induced PI3K translocation and activation is significantly reduced in latrunculin-treated cells, but localized Ras activation occurs independently of a functional cytoskeleton. Importantly, directional Ras activation was found to occur in the absence of PTEN as well as to precede and occur independently of PI3K activation, whereas PI3K activation requires an intact Ras-binding domain (RBD), which suggests that Ras might directly regulate PI3K activity [6,28]. These data thus strongly suggest that Ras is an integral component of the directional-sensing machinery upstream of, and regulating, the localized PtdIns(3,4,5)P_3 production (Figure 2). Other evidence pointing to the involvement of Ras GTPases in PI3K activation includes the findings that both *rasC*$^-$ and *rasG*$^-$ cells exhibit reduced chemoattractant-stimulated activation of Akt/protein kinase B, a downstream effector of PI3K, and that overexpression of a constitutively active RasG mutant prolongs cAMP-induced PtdIns(3,4,5)P_3 accumulation [28,33,34]. Also, the observation that *aleA*$^-$ cells fail to promote the formation of a second peak of chemoattractant-stimulated F-actin polymerization [31], which is sensitive to PI3K activity and linked to pseudopod extension [35], further suggests a link between Ras and PI3K activities. Finally, the finding that H-Ras directly binds and activates PI3Kγ in mammalian cells [36] raises the possibility that Ras also regulates PI3K activation in chemotactic leucocytes.

Interestingly, the directionality defect caused by the inhibition of Ras signalling in *Dictyostelium*, through the overexpression of a constitutively active Ras mutant, is significantly more severe than that of cells treated with the PI3K inhibitor LY294002 or of *pi3k1/2*$^-$ cells [6,28]. This observation suggests that Ras may control directional sensing by acting through other effectors in addition to PI3K, effectors that are likely to be involved in the as-yet-unidentified PI3K-independent gradient-sensing pathways.

Even though Rac and Cdc42 bind and modulate the activity of PI3K *in vitro* [37,38], no evidence supports a direct activation of PI3K by Rho GTPases in living cells. On the other hand, one study reports that murine neutrophils lacking Rac1 fail to spatially constrain the chemoattractant-induced accumulation of PtdIns(3,4,5)P_3 at the leading edge, consequently causing directionality defects [39]. However, as discussed below, this effect could be indirect and result from the role of Rac in F-actin polymerization or other Rac-dependent cytoskeleton rearrangements. For example, in *Dictyostelium*, F-actin polymerization promotes the translocation of PI3K to the plasma membrane [28].

Rho GTPases in directional sensing and PTEN regulation

Various findings suggest that Rho GTPases are also involved in directional sensing. Notably, inhibition of Cdc42 by overexpression of dominant-negative mutants in neutrophils produces cells that have an irregular front and frequently form multiple leading edges [40]. Consequently, cells with impaired Cdc42 signalling are unable to perform chemotaxis, even though cell motility appears intact, which suggests that Cdc42 is required for gradient sensing [40,41]. Cdc42 localizes at the leading edge of chemotactic neutrophils, where it is activated by the PAK (p21-activated kinase)-associated RacGEF PIXα in a $G_{\beta\gamma}$ (subunit of heterotrimeric G-proteins)- and PAK1-dependent fashion. Independently of its kinase activity, PAK1 was shown to promote the $G_{\beta\gamma}$-dependent stimulation of PIXα and consequently Cdc42, which then activates PAK1 [42,43]. In turn, PAK activity is essential for the spatial restriction of Rac-dependent actin polymerization to the leading edge of migrating fibroblasts [44]. These observations suggest that Cdc42 signalling is involved in gradient sensing, translating the external cues into the spatial localization of active Rac and consequently of F-actin polymerization.

Recent studies indicate that Rho GTPases regulate the localization and activation of PTEN in chemotactic cells, providing another potential mechanism by which they control directional sensing [45]. *Dictyostelium* cells that lack PTEN have altered levels of PtdIns(3,4,5)P_3, which generates a broad irregular front with multiple pseudopodia giving rise to directionality defects [6,7]. Depletion of PTEN through siRNA (small interfering RNA) knockdown inhibits the chemotaxis of neutrophils and Jurkat cells [45,46]. Moreover, mutants of *Dictyostelium* and mammalian PTEN that are unable to interact with the membrane fail to rescue *pten*$^-$ cells, suggesting that membrane localization is required for the function of PTEN in chemotaxis [7,47]. Findings from a number of studies suggest that regulation of PTEN is very complex and involves multiple phosphorylation events, as well as lipid and protein interactions that modulate PTEN's activity and localization [48,49]. As mentioned above, *Dictyostelium* PTEN

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localizes to the plasma membrane and is delocalized from the front of the cell upon gradient sensing [6,7]. PTEN localization in neutrophils is more controversial. Although the absence of enrichment of PTEN at the plasma membrane of chemotactic leucocytes is universally observed, one group reported that PTEN is excluded from the leading edge [43,45], while in two other studies such a polarized localization of GFP (green fluorescent protein)–PTEN in chemotaxing neutrophils was not observed [46,50]. However, Li et al. [45] found that only PTEN, and not GFP–PTEN or PTEN–GFP, exhibited a polarized distribution, which suggests that a GFP tag can affect PTEN's localization. A recent study using single-molecule imaging techniques provides evidence that mammalian PTEN acts through dynamic interactions with the plasma membrane [47]. It was found that PTEN has a higher affinity for retracting membranes, which includes the rear of highly polarized cells, thus raising the possibility that polarized membrane distribution of PTEN is a conserved mechanism.

The first result supporting a role for Rho GTPases in the regulation of PTEN is the observation that Cdc42 signalling at the leading edge is required for the exclusion of PTEN, in addition to the localized F-actin polymerization [43]. Alternatively, other studies suggest that PTEN is regulated by RhoA, which is also excluded from the leading edge of migrating neutrophils, a process that involves its downstream effector ROCK (p160-Rho-associated coil-containing protein kinase) [45,51–53]. Chemoattractant-induced RhoA activation occurs in a $G_{\alpha 12/13}$-dependent fashion, and was recently suggested to involve the $G_{\alpha 12/13}$-binding RhoGEF Lsc/p115 [50,54]. However, recent work indicates that Cdc42 regulates the activity and localization of RhoA in migrating neutrophils and fibroblasts [45,55], which could represent the mechanism by which Cdc42 promotes the exclusion of PTEN from the leading edge. The finding that Cdc42-dependent signalling might target RhoA for proteolytic degradation provides a potential molecular mechanism underlying the absence of RhoA signalling at the leading edge and consequently of PTEN [56]. In addition, several studies suggest that the G_i-dependent activation of Rac1 at the leading edge also counteracts RhoA activity [50,57–62]. In fibroblasts, the Rac1-promoted down-regulation of Rho was recently found to involve the Tiam-1/Rac1-mediated activation of p190-RhoGAP [63]. Another study recently proposed that Rac-dependent activation of RhoGAPs at the leading edge could occur through the Rac effector WAVE2 [Wiskott–Aldrich syndrome protein (WASP) family verprolin-homologous protein 2], based on the finding that the WAVE2 scaffold Hem-1 (haemopoietic protein 1) interacts with RhoGAPs [64]. Rho–ROCK signalling is also found to antagonize Rac activity, which was recently suggested to involve the activation of FilGAP, a filamin-binding RacGAP [50,65,66].

These data support a model of directional sensing that proposes that the mutual inhibition of the 'frontness' and 'backness' signals underlie the chemoattractant-induced cell polarization [50,51]. However, the recent findings that PtdIns(3,4,5)P_3 and Cdc42, as well as Rac1 signalling at the front, have a positive effect on RhoA activity at the back of chemotactic neutrophils suggests that the different small GTPase signalling pathways also co-operate to establish and stabilize cellular polarity [55,67].

Very little is known about PTEN regulation in *Dictyostelium* and, to our knowledge, the involvement of Rho GTPases in this process has not been investigated. Although no genes encoding Cdc42 or Rho proteins are present in this organism, it is thought that some of the multiple Rac GTPases are functional homologues of Cdc42 and RhoA. *Dictyostelium* RacB was found to be required for full activation of PAKc, a PAK1 homologue [68,69]. Interestingly, cells lacking PAKc or expressing a mutant PAKc impaired in RacB binding produce multiple pseudopod extension and membrane retractions on all regions of the plasma membrane independently of the direction of the chemoattractant gradient, which hints that these cells have directional-sensing defects. The presence of a conserved putative $G_{\beta\gamma}$-binding domain suggests that, like PAK1, PAKc may be regulated by the $G_{\beta\gamma}$ subunit of heterotrimeric G-proteins activated downstream of the chemoattractant receptors [43,68]. Even though PTEN localization in *racB*$^-$ and *pakc*$^-$ cells was not assessed in these studies, the above-mentioned considerations raise the possibility that *Dictyostelium* RacB plays a role similar to that of Cdc42 in the control of directional sensing.

SMALL GTPases CONTROL MOTILITY AND POLARITY BY REGULATING CYTOSKELETON DYNAMICS IN CHEMOTAXING CELLS

The polarized intracellular response resulting from gradient sensing leads to major cytoskeletal reorganization, which results in global cell polarization and the initiation of directional movement. It is well established that cellular motility results from the co-ordination of leading-edge protrusion, the establishment of new adhesion sites at the front, and cell-body contraction co-ordinated with the detachment of adhesion sites at the rear. Every step involves the assembly, disassembly or reorganization of the actin cytoskeleton, and each must be co-ordinated in both space and time to generate productive net forward movement [70]. It is equally well recognized that small GTPases, especially those of the Rho family, regulate these cytoskeleton dynamics, although it was only recently that we started to uncover the molecular mechanisms implicated in these processes. Microtubules also play an important role in directional cell migration in many cell types, and recent studies indicate they are also regulated by Rho GTPases [180].

Regulation of F-actin polymerization at the leading edge

Whereas Cdc42 seems to be implicated in directional sensing and in determining the site of F-actin polymerization, the primary role of Rac seems to be to generate the protrusive force required for cell motility, through the regulation of F-actin polymerization at the leading edge [70]. Cdc42 also induces F-actin polymerization, but is more closely associated with the formation of filopodia, which have been ascribed sensory or exploratory functions, often seen at the front of chemotactic cells [71]. Rac and Cdc42 regulate F-actin polymerization through the activation of the SCAR (suppressor of cAMP receptor)/WAVE and WASP proteins respectively, as well as through the Rac/Cdc42 effector IQGAP1 (IQ-motif-containing GTPase-activating protein 1) [72,73] (Figure 3). SCAR/WAVE and WASP are scaffold proteins that, once activated, relay the signal from Rac/Cdc42 to the actin-nucleation machinery, the Arp2/3 complex (actin-related protein 2/3 complex) [74]. IQGAP1 is also a scaffold that seems to mediate many of Rac/Cdc42 cytoskeletal functions [73]. Rho promotes actin polymerization through its interaction with mDia (formin protein mammalian homologue of Diaphanous), and this has been suggested to contribute to membrane extension during fibroblast migration [75,76].

The importance of Rac in cellular motility was put forward following the finding that *Dictyostelium* cells expressing a dominant-negative Rac1B mutant do not have a well-defined F-actin-rich leading edge and do not protrude pseudopodia, resulting in very poor motility [77]. Cells expressing constitutively active forms of Rac1A, Rac1B or Rac1C display motility defects, which supports the importance of regulated Rac activity during cell migration [77,78]. These Racs appear to act in part through the IQGAP-related protein DGAP1 (*Dictyostelium* GAP1), which interacts with the GTP-bound Racs and is involved in the modulation of the

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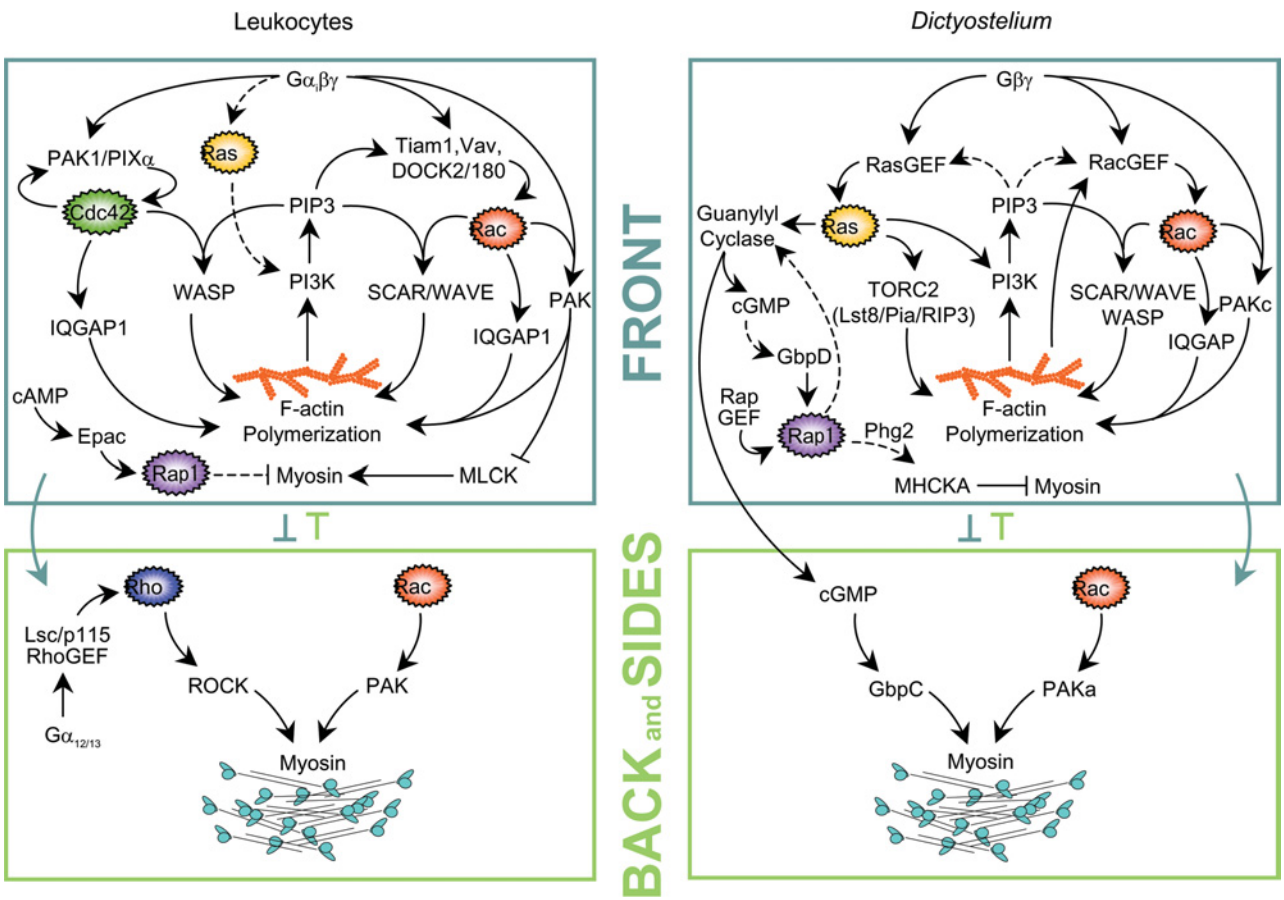

Figure 3 Small GTPases similarly regulate actin and myosin dynamics in chemotactic leucocytes and *Dictyostelium*

At the leading edge, Rac/Cdc42 and Ras signalling control F-actin polymerization, whereas Rap regulates myosin. Rac and Rho signalling at the back and sides of chemotactic cells control myosin assembly (see the text for details). Signalling events at the front of chemotactic cells inhibit 'backness' signals and vice versa, although signals at the front also seem to positively regulate signalling at the back, leading to enhanced polarity. Several signalling molecules are expected to play similar roles in both leucocyte and *Dictyostelium* chemotaxis (such as TORC2), but, for simplification, only those that have been studied in either one of the systems are illustrated. Broken arrows represent unconfirmed links. PIP3, PtdIns(3,4,5)P_3.

cytoskeleton and control of cell motility [78,79]. *Dictyostelium* RacB, which is closely related to Rac1, was demonstrated to play a role in regulating chemoattractant-mediated F-actin polymerization [69]. The profile of RacB activation shows the same bimodal kinetics as does that for F-actin polymerization, suggesting a linkage between the two. *racB*$^-$ cells exhibit an approx. 50 % reduction in chemoattractant-mediated F-actin polymerization. These cells are less polarized and move significantly more slowly than wild-type cells. Recently, an atypical *Dictyostelium* Rac protein, RacG, was found to promote F-actin polymerization and was suggested to regulate chemotaxis as well as cell motility; cells lacking RacG display reduced migration speed and directionality, and cells expressing a constitutively active RacG mutant present severe motility defects [80].

In neutrophils, the expression of a dominant-negative Rac mutant inhibits chemoattractant-stimulated accumulation of F-actin and polarization [40]. Two mammalian Rac isoforms, Rac1 and Rac2, play key roles in neutrophil motility and chemotaxis [81,82]. Rac1 is ubiquitiously expressed, while Rac2 expression is relatively restricted to cells of the haematopoietic lineage [83]. Although Rac2 is the predominant isoform in human neutrophils, murine neutrophils express similar amounts of Rac1 and Rac2 [84,85]. Interestingly, leucocytes from Rac2 knockout mice display markedly reduced F-actin assembly and are unable to migrate efficiently, even if directional sensing appears intact [39,85,86]. In turn, murine neutrophils lacking Rac1 display abnormal polarization and are unable to migrate towards the source of chemoattractant [39].

Activated Rac localizes with F-actin at the front of chemotactic neutrophils and *Dictyostelium* cells [87,88], where accumulating findings suggest the involvement of Rac in auto-amplifying positive-feedback loops that cause massive F-actin assembly and PtdIns(3,4,5)P_3 production at the leading edge [29] (Figure 3). Interestingly, in *Dictyostelium*, the kinetics of chemoattractant-induced RacB activation, PtdIns(3,4,5)P_3 production and F-actin polymerization all share a similar biphasic profile, namely a strong and rapid first peak at about 5 s after chemoattractant stimulation, followed by a second, lower, but much broader, peak at about 30–60 s [35,69,89–91]. The first rapid response was suggested to represent the turning 'on' of the signal-transduction machinery, whereas the second slower response, which most likely results from positive-feedback signalling, was linked to pseudopod extension [29,35,89–92]. In addition, the second phase of cAMP-induced RacB activation, as well as F-actin polymerization, were found to be sensitive to perturbations in PI3K activity (reduced in *pi3k1/2*-null cells or cells treated with LY294002, and increased in *pten*$^-$ cells), suggesting that they are both regulated by PtdIns(3,4,5)P_3 [35,69].

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The initial enrichment of PtdIns(3,4,5)P_3 at the front of chemotactic cells promotes the local recruitment and activation of PH (pleckstrin homology)-domain-containing-proteins as well as other proteins that selectively bind to PtdIns(3,4,5)P_3, which include RacGEFs as well as SCAR/WAVE and WASP proteins. The mammalian RacGEFs Tiam-1, Vav and P-Rex1 all bind PtdIns(3,4,5)P_3 through their PH domains and regulate the chemotaxis of various cell types [93–97]. Members of the CDM (Ced-5, Dock180, Myoblast city) or DOCK family of RacGEF were also found to regulate Rac activity during chemotaxis [98–101]. DOCK2 and DOCK180, as well as *Dictyostelium* DOCKH3 and DOCKA, localize to the leading edge of chemotactic cells in a PI3K-dependent manner, and DOCK180 was shown to bind PtdIns(3,4,5)P_3 specifically through its DHR-1 domain [100–102] (M. Krischke and R.A. Firtel, unpublished work). The Rac effector WAVE2, which is crucial for lamellipodium formation at the leading edge of chemotactic neutrophils [103–106], is also recruited to the plasma membrane in a PtdIns(3,4,5)P_3-dependent manner [107]. N-WASP (neuronal WASP) as well as *Dictyostelium* WASP bind PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, and PtdIns(3,4,5)P_3 binding promotes the localization of *Dictyostelium* WASP at the leading edge of chemotactic cells [107,108].

The considerations mentioned above suggest that PtdIns(3,4,5)P_3 directs the assembly of Rac signalling complexes at the leading edge, which leads to the localized polymerization of F-actin. In turn, F-actin promotes the recruitment of additional PI3K to the plasma membrane [28] and positively regulates RacGEFs. *Dictyostelium* RacGEF1 was found to localize to sites of F-actin polymerization, and this localization is required for full RacB activation, which promotes further F-actin assembly [69]. The localization of RacGEF1 with F-actin requires its actin-binding calponin homology domain, which suggests that RacGEF1 directly interacts with actin. Similarly, a recent study revealed that Tiam-1 interacts with the Arp2/3 complex, linking the activation of Rac1 to F-actin polymerization in fibroblasts [109]. By promoting F-actin polymerization, Rac plays an important role in the local amplification of the PtdIns(3,4,5)P_3 signal. This conclusion is supported by the observation that inhibition of Rac signalling in neutrophils prevents chemoattractant-induced accumulation of PtdIns(3,4,5)P_3 [40,110].

Through the regulation of PI3K, Ras is suggested to play a part in leading edge formation as part of the feedback signalling pathways that amplify the PtdIns(3,4,5)P_3 signal and promote F-actin polymerization [28,29] (Figure 3). Although chemoattractant-induced activation of Ras occurs independently of F-actin, the finding that Ras activity is reduced in latrunculin-treated cells suggests that Ras may be regulated in part by F-actin [28]. Supporting this hypothesis, a recent study has demonstrated that the stabilization of the actin-cytoskeleton in yeast, through the deletion of the actin regulatory proteins Sla1p or End3p, leads to the hyperactivation of the Ras signalling pathway [111].

The finding that the active form of *Dictyostelium* RasG interacts with a component of TORC2 (target of rapamycin complex 2), RIP3 (Ras-interacting protein 3), suggests that Ras signalling can also regulate the actin cytoskeleton independently of PI3K [112,113]. The TORC2 complex is highly conserved from *Dictyostelium* and yeast to mammals, and accumulating evidence strongly indicates that TORC2 modulates the actin cytoskeleton, most likely by acting through the Rho family small GTPases [114]. Consistent with the involvement of RasG and TORC2 in directed cell movement, cells lacking RasG or any of the TORC2 proteins Lst8, RIP3 and Pia (Pianissimo) all exhibit defects in cell polarity, motility, and directionality [112,113,115,116]. Furthermore, the finding that a RIP3 mutant unable to bind RasG fails to effectively complement the null mutation suggests that Ras plays an important role in mediating TORC2 function [113]. Since yeast and mammalian TORC2 were suggested to promote the activation of Rho GTPases [117,118], the above considerations suggest that TORC2 could allow for a Ras-promoted regulation of Rho GTPases, although indirectly. Cross-talk between Ras and Rho GTPases in mammalian cells can occur through the RacGEFs Tiam-1 and Vav2, which both bind, and are activated by, Ras and/or Rap1 [119–121]. Interestingly, the Ras-promoted activation of Rac1 by Tiam-1 plays an essential role in Schwann-cell migration [122].

Regulation of actin-myosin assembly

Conventional myosin II forms a hexamer, composed of two MHCs (myosin heavy chains), as well as two pairs of essential and regulatory MLCs (myosin light chains), which assembles into bipolar filaments that have high ATPase activity and are capable of interacting with actin [123]. The assembly of actin–myosin filaments is suggested to stabilize the actin cytoskeleton and consequently enhance cortical rigidity, as well as to provide the motor activity necessary for efficient cell migration, through the ATP-driven translocation of actin filaments [124]. Myosin II localizes to the sides and rear of chemotactic neutrophils and *Dictyostelium* cells, where it prevents the formation of lateral pseudopodia and promotes cell-body contraction and posterior retraction [50,125–129]. Despite its conserved role, myosin II appears to be differently regulated in mammalian and *Dictyostelium* cells [123,124,130]. However, a growing body of evidence suggests that small GTPases play important roles in the regulation of myosin II assembly in each cell type (Figure 3).

In neutrophils, findings indicate that RhoA plays a crucial role in cell polarity and motility that seems independent of its role in PTEN regulation. The use of dominant-negative and constitutively active RhoA mutants coupled to the pharmacological inhibition of ROCK suggested that RhoA signalling, at the back and sides of chemotactic leucocytes, inhibits the formation of lateral pseudopodia through the regulation of myosin II in addition to the inhibition of Rac signalling [50]. Xu et al. [50] showed that RhoA–ROCK signalling mediates chemoattractant-induced MLC phosphorylation, which promotes the assembly of actin–myosin filaments. MLC phosphorylation is thought to be mediated by the ROCK substrate MBS [myosin-binding subunit; also known as MYPT (myosin phosphatase target subunit)], leading to the inhibition of the MLC phosphatase activity, which results in increased MLC phosphorylation [131,132]. However, ROCK could also regulate the assembly of actin–myosin filaments by acting on other substrates, many of which are involved in the remodelling of the actin cytoskeleton [133].

Activated Rac is enriched at the leading edge of chemotactic neutrophils, where it promotes F-actin polymerization; however, some data indicate that Rac could also play a role at the back of the cell. Using a FRET (fluorescence resonance energy transfer)-based biosensor for Rac activity in living cells [134], activated Rac was detected in the retracting tail in addition to the leading edge of migrating neutrophils [87]. Moreover, Rac1 deficient neutrophils were reported to display a consistent inability to retract their tails [39]. The recent finding that Rac1 not only inhibits Rho signalling at the leading edge but also positively regulates RhoA-myosin II function at the back of chemotactic neutrophils provides a possible explanation for the effects of Rac1 on trailing-edge retraction [67]. Alternatively, Rac could regulate myosin II through PAK, which was found to promote contractility by direct phosphorylation of MLC [135–137] or phosphorylation of the actin-regulatory proteins desmin and caldesmon [138–140]. Interestingly, evidence suggests that Rac signalling is also implicated

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in the negative regulation of myosin at the leading edge. Rac promotes MHC phosphorylation leading to actin–myosin disassembly and PAK phosphorylates and inhibits MLCK (MLC kinase) [60,141,142]. Spatiotemporal regulation of Rac–PAK signalling is most likely to be central to the co-ordination of membrane protrusion at the front and posterior retraction during cell migration [143].

In *Dictyostelium*, myosin II is also regulated by phosphorylation, although in this case filament formation is inhibited by phosphorylation of the heavy chain by MHCKs (MHC kinases) and phosphorylation of the regulatory light chain by MLCKs enhances motor activity, whereas spontaneous filament formation occurs upon dephosphorylation of the heavy chain by protein phosphatase 2A [124]. *Dictyostelium* encodes four MHCKs (MHCKs A–D) and the *mhck* $A^-/B^-/C^-$ triple gene disruption revealed that these three MHCKs are responsible for the majority of the MHCK activity in cells [144]. Whereas MHCK-B and MHCK-C mostly localize in the cytosol and posterior of migrating cells respectively, MHCK-A is recruited to actin-rich protrusions in *Dictyostelium*, providing a mechanistic basis for the lack of myosin II filaments at the leading edge [145]. Two major signal-transduction pathways that regulate myosin II function in chemotactic *Dictyostelium* cells involve the PAK homologue PAKa and cGMP production [124]. Cells deficient in cGMP production or that lack PAKa fail to promote the chemoattractant-induced association of myosin II with the cell cortex, which most likely underlies the extension of lateral pseudopodia and inefficient uropod retraction observed in migrating cGMP or *paka*-null cells [146–149]. PAKa co-localizes with myosin II at the posterior of migrating cells. Even though the activation of PAKa by Rac was not directly tested, the finding that the GTP-bound form of Rac1B specifically binds to the PAKa CRIB (Cdc42/Rac-interacting binding) domain strongly suggests that Rac regulates PAKa, as was found to be the case for PAKc [68,69] and the mammalian PAK1 [150,151]. The mechanism by which PAKa regulates myosin II is unknown, although PAKa was not found to phosphorylate myosin II, which is consistent with its role in the assembly of myosin II filaments and not their dissociation [148].

The cGMP-dependent control of myosin II in *Dictyostelium* chemotaxis seems to be independent of, but possibly co-operating with, other pathways, as cGMP production appears to be regulated by Ras subfamily GTPases rather than by Rac. cGMP plays an important role in *Dictyostelium* chemotaxis, and is predominantly produced by a soluble guanylate cyclase [146,147,152,153]. Evidence for a regulatory role of cGMP in the function of myosin II includes the identification of an MLCK that is indirectly activated by cGMP, involving GbpC (cGMP-binding protein C) [154–157]. This suggests that, in addition to promoting the above-mentioned assembly of actin-myosin filaments, cGMP also regulates the phosphorylation-dependent motor activity of myosin II. The regulation of cGMP production by small GTPases was suggested following the finding that GTP[S] (guanosine 5′-[γ-thio]-triphosphate) stimulates guanylate cyclase activity independently of heterotrimeric G-proteins, even though chemoattractant-induced guanylate cyclase activation requires heterotrimeric G-protein signalling [158]. This hypothesis is supported by the observation that cells lacking the Aimless RasGEF or the Ras effector RIP3 display a significantly reduced chemoattractant-induced cGMP response, although it is unclear if this effect is direct or indirect [112]. Furthermore, *rip3*-null cells exhibit a marked polarization defect, migrate more slowly than wild-type cells and extend lateral pseudopodia during chemotaxis, which is reminiscent of cells lacking myosin II (*mhcA*$^-$ cells) [125,128]. Consistent with the involvement of Ras in guanylate cyclase activation, a recent study indicates that cells lacking either RasG or RasC display reduced cAMP-induced cGMP production, whereas cells that lack both Ras proteins completely fail to produce a cGMP response [34]. Additionally, the soluble guanylate cyclase was found to localize to, and to be activated at, the leading edge of chemotactic cells, hence in the same compartment as active Ras [159].

The small GTPase Rap1 was also found to promote cGMP production, although this was reported to occur in response to osmotic stress [160]. A role for Rap1 in the regulation of guanylate cyclase activity during chemotaxis therefore remains to be investigated. Interestingly, we have evidence suggesting Rap1 controls the inhibition of myosin II at the leading edge of chemotactic *Dictyostelium* cells (T.-J. Jeon, D.-J. Lee, S. Merlot, K. Takeda, G. Weeks and R. A. Firtel, unpublished work). Rap1 is well known for its control of cell adhesion in mammalian cells and appears to play a similar role in *Dictyostelium* [161,162], but the results from several studies suggest that Rap1 also regulates cell polarity and motility by controlling myosin II function. Rap1 is rapidly activated in response to chemoattractant stimulation. Whereas this small GTPase is predominantly associated with intracellular membranes, activated Rap1 is specifically detected at the plasma membrane and found to be enriched at the leading edge of migrating *Dictyostelium* cells (T.-J. Jeon, D.-J. Lee, S. Merlot, K. Takeda, G. Weeks and R. A. Firtel, unpublished work), which is similar to the reported localization and activation of mammalian Rap1 in fibroblasts and leucocytes [163,164]. Evidence hinting that Rap1 controls myosin II includes the observation that the azide-promoted myosin II-dependent cell contraction is inhibited in cells that overexpress Rap1 or a constitutively active Rap1 mutant (Rap1G12V) [165] (T.-J. Jeon, D.-J. Lee, S. Merlot, K. Takeda, G. Weeks and R. A. Firtel, unpublished work). Chemoattractant-induced myosin II assembly and cortical localization is strongly reduced in cells expressing Rap1G12V, whereas myosin II phosphorylation as well as the basal level of F-actin are significantly increased (T.-J. Jeon, D.-J. Lee, S. Merlot, K. Takeda, G. Weeks and R. A. Firtel, unpublished work). These results suggest that Rap1 negatively regulates myosin II assembly at the cell cortex by promoting the phosphorylation of myosin II, most likely through the regulation of MHCK-A.

Rap1's function in *Dictytostelium* chemotaxis most likely involves the recently identified Rap1 effector kinase Phg2 as well as the putative cGMP-binding protein GbpD, which was found to be a specific Rap1 GEF [162]. The latter finding supports the possibility that cGMP controls myosin II function at the leading edge by preventing its assembly at this site, in addition to promoting myosin II assembly and contraction at the rear of chemotactic cells. Even if cGMP binding to GbpD could not be detected [156], this does not exclude the possibility that GbpD binds cGMP with very low affinity, which would allow detecting and counteracting the effect of high cGMP concentrations at the front, preventing the assembly of actin–myosin II filaments at the leading edge. However, Rap1 is likely to be regulated by more than one GEF. Phg2 was reported to promote the Rap1-regulated cell adhesion [162], but we have evidence suggesting that Phg2 also regulates the chemoattractant-induced phosphorylation of myosin II (T.-J. Jeon, D.-J. Lee, S. Merlot, K. Takeda, G. Weeks and R. A. Firtel, unpublished work).

Mammalian Rap1 has attracted much attention because of its involvement in several aspects of cell adhesion [161]. However, recent data suggest that chemoattractant-induced Rap1 signalling at the leading edge is critical for lymphocyte polarity and migration [166,167]. Evidence of a role for mammalian Rap1 in cell migration and polarity includes the observations that lymphocytes expressing a constitutively active Rap1 mutant undergo

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spontaneous polarization and display increased cell migration, in addition to enhanced adhesion [168] and that *rap*$^{-/-}$ T-cells present polarization defects [169]. The Rap1-mediated control of cell motility and polarity most likely involves its regulation of adhesion as well as the actin cytoskeleton. Rap1 controls cell adhesion by regulating the β1, β2 and β3 family of integrins, which are associated with the actin cytoskeleton [170], in part through the Rap1-binding protein RapL [171,172]. On the other hand, a number of Rap1 effectors are implicated in the control of actin dynamics, including the adaptor proteins Riam [173] and lamellipodin [174], as well as Arap3 [175,176], which were shown to regulate lamellipodia formation. The Rap1 effector and adaptor protein afadin may also link Rap1 to actin, since it was found to interact with the actin-binding protein profilin [177]. Rap1 could also indirectly regulate the actin cytoskeleton through cross-talk with Rac signalling, since Rap1 interacts with the RacGEFs Vav2 and Tiam-1 and promotes Rac activation [121,178].

Interestingly, a recent study reveals that cAMP regulates Rap1 signalling in different types of haemopoietic cells through Epac1 (exchange protein activated by cAMP-1) [179]. Activated Epac1 promotes Rap1 activation, cell adhesion and cell polarization, and enhances leucocyte chemotaxis. Even though the Rap1-mediated regulation of myosin II was not investigated in these studies, the findings mentioned above suggest that Rap1 signalling may be similarly regulated by cyclic nucleotides and play comparable roles in migrating leucocytes and *Dictyostelium* cells. Altogether, these results point to a central role for Rap1 in coordinating the signals regulating cell polarity and motility as well as adhesion during chemotaxis.

Regulation of microtubules

In many cell types, the Rho-GTPase-regulated organization of microtubules makes an important contribution to cell polarity as well as directionality during migration, a process that was recently reviewed elsewhere [180]. Surprisingly, microtubules were suggested to suppress polarity in neutrophils, through the inhibition of RhoA signalling, but to enhance their directional migration [181,182]. Interestingly, in both neutrophils and *Dictyostelium*, the MTOC (microtubule-organizing centre) re-orients towards the direction of migration, after the extension or re-orientation of a pseudopod [182,183]. The observations that disruption of the microtubules in neutrophils causes directionality defects and that lack of MTOC repositioning results in pseudopod retraction in migrating *Dictyostelium* cells led to the suggestion that the microtubules do not direct cell migration but stabilize a chosen direction of movement.

Rho GTPases are found to regulate microtubule dynamics in different ways [24,180,184]. Consistent with its reported localization at the front of migrating fibroblasts, RhoA was shown to promote the formation of stabilized microtubules at the leading edge through its effector mDia in these cells [185–187]. In different cell types, Rac1 and Cdc42 promote microtubule stabilization at the leading edge through the PAK-mediated phosphorylation and inhibition of the microtubule-destabilizing protein stathmin [188,189]. Rac and Cdc42 also promote the interaction of the plus ends of microtubules with proteins at the cell cortex, termed 'microtubule plus end capture', by influencing the activity of plus-end-binding proteins such as CLIP170 (cytoplasmic linker protein-170) and APC (adenomatous polyposis coli) [190,191]. This process was found to involve the Rac/Cdc42 effector IQGAP1, which is reported to interact with CLIP170 as well as with APC [73,192,193]. IQGAP1 was originally identified as a Rac/Cdc42 effector that promotes the cross-linking of F-actin [194], thus representing a possible link between the microtubule and actin cytoskeletons, which functionally co-operate in a variety of processes, including cell migration [195].

The regulation of microtubules during *Dictyostelium* chemotaxis remains unstudied. Similar to IQGAP1, the IQGAP-related *Dictyostelium* protein DGAP1 is involved in the Rac-dependent modulation of the actin cytoskeleton, but a role for DGAP1 in microtubule dynamics has never been investigated [79].

SIGNAL RELAY

Signal relay has been studied most intensively in *Dictyostelium*. As mentioned above, signal relay in *Dictyostelium* cells promotes their aggregation, which leads to the formation of multicellular structures that allow aggregates to survive starvation. Starved *Dictyostelium* cells secrete cAMP, which acts as a chemoattractant and induces its own production by binding and activating the cAMP receptors cAR1 and cAR3, which then promote the activation of the cAMP-synthesizing enzyme ACA (adenylate cyclase for aggregation) [17]. cAMP-induced ACA activation requires a heterotrimeric G-protein and a PH-domain-containing cytosolic protein, CRAC (cytosolic regulator of adenylate cyclase) [158,196–198]. CRAC binds PtdIns(3,4,5)P_3-enriched membranes, which promotes its localization at the leading edge of chemotactic cells, and this is required to promote ACA activation [199,200].

Accumulating evidence suggests that Ras-dependent signalling pathways tightly control ACA activity. Receptor-mediated cAMP production was shown to be abolished in cells lacking the RasGEF Aimless or RasC [31,33,34], the latter having been found to be specifically activated by Aimless (H. Kae and G. Weeks, unpublished work referred to in [34]; P. G. Charest and R. A. Firtel, unpublished work). Interestingly, the Aimless/RasC pathway seems to regulate ACA independently of CRAC, which suggests that different signals are required for ACA activation [31,33]. However, since Ras regulates PI3K and since PtdIns(3,4,5)P_3 is required for CRAC's function, Ras also indirectly regulates CRAC. Several findings indicate that Ras-dependent ACA activation could also be mediated by TORC2. Indeed, cells lacking any of the TORC2 components (Lst8, RIP3 or Pia) are severely impaired in chemoattractant-induced activation of ACA [112,113,116]. Since RIP3 was found to specifically interact with RasG and, as previously discussed, Ras binding to RIP3 seems required for most of TORC2's function, it could be extrapolated that RasG also contributes to ACA activation. However, one cannot exclude the possibility that RasC also regulates TORC2 *in vivo*, since some data indicate that RasC is the primary Ras protein regulating ACA activity. In fact, recent comparative studies show that, whereas *rasG*-null cells display reduced cAMP production, cells that lack RasC completely fail to promote chemoattractant-induced ACA activation [33,34].

Interestingly, studies also suggest that RasG negatively regulates ACA activity. Expression of a constitutively active RasG mutant (RasG-G12T) inhibits the ability of cells to aggregate upon starvation, due to a lack of cAMP production and signal relay [201]. This was suggested to result from the RasG-promoted inhibition of ERK2 (extracellular-signal-regulated kinase 2), whose activating phosphorylation was markedly reduced in strains overexpressing RasG-G12T [202]. ERK2 inhibits the cAMP-specific phosphodiesterase RegA during the time that cAMP is being produced [203,204]. By inhibiting ERK2, RasG would activate RegA and thus promote cAMP degradation. Alternatively, RasG-G12T could affect ACA activation through TORC2 by affecting the Ras cycle. These observations, coupled with the fact that cells lacking RasG display reduced cAMP production, could point to a

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role for RasG signalling in regulating the balance between cAMP production and adaptation of the response, which is crucial to maintain proper chemoresponsiveness [205]. Recently, PI3K was suggested to play such a role, controlling the chemoattractant-mediated activation and adaptation of ACA [206], providing another potential mechanism by which RasG could regulate both processes.

CONCLUSION

The involvement of small GTPases in directed cell migration might have been predictable, yet the recent findings that they control essentially every processes underlying chemotaxis, including directional sensing, signal relay, cell polarity and motility, is rather unexpected. Research of the last few years also began uncovering the molecular mechanisms by which small GTPases control eukaryotic chemotaxis. These mechanisms will most likely apply to cancer-cell migration, which also involves small GTPase signalling [25,207,208]. The participation of small GTPases in cancer progression is supported by the findings that deregulated expression or function of different small GTPases and many of their regulators or effectors have been associated with malignancy [209,210]. A better understanding of the function of small GTPases in cell motility and chemotaxis is particularly important, as it could lead to the development of new therapeutic approaches targeting invasive tumours.

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