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REVIEW

Moving towards a paradigm: common mechanisms of chemotactic signaling in *Dictyostelium* and mammalian leukocytes

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Abstract Chemotaxis, or directed migration of cells along a chemical gradient, is a highly coordinated process that involves gradient sensing, motility, and polarity. Most of our understanding of chemotaxis comes from studies of cells undergoing amoeboid-type migration, in particular the social amoeba *Dictyostelium discoideum* and leukocytes. In these amoeboid cells the molecular events leading to directed migration can be conceptually divided into four interacting networks: receptor/G protein, signal transduction, cytoskeleton, and polarity. The signal transduction network occupies a central position in this scheme as it receives direct input from the receptor/G protein network, as well as feedback from the cytoskeletal and polarity networks. Multiple overlapping modules within the signal transduction network transmit the signals to the actin cytoskeleton network leading to biased pseudopod protrusion in the direction of the gradient. The overall architecture of the networks, as well as the individual signaling modules, is remarkably conserved between *Dictyostelium* and mammalian leukocytes, and the similarities and differences between the two systems are the subject of this review.

Keywords Chemotaxis · Amoeboid migration · Signal transduction · Cytoskeleton · Polarity · GPCR

Introduction

Role of chemotaxis in health and disease

Directed migration of a cell along a chemical gradient, or chemotaxis, is a fundamental process that is conserved from bacteria to eukaryotes. The ability to sense small differences in the chemoattractant concentration is essential for a variety of physiological and pathophysiological conditions. During embryogenesis, chemotaxis orchestrates the migration of many cells, including neural crest and primordial germ cells [1, 2]. Chemotaxis is also critical for the intricate trafficking of immune cells and their recruitment to sites of inflammation [3–5]. Importantly, inappropriate chemotaxis of leukocytes contributes to chronic inflammatory diseases, including arthritis, asthma, and atherosclerosis [3, 6]. Furthermore, the leading cause of deaths from cancer is metastasis, which is the dissemination of tumor cells to secondary sites, a process that is driven by chemotaxis [7].

Dictyostelium and leukocytes display amoeboid migration

Most of our understanding of the signaling pathways regulating chemotaxis comes from studies of the soil amoeba *Dictyostelium discoideum*, which relies on chemotaxis during various stages of its life cycle. Vegetative cells locate food sources by migrating toward products such as folic acid derived from bacteria or yeast. During periods of starvation cells enter a developmental program in which they acquire the ability to sense and migrate toward locally secreted cAMP by upregulating the expression of cAMP receptors (cARs) and related signal transduction components. Chemotaxis toward self-generated cAMP gradients leads to the formation of a multicellular organism

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that undergoes several morphological changes, eventually resulting in the formation of spores, which can survive unfavorable conditions. The robust chemotactic responses of *Dictyostelium* cells, combined with the powerful genetic approaches this haploid organism offers, make it a powerful system for the study of chemotaxis.

Eukaryotic cells exhibit several distinct modes of migration. *Dictyostelium* cells, leukocytes, as well as metastatic tumor cells use amoeboid migration, which is characterized by rapid protrusion and retraction of pseudopods driven by actomyosin contractility, weak cell-substrate interactions, and a lack of matrix degradation [8]. As a result, amoeboid migration is extremely fast, with speeds reaching 10–25 μm/min [9]. In contrast, mesenchymal migration seen in fibroblasts and some tumor cells is slower (~ 0.1-1 μm/min) and requires strong interaction with the substrate, as well as proteolysis of the extracellular matrix [10]. In addition to single-cell migration, cells can migrate as a group in a process known as collective cell migration [11, 12]. Multicellular migration is observed in certain cancers and during neural crest migration, for example, as well as in aggregation-competent *Dictyostelium* cells, which use “streaming” to relay the chemotactic signal and improve the recruitment range. This review will focus exclusively on amoeboid migration during chemotaxis of individual leukocytes and *Dictyostelium* cells.

Chemotaxis can be thought of as integrating processes of motility, directional sensing, and polarity. Motility refers to the ability of cells to extend pseudopods and move around randomly in the absence of cues [13–15]. Directional sensing refers to the ability of a cell to sense and move along a gradient, and even when immobilized, to direct its signaling events towards the high side [16–18]. Polarity refers to a semi-stable state where signaling and cytoskeletal events occur preferentially at the front or back of a cell, allowing a cell to move persistently in the same direction even without an external cue. *Dictyostelium* cells can have more or less intrinsic polarity, with later stages of development having strong polarity similar to neutrophils.

Chemotactic networks of *Dictyostelium* and leukocytes

It is convenient to think about the molecular events regulating motility, directional sensing, and polarity in terms of interacting networks. Figure 1 shows the interconnections between the receptor/G protein, signal transduction, actin cytoskeleton, and polarity networks [17]. The receptor/G protein network involves the chemoattractant receptors, G proteins, and additional upstream components that detect the gradient and transmit a bias to the signal transduction network. The signal transduction network consists of a large number of interacting pathways that amplify the directional bias and transmit the signal to the cytoskeleton network. The actin cytoskeleton network generates a protrusive force to move the cell and also provides feedback to the signal transduction network. Finally, the polarity network depends on the cytoskeleton and like the gradient sensing network provides a bias to the signal transduction network. Thus, the signal transduction network occupies a central location among the interacting networks that bring about chemotaxis. Therefore, in this review we focus on the similarities and differences in the topology of the signal transduction networks of *Dictyostelium* and leukocytes, while only briefly outlining the other networks.

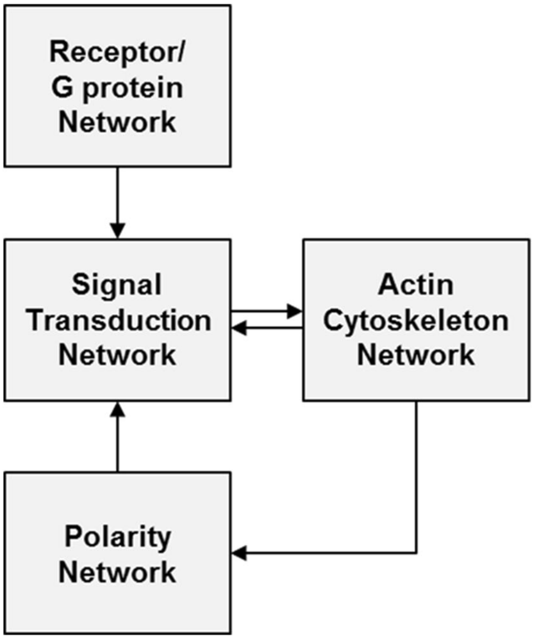


Fig. 1 Overview of the networks contributing to chemotaxis. The four proposed networks required for amoeboid chemotaxis with arrows representing the interactions between them

Genetic analysis in *Dictyostelium* and leukocytes has revealed that there are hundreds of proteins involved in chemotaxis. It appears that most of these are in the signal transduction and cytoskeleton networks. The topologies of the networks have been mostly derived from observations of the responses of living cells in a gradient or with uniform stimulation (see Fig. 2). Biosensors for critical activities are compared between wild-type cells and those expressing single or multiple constitutively active or dominant-negative versions of proteins of interest, or cells with reduced amounts of proteins either via knock-down or knock-out approaches. Especially in *Dictyostelium* multiple genes can be deleted to generate combinations of deficiencies. While the positive interactions are clear, the lack of interaction may be due to the fact that it has not been experimentally examined. Within the more complex networks, such as those for signal transduction and actin cytoskeleton, it

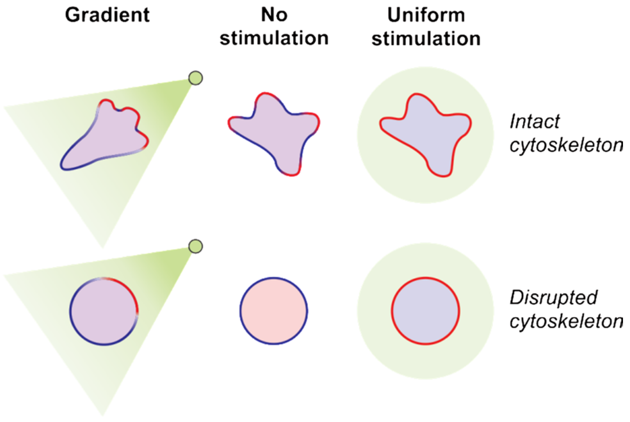


Fig. 2 Spatiotemporal regulation of "front" and "back" proteins with chemoattractant stimulation. Many proteins involved in chemotaxis are differentially localized to the front or back of migrating cells and this helps establish the balance between protrusion and retraction that leads to directed cellular migration. Thus in a cell exposed to a chemoattractant gradient the "front" proteins (shown in *red*) localize to the pseudopods, which are oriented toward the gradient, whereas the "back" proteins (shown in *blue*) line the back and sides of the cell. In a round cell with a disrupted cytoskeleton, for example, via actin-depolymerizing drugs, the "front" proteins localize to the high side of the gradient in a "crescent", whereas the "back" proteins have opposing localization. In a resting cell or in a cell with a disrupted cytoskeleton in the absence of a chemoattractant the "back" proteins are localized uniformly along the membrane or cortex, whereas the front proteins are in the cytosol. When a cell makes a protrusion, the back proteins dissociate from that region, and the front proteins associate with that extending region of the cell periphery [396, 397]. With a uniform stimulation, the "front" proteins transiently re-localize to the entire membrane and "back" proteins transiently dissociate. These protein translocations after chemoattractant stimulation occur within 10–30 s and then return to basal state after about 30-60 s [19, 85, 397]. The precise kinetics of specific signaling components in different contexts will be addressed further in this review. Chemoattractants also trigger activation of some proteins at the front or the back of a cell without affecting localization of those proteins. In this case, fluorescently-tagged biosensors that recognize activated versions of the protein or their enzymatic products act as "front" or "back" proteins

is convenient to separate the components into modules. This is clearly an oversimplification as there are numerous points of overlap between the modules. We have chosen to discuss several modules which have the strongest effects on chemotaxis, are the most studied, and have clear homologies across both *Dictyostelium* and leukocytes.

Receptor/G protein network

Chemoattractants can act through several classes of conserved receptors, each having specific properties and functions in various cell types. In many cases this cellular directional sensing is accomplished through G protein-coupled receptors (GPCRs). Several lines of evidence suggest that these receptors and their functional interactions with the heterotrimeric G proteins (G_α, G_β, G_γ) share a common ancestor to all eukaryotes [19]. The GPCR systems share even further homologies within the evolutionary clade that includes Animals, Fungi, and Amoebozoans. Within these related phylogenetic Kingdoms, ligand binding to the GPCR activates the G_α guanine nucleotide exchange factor (GEF) activity of the receptor (releasing the $G_{\beta\gamma}$) and/or G protein-independent signaling leading to the activation of downstream pathways [19–23].

There are many striking similarities when comparing the roles GPCRs play in the amoeboid migration of *Dictyostelium* and leukocytes, but there are also some differences. First, the pertussis toxin-sensitive $G_{\alpha i}$ proteins in leukocytes are the predominant heterotrimeric G proteins shared by many receptors, although some evidence suggests that $G_{12/13}$ proteins may signal to the rear of the cell [23–25]. In *Dictyostelium,* different receptors for cAMP or folic acid utilize different G_α subunits [26]. Second, leukocytes can utilize numerous combinations of the multiple G_β/G_γ genes during directed cell migration, whereas *Dictyostelium* express only one G_β and one G_γ in their genome [27]. Last, in both cell types ligand binding to chemoattractant GPCRs induces phosphorylation of the intracellular C-terminus of the receptors. Attraction to chemokines in leukocytes has been shown to utilize both β-arrestins and receptor phosphorylating G protein receptor kinases (GRKs) to regulate migration [28, 29]. Receptor phosphorylation is also important in regulating ligand affinity for the cAR1 receptor in *Dictyostelium*, although there are contradictory reports as to what effects this phosphorylation may have on downstream signal transduction and adaptation [30–32]. Although the intricate details of receptor and G protein function, including receptor-ligand interaction and the regulation of the heterotrimeric G protein cycle, are not addressed here, excellent reviews on these topics can be found in [33–37].

Compared to *Dictyostelium*, there is a rich repertoire of receptors and ligands controlling directed migration in many different mammalian cells, including leukocytes [38]. As part of the innate immune system neutrophils are the first responders to tissue damage and bacterial infection. *N*-formylmethionyl-leucyl-phenylalanine (fMLP) is a tripeptide produced by bacteria that serves as a chemoattractant. The GPCR *N*-formyl peptide receptor, FPR1, responds to and relays this bacterial cue [39]. Stromal cell-derived factor 1 (SDF-1), also known as chemokine 12 (CXCL12), is a strong chemoattractant for lymphocytes and monocytes [40]. Leukocytes also respond to C5a, Interleukin-8 (IL8), PAF, and LTB4 with specific chemokine GPCRs [34]. In contrast, only a few chemoattractants have been identified in *Dictyostelium*, including folic acid utilized in foraging and cAMP used in cell to cell communication.

The receptor/G protein network provides the initial spatial detection of the extracellular chemoattractant gradient.

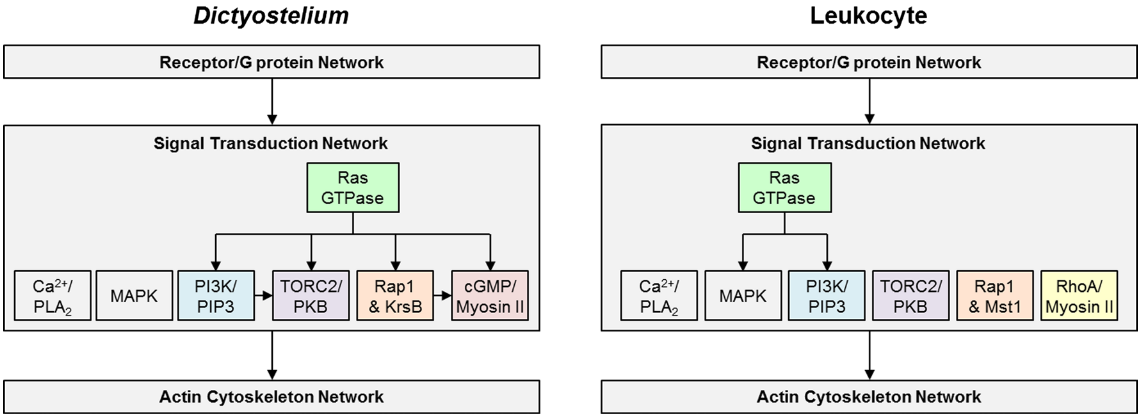


Fig. 3 Topology of the signal transduction network in *Dictyostelium* and mammalian leukocytes. The signal transduction network is placed between the receptor/G protein and the actin cytoskeleton networks, while the polarity network is omitted from this figure. The individual modules within the Signal Transduction Network are represented by a specific color and these colors will be used to represent the modules in subsequent figures. The arrows depict interactions between the modules that are strongly supported in the literature

The directional signal is then transferred inside the cell to the numerous modules in the signal transduction network. The signaling modules downstream of the receptor/G protein network provide the cell with intricate control of its behavior in a chemoattractant gradient. They not only allow for the integration of numerous environmental stimuli simultaneously but also provide the amplification and adaptation mechanisms that provide the great sensitivity that is seen in amoeboid directed migration.

Signal transduction network

The following signaling modules are downstream of the receptor/G protein network and will be discussed in this section: Ras GTPase, PI3K/PIP3, TORC2/PKB, PLA_2, Ca^{2+}, MAPK, Rap1 and KrsB/Mst1, cGMP/Myosin II and RhoA/Myosin II (Fig. 3).

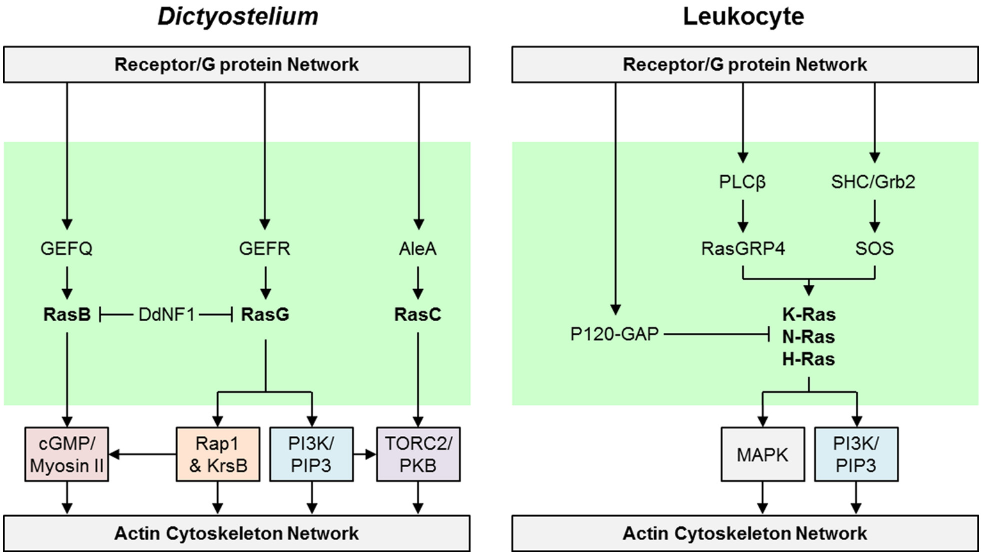
Ras GTPase Module

Dictyostelium *Dictyostelium* cells express several members of the Ras small GTPases family that are central to directed cell migration, but the precise functions that Ras proteins play is still under investigation. There are 14 Ras family genes in the *Dictyostelium* genome and the 5 characterized isoforms RasS, RasD, RasB, RasC, and RasG share similarities with mammalian H-Ras and K-Ras [41, 42]. Evidence suggests that RasS modulates endocytosis and negatively regulates cell speed [43]; RasD mediates multicellular slug thermotaxis and phototaxis [44]; RasB controls the contractile pathway during chemotaxis through myosin heavy chain kinase A (MHCKA) and is also involved in mitosis [45, 46]. In contrast to the other Ras G proteins, RasC and RasG proteins appear to be particularly important for chemotaxis.

Due to the overlapping functions it has been difficult to establish the specific roles of RasC and RasG [42]. Expression of constitutively active RasC leads to the activation of many biochemical pathways, which results in a dramatic increase in cell spreading and cytoskeletal activity, consequently altering migration [47]. Similarly, expression of constitutively active RasG or deletion of its GTPase activating protein (GAP) DdNF1 leads to overactivation of phosphoinositide 3-kinase (PI3K) and a defect in migration [48]. However, while studies of cells with deletions of RasG, RasC, or RasC/G have suggested that these proteins represent an essential basal signaling module necessary for chemotaxis, others indicate that chemotaxis can occur in the absence of these proteins, at least in steep gradients [49]. These discrepancies may be due to possible compensatory expression or activity of many different proteins, for example of RasD in RasG-null cells [44]. In addition to RasC and RasG having both overlapping and unique functions, RasD and RasB also show functional overlap with RasG [42, 50, 51]

The Ras module is downstream of the receptor and heterotrimeric G proteins, and studies using a combination of genetic mutations and pharmacological inhibitors suggest that it is upstream of several other signal transduction modules (Fig. 3) [42, 52]. First, it has been shown that in strains deficient in cAMP signaling, such as the *car1/3*$^-$ and $g\beta^-$ strains, there is no chemoattractant-mediated Ras activation [53]. In $g\alpha 2^-$ cells there is a brief, weak Ras activation [54]. Second, RasC and RasG proteins activate separate downstream pathways (Fig. 4): RasC-GTP activates TORC2, whereas RasG-GTP and RasD-GTP interact with and activate PI3K1 and PI3K2 [47, 55, 56]. In addition, RasG is the major Ras protein that regulates Daydreamer (DydA), which localizes to the leading edge and is required

Fig. 4 Ras GTPase module in *Dictyostelium* and mammalian leukocytes. The arrow and bar lines represent positive and inhibitory links, respectively, and the lines shown depict interactions that are strongly supported in the literature



for proper directed cell migration [57, 58]. Thus, RasC/G signaling pathways provide a link between heterotrimeric G proteins and downstream pathways.

In many of the studies referenced above Ras activation has been monitored with a tagged Ras-binding domain (RBD) peptide. This probe utilizes the RBD of Raf1, which binds activated Ras-GTP and was originally tagged with GST to monitor mammalian cell cycle progression [59]. In *Dictyostelium* cells this probe reports localized Ras activity, although it preferentially binds RasG-GTP and not RasC-GTP [53]. During random migration the RBD-GFP probe localizes to sites of membrane protrusions. With uniform cAMP stimulation the probe relocalizes transiently from the cytosol to the entire membrane with a peak at ~ 3-6 s, and in a gradient it localizes to the leading edge, establishing its classification as a “front” protein [42]. When a gradient of chemoattractant is applied to the cell, the activation of Ras goes through phases of an initial uniform response, symmetry breaking of the response to one region of the cell, and then confinement of this activated Ras to a more restricted region of the cell membrane; with the whole process taking up to 90 s to complete [54]. Ras activation is independent of cytoskeletal activity: Patches of active Ras can be observed in unstimulated cells, uniform stimulus results in a uniform response, and cells in a gradient of chemoattractant elicit a crescent towards the high side of the gradient (see Fig. 2) [42].

Precise regulation of Ras activity in *Dictyostelium* is most likely due to the direct activity of numerous GEF and GAP proteins. In fact, the first suggestion that Ras proteins were downstream of receptor in GPCR-mediated chemotaxis came from the discovery that Aimless (AleA, GEFA), a homolog of mammalian Son of Sevenless (SOS) RasGEF, was required for *Dictyostelium* cells to aggregate [60, 61]. It is now known that GEFA is part of a complex (Sca1 complex) that includes Sca1, GEFH, PHR, and protein phosphatase 2A (PP2A) [62]. It has been shown in vitro that GEFA is the major activator of RasC nucleotide exchange, while GEFR is required for the activation of RasG [63]. There are 25 genes encoding Ras GEFs in the *Dictyostelium* genome, and their exact functions are complex and difficult to classify. Based on the studies of null cell lines, different GEFs have distinct roles in the initial uniform response, symmetry breaking, and crescent confinement in RBD-GFP localization experiments [54]. The RasGAP DdNF1 is distributed uniformly on the membrane, negatively regulating both RasB and RasG activity, which helps lead to symmetry breaking and confinement in chemotaxis [48, 54]. Moreover, some evidence suggests RasGAPs might act as global signaling inhibitors that are activated upstream of the Ras G proteins [64].

Leukocytes In mammalian cells Ras oncogenes are well known to regulate proliferation, cell growth, survival, and energy metabolism; however, Ras involvement in leukocyte chemotaxis has not been extensively studied [65]. The three most commonly studied mammalian Ras genes encode four homologous proteins with slightly different post-translational modifications on their carboxyl terminals: H-Ras, N-Ras, KA-Ras, and KB-Ras [66]. In mice, genetic mutations have shown that only the K-Ras gene is essential for embryonic development, suggesting unique functions of the Ras gene products [67]. Interestingly, K-Ras has previously been shown to be mutated in ~ 30 % of all human tumors [68].

Leukocyte chemokine and fMLP GPCRs have both been shown to activate and utilize Ras protein signaling in directed cell migration. One of the first observations that Ras proteins were acting downstream of a chemoattractant receptor was in neutrophils [69]. Worthern et al. reported that the Ras/Raf/MEK pathway was activated

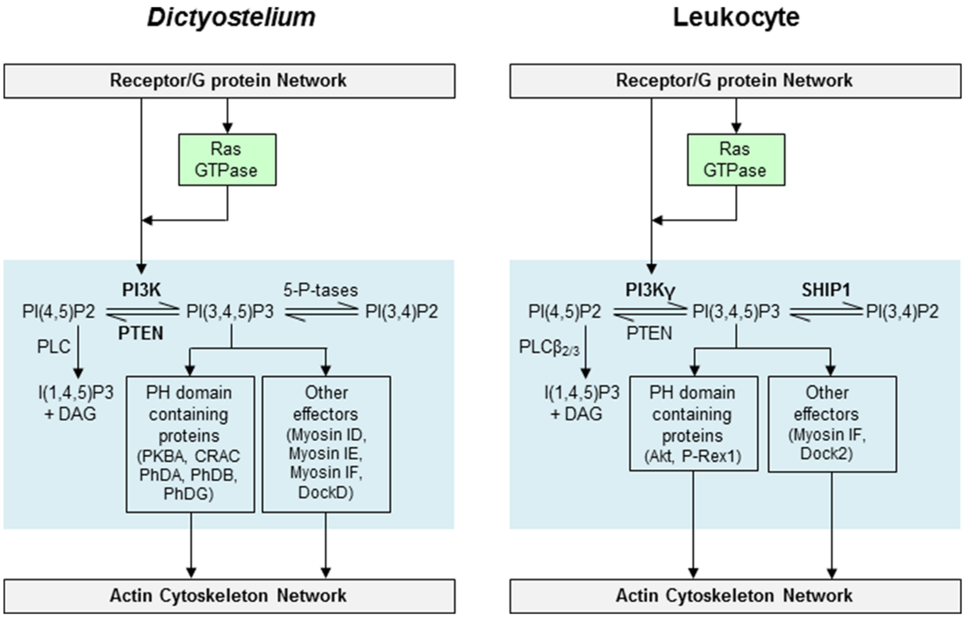


Fig. 5 PI3K/PIP3 module in *Dictyostelium* and mammalian leukocytes. The arrow and bar lines represent positive and inhibitory links, respectively, and the lines shown depict interactions that are strongly supported in the literature

by fMLP stimulation in a pertussis toxin-sensitive manner, with a maximum response in ~ 2 min. In addition, neutrophil chemotaxis mediated by interleukin and integrin signaling was shown to be regulated by Ras activity [70, 71]. In Jurkat T cells, H-Ras is reportedly involved in directed transendothelial migration and integrin signaling in response to SDF-1α [72]. It is also reported that Ras is activated when CCR7 (an additional receptor for SDF-1α) is occupied in primary T cells [73]. In addition to G protein-mediated responses, an SDF-1α receptor CXCR4 transactivates receptor tyrosine kinases (RTKs) leading to the recruitment of a RasGEF SOS to induce chemotaxis responses [74].

The ways in which chemoattractant GPCRs regulate the Ras module is only beginning to be elucidated in mammalian leukocytes. The p110γ subunit of PI3Kγ, which is highly expressed in neutrophils, has a Ras-GTP binding domain and requires H-Ras for optimum activity [75]. This places Ras protein signaling, at least in part, upstream of the PI3K/PIP3 module in leukocytes (Fig. 4). A recent report suggests that PLCβ mediates Ras activation by releasing diacylglycerol, which activates RASGRP4 (Ras GEF). Compared to wild type, fewer RasGRP4-null neutrophils migrate toward the chemoattractant [76]. However, this GEF can also activate another small GTPase Rap1, which may contribute to the observed phenotype. There is additional evidence that suggests that fMLP-stimulated inhibition of Gap120 (Ras GAP) enhances K-Ras and N-Ras signaling in neutrophils, leading to increased directed migration [77]. The Ras GAP neurofibromin (NF1) is highly expressed in leukocytes, but the role NF1 plays in leukocyte cell biology remains to be studied [78].

PI3K/PIP3 Module

Dictyostelium One of the hallmarks of a migrating cell is the establishment of a phosphatidylinositol (3,4,5)-trisphosphate (PIP3) gradient across the cell. PIP3 levels are regulated by the action of PI3K, which converts PI(4,5)P2 into PIP3, and a 3-phosphatase PTEN, which mediates the opposite reaction (Fig. 5). Although PIP3 can be degraded by 5-phosphatases to generate PI(3,4)P2, this process does not appear to play a major role in chemotaxing cells as PI(3,4)P2 does not accumulate in response to cAMP stimulation, nor does deletion of any of the four inositol-5-phosphatases either alone or in combinations of two reduce the cells' ability to chemotax [79, 80]. Thus, conversion of PIP3 to PI(4,5)P2 by PTEN appears to be the predominant route for PIP3 degradation. In a migrating cell PI3K localizes to and is activated at the leading edge, while PTEN is excluded from the front and instead localizes to the back and sides. Treatment with cAMP stimulates rapid activation of PI3K. This is thought to be achieved by the interaction of membrane-associated PI3K with RasG, as well as F-actin-dependent recruitment of PI3K to the plasma membrane [42, 56]. However, while PI3K recruitment is impaired in cells with a disrupted cytoskeleton due to Latrunculin A treatment, PIP3 is generated effectively. At the same time, PTEN dissociates from the plasma membrane, possibly due to depleted PI(4,5)P2 levels [81]. The main enzyme mediating PI(4,5)P2 degradation is phospholipase C (PLC), which generates inositol (1,4,5)-trisphosphate (IP3) and diacylglycerol in response to cAMP treatment [82]. If PLC were activated at the front of a cell, it could lead to a reduction in PI(4,5)P2, which would favor local PTEN dissociation [83]. Although PLC-null cells do not have defects in chemotaxis, overexpression of PLC mimics the phenotype of PTEN-null cells, as will be discussed below. Together, this spatial and temporal control leads to the accumulation of PIP3 at the leading edge.

Aberrant chemotaxis in cells with excessive PIP3 accumulation highlights the importance of the PI3K/PIP3 pathway. PTEN-null cells have increased PIP3 around the cell perimeter. These cells have many protrusions that are not restricted to the leading edge, and consequently they move and chemotax poorly. Disruption of another negative regulator of the PI3K/PIP3 pathway, the dual-specificity kinase SHK1, also results in cells that lack polarity and consequently move slowly [84]. Interestingly, unlike PTEN, these cells do not have increased PIP3 levels basally, but instead have a prolonged peak of cAMP-stimulated PH domain recruitment and PKBA activation. Similarly, expression of PI3K with a myristoylation tag that targets it constitutively to the plasma membrane also leads to additional PIP3 accumulation, extra protrusions, and defective chemotaxis [56].

PIP3 interacts with a number of downstream targets with diverse roles. Most proteins interact with PIP3 via pleckstrin homology (PH) domains, including PKBA, CRAC, and the PhD family of proteins. All of these proteins are recruited to the leading edge during chemotaxis, or to the cortex with global cAMP stimulation. Cells lacking PIP3-interacting proteins have variable defects in chemotaxis. For example, one report indicates that cells lacking the Akt homologue PKBA do not polarize and have reduced migration speed in gradients of cAMP [85]. Another indicates that deletion of PKBA has little consequence, but in cells lacking PTEN deleting this kinase rescues the aberrant chemotaxis phenotype [86]. Once recruited to the plasma membrane, PKBA is activated by phosphorylation by phosphoinositide-dependent protein kinase (PDK) and TORC2. A close homolog of PKBA, PKBR1, is myristoylated and thus constitutively membrane-bound, making its activation PIP3-independent but TORC2-dependent. Together the two PKBs transiently phosphorylate downstream substrates, including p21-activated kinase (PakA), talin, Ras GEFs N and S, a scaffold protein supporting another Ras GEF A, Rho GAPs G and Q, and PI4P 5-kinase, possibly providing links to the cytoskeleton and generating feedback loops [55, 62, 86, 87]. PakA, which has been shown to play a role in myosin II dynamics, appears to be an important target of PKBA since its deletion rescues the phenotype of PTEN-null cells [86, 88]. Interestingly, phosphorylated PakA localizes to the back of a migrating cell similarly to PTEN and myosin II. How activated PKBA, which localizes to the leading edge, is able to activate a back protein remains unclear.

Other PH domain-containing proteins have diverse roles in chemotaxis. The PH domain-containing protein cytosolic regulator of adenylyl cyclase (CRAC) was originally identified as a critical regulator of cAMP-stimulated adenylyl cyclase activation [89, 90]. The chemotactic defect of *crac*⁻ cells is independent of their deficiency in AC activity, since C-terminal truncations that lack AC activity are able to rescue the aberrant chemotaxis of *crac*⁻ cells [91]. The Phd family of proteins interact with PIP3 via their PH domains, and four Phd proteins (A, B, G, I) have been identified [92–94]. Cells lacking PhdA, PhdB, and PhdG exhibit chemotaxis defects. Consistent with the PIP3-interacting property of PH domains, PhdA and PhdI localize to the leading edge of a migrating cell, and are recruited to cell cortex with global cAMP stimulation [92, 93]. The affinity of PhdG for PIP3 appears to be quite low; however, it associates with the plasma membrane when PIP3 levels are elevated, for example, in PTEN-null cells [93]. PhdB, which is recruited to the plasma membrane by both PIP3-dependent and PIP3-independent means, is reported to act as a GAP for Rap1 (RapGAP3) [94]. Its localization is not clear since one group reported localization at the leading edge, and another at the lagging edge of a migrating cell [93, 94].

In addition to recruiting downstream targets via PH domains, PIP3 can also interact with several other domains. Actin-based motor proteins myosins ID, IE, and IF are recruited to PIP3 at the leading edge via their tail homology 1 (TH1) domain [95]. Cells lacking all three of these myosins have reduced cAMP-stimulated actin polymerization and defective chemotaxis. Two Dock180-related RacGEFs (DockA and DockD) also regulate actin dynamics, cAMP-stimulated polarization, and motility [96]. Similarly to mammalian Dock180, DockA and DockD possess CDM-zizimin homology (CZH1) domains that interact with phospholipids. In particular, DockD is recruited to the cortex in response to chemoattractant treatment, and this recruitment depends on PIP3 generation. Thus, DockD, together with functionally redundant DockA, might link PIP3 signaling to actin polymerization.

Despite the importance of localized PIP3 generation, the PI3K/PIP3 pathway is not essential for chemotaxis. *Dictyostelium* possesses five class I PI3K genes, with PI3K1-3 being most similar to mammalian p110 PI3Ks. Hoeller and Kay created cells lacking the five PI3K genes as well as PTEN. PIP3 levels in these cells were undetectable by PH domain recruitment, although PKBA activation in response to cAMP was reported, and PIP3 was biochemically detectable [97, 98]. These gene disruptions minimally impaired the ability to sense a chemotactic gradient, although there was a marked reduction in speed both during chemotaxis and random motility, suggesting that PIP3 signaling plays a critical role in this process [98]. Similar observations were made either using chemical inhibition of PI3K with 60 μM LY294002 or by disrupting PI3K1 and 2, which nearly abolishes cAMP-stimulated PIP3 production. Although both of these conditions reduced motility speed, they did not affect cells' directionality in a gradient [98–103]. It is important to note that the findings that PI3K is important for motility but not directionality are based on experiments using steep gradients. In contrast, in shallow gradients the PI3K/PIP3 pathway is required for proper cell orientation in the direction of the chemoattractant source [101, 103]. One possible reason for why excessive PI3K/PIP3 pathway can induce pseudopod production but its disruption does not always impair chemotaxis is that cells possess several parallel pathways that transmit input from the receptor to the actin cytoskeleton. Some of these pathways, including those involving TORC2, PLA_2 and cGMP, will be discussed below.

Leukocytes Chemoattractants trigger activation of PI3K and consequent generation of PIP3 in leukocytes (Fig. 5). The PH domain of Akt, which binds PIP3 and its by-product PI(3,4)P2, localizes to the leading edge of migrating neutrophil-like HL-60 cells [104]. Mammalian class I PI3K is divided into two types: IA (α, β, δ) and IB (γ). Although leukocytes express all 4 class I PI3Ks, PI3Kδ and PI3Kγ are predominant. Mammalian PI3Kγ, or p110γ is activated by $G_{\beta\gamma}$ subunit following GPCR activation by a chemoattractant. In addition, PI3Kγ activation depends on interaction with Ras [105]. Neutrophils from PI3Kγ-null mice showed no chemoattractant-stimulated PIP3 accumulation, both biochemically and using the PH-Akt probe, suggesting this is the main isoform activated in this cell type [106–109]. In contrast, class IA PI3Ks are typically activated downstream of RTKs; however, GPCRs can also trigger activation of this class of PI3Ks.

As in *Dictyostelium*, the PI3K/PIP3 pathway is important, but not essential for leukocyte chemotaxis. The role of the PI3K/PIP3 pathway in leukocytes, including neutrophils, T cells, and natural killer cells, was first examined using general PI3K inhibitors, wortmannin and LY294002 [110–114]. Although these inhibitors resulted in significantly reduced chemoattractant-induced migration, the inhibition was not complete. Similarly, neutrophils and macrophages from PI3Kγ-null mice showed reduced motility, although they were still able to chemotax [106–108, 115, 116]. It appears that PI3Kγ might regulate motility by affecting integrin-based adhesion and F-actin accumulation, but not the ability of cells to sense direction once they are migrating [109]. Even though PI3Kγ is the main GPCR-activated isoform, PI3Kδ is also involved in regulation of directed cell migration, and the importance of a particular PI3K isoform might depend on the cell type examined. Using cells from mice lacking specific PI3K isoforms, PI3Kγ appears to be involved in T cell chemotaxis, whereas PI3Kδ is important in B cells [117]. Other reports indicate that both class 1A and 1B PI3Ks are important for T cell migration [118]. A study using isoform-specific inhibitors suggests that PI3Kδ also plays a significant role in neutrophil migration [119]. Interestingly, one study showed temporal regulation of the different classes. Using dominant negative p85α and p110γ to disrupt class IA and IB PI3K, respectively, Boulven et al. found that PI3Kγ activity is important for the first peak of PIP3 generation (30 s), whereas class IA PI3Ks are important for the second prolonged peak (120 s) in a neutrophil-like cell line [120]. Only the second peak was deemed important for chemotaxis in this system. Another study showed that PI3Kγ and PI3Kδ mediate short-term (90 min) and long-term (several hours) neutrophil emigration, respectively, in vivo [121].

As in *Dictyostelium*, PI3K activation in leukocytes causes recruitment of PIP3-binding effectors. Some of the effectors, including Akt/PKB, DOCK180-related RacGEFs and myosin I, are clear homologs between the two systems. Others, such as p-Rex1, have only been found in leukocytes so far. Recruitment of Akt, via its PH domain, is often used as a read-out of PIP3 generation [104]; however, despite its robust localization to the leading edge, the exact role of Akt in chemotaxis is not clear. Neutrophil chemotaxis is slightly reduced using a specific Akt inhibitor, but only under certain assay conditions [122]. Mammalian Akt has 3 isoforms (Akt1-3), with Akt1 and Akt2 being most abundant in leukocytes. Using single knockouts, Chen et al. demonstrated that in neutrophils Akt2, but not Akt1, is recruited to the leading edge and is required for migration [123]. It appears that Akt might regulate cell motility by stimulating the production of F-actin in response to fMLP [124]. In fact, some evidence for direct links between Akt and the cytoskeleton is beginning to accumulate. One example is Akt-mediated phosphorylation and inhibition of GSK3β, which leads to activation of an actin-binding and depolymerizing protein cofilin by activation of its phosphatase slingshot2 [125]. Analogously to *Dictyostelium*, Pak might also be a potential downstream target of Akt. In leukocytes Pak, along with Pak-associated GEF (PIXα), modulates actin polymerization, polarization, adhesion, and chemotaxis [126–128]. Although it is not known whether Pak is an Akt substrate in leukocytes, Akt does phosphorylate and activate Pak in other mammalian cell types [129].

The PI3K/PIP3 pathway can also modulate cytoskeleton via PIP3-mediated recruitment of RacGEFs. P-Rex1, a RacGEF with preference for Rac2, is synergistically activated by PIP3 and $G_{\beta\gamma}$ via interaction with PH and Dbl-homology domains, respectively [130, 131]. DOCK2, a DOCK180-related protein expressed primarily in cells of hematopoietic origin, interacts with PIP3 via its DOCK homology region 1 domain, and this association is enhanced by ELMO1 [132]. Both DOCK2 and P-Rex1 are recruited to the front of chemotaxing neutrophils [132, 133]. Interestingly, DOCK2 slowly accumulates at the leading edge even in the absence of PI3Kγ by associating with phosphatidic acid generated by phospholipase D [134]. Neutrophils from $DOCK2^{-/-}$ or $P\text{-}Rex1^{-/-}$ mice have reduced migration speed, although the defects are not complete given the functional redundancy of the two RacGEFs [131, 132]. DOCK2 might have a more prominent role in leukocyte chemotaxis, since DOCK2$^{-/-}$ neutrophils also had defects in directional sensing [132]. Another connection between PIP3 and the cytoskeleton is myosin IF, which is recruited to PIP3 at the leading edge via its TH1 domain, similarly to the homologous myosin ID, IE and IF in *Dictyostelium* [95].

Negative regulation of PI3K/PIP3 signaling can be achieved by several means, including degradation of the PI3K substrate PI(4,5)P2 and its product PIP3, as well as direct inhibition of PI3Kγ activation. The latter is achieved by a $G_{\beta\gamma}$-binding protein RACK1, which competes with PI3Kγ and other effectors for binding to $G_{\beta\gamma}$ [135]. Consistent with this role, knock-down of RACK1 enhances, whereas overexpression dampens chemotaxis of Jurkat T cells and differentiated HL-60 cells. Regulation by phosphoinositide degradation is complex and appears to be cell-type and context-dependent. The main PI(4,5)P2-degrading enzyme in leukocytes, PLCβ–$_{2/3}$, is required for chemotaxis of T lymphocytes, but not neutrophils [115, 136, 137].

PIP3 can be dephosphorylated at the 3’ position by PTEN and at the 5’ position by SHIP1 phosphatases. In *Dictyostelium* the opposing localization of PI3K and PTEN clearly establishes the PIP3 gradient in a migrating cell. In contrast, in leukocytes there are conflicting reports of PTEN localization to the back of a cell [126, 138–140]. The reasons for the differences in observations are unclear. Disrupting PTEN by knock-out or knock-down approaches leads to slightly enhanced cell speed and very minor, if any, effects on gradient sensing [141–143]. Overexpression of PTEN slightly decreases cell speed without affecting directionality [138]. Consistently, in vivo recruitment of PTEN-null neutrophils is also improved [143, 144]. In contrast to the weak phenotype of PTEN-null leukocytes, disruption of the 5-phosphatase SHIP1, which causes highly elevated levels of PIP3, leads to a phenotype that is similar to the PTEN-null *Dictyostelium* cells [116]. SHIP1-null neutrophils display a broad flattened morphology, defects in polarization, increased basal F-actin, and reduced migration, and their directionality is not affected [116]. Increased spreading and reduced motility might be due to enhanced adhesion of *ship1*$^{-/-}$ cells to the substrate [145]. Similarly to the observations in neutrophils, SHIP1-null macrophages and T lymphocytes also have reduced motility but not directionality [116, 146]. The situation in vivo is complicated by the presence of multiple chemoattractants, and evidence exists that PTEN might be important for prioritizing these signals [140]. Presumably inhibition of PTEN leads to PIP3 localization around the entire cell perimeter, instead of at the leading edge, allowing for activation of parallel pathways, such as PLA_2 and p38 MAPK, which are important for the recognition of bacterially derived ‘end-target’ chemoattractants [140, 147].

TORC2/PKB Module

Dictyostelium As previously mentioned in the PI3K/PIP3 section, PKBA and PKBR1 are a central part of a signaling hub, are responsible for the relay of extracellular signals in *Dictyostelium* chemotaxis, and phosphorylate numerous downstream substrates important for migration. These AGC family kinases are homologs of mammalian PKB (also known as Akt) [85, 148]. Akt activation occurs when levels of PIP3 increase causing the N-terminal PH domain to localize the protein to the membrane [149]. PKBA in *Dictyostelium* also contains a PH domain and transiently translocates to the plasma membrane upon cAMP stimulation [85]. In contrast, the homologous PKBR1 lacks a PH domain but has a myristoylation site that keeps the protein constitutively at the membrane [148]. PKBR1 appears to be more important as *pkbR1*$^-$ cells display greater defects in chemotaxis than *pkbA*$^-$ cells [85, 148]. Importantly, PKBR1 provides a pathway to phosphorylate PKB substrates independently of PIP3 and thus a rationale for understanding PIP3-independent chemotaxis. In contrast to the stronger activation of PKBR1 induced by cAMP, folate appears to mainly activate PKBA [97]. Expression of PKBA in *pkbR1*$^-$ cells and *pkbA*$^-$/*pkbR1*$^-$ double knockout cells further shows that these two proteins have overlapping and distinct functions for chemotaxis [55, 148].

PKB signaling in *Dictyostelium* chemotaxis receives input from RasC and RasG heterotrimeric G protein-dependent pathways (Fig. 6). Ras interacting protein 3 (Rip3), so designated because it interacts with human H-Ras in 2-hybrid assays, was isolated in a screen for chemotaxis mutants in *Dictyostelium* [150]. Since RIP3-null cells share many similar chemotaxis defects as GEFA-null cells, Rip3 appears to be an additional RasG regulatory protein [150]. Another gene isolated in a mutagenesis screen was pianissimo (PiaA), which when disrupted leads to the silencing of chemoattractant-mediated responses [151]. PiaA and Rip3 are highly conserved in evolution and were later discovered to be associated with target of rapamycin (TOR) kinases in yeast and *Drosophila*, defining a complex designated as TORC2 (TOR Complex 2) [152, 153]. The mammalian homologues of PiaA and Rip3 were designated as Rictor and mSIN1, respectively. The proteins appear to form a complex in *Dictyostelium* when examined by co-immunoprecipitation, but it is unstable and has not been purified [154]. Evidence suggests that TORC2 is activated by RasC since phosphorylation at specific TORC2 sites on the hydrophobic motifs of PKBs is low in *rasC*$^-$ cells [47, 155]. Moreover, the immunoprecipitated complex can restore RasC-activated phosphorylation of the hydrophobic motif in vitro. There is also evidence for negative feedback from PKB signaling to the upstream pathways as TORC2 or PKB signaling disruption leads to an increase

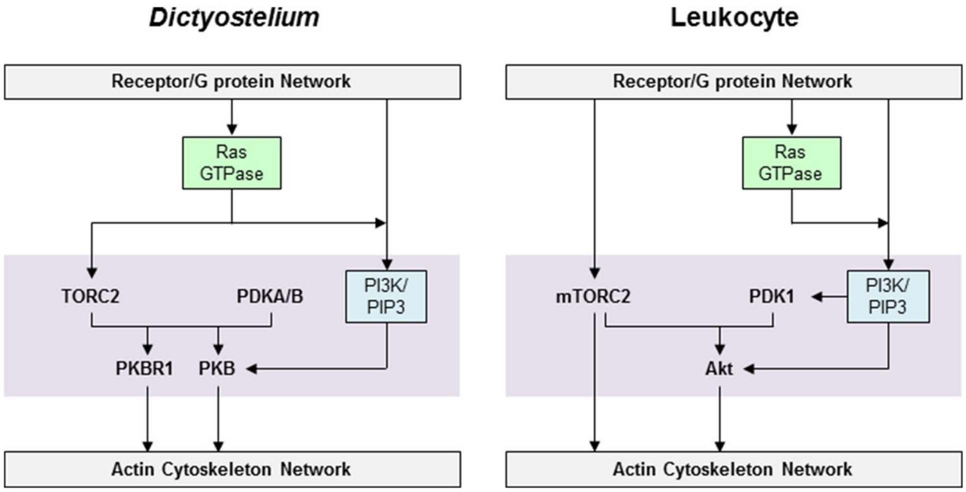


Fig. 6 TORC2/PKB module in *Dictyostelium* and mammalian leukocytes. The arrow and bar lines represent positive and inhibitory links, respectively, and the lines shown depict interactions that are strongly supported in the literature

in RasC activity [62]. In addition to the importance of the RasC pathway, the previously discussed RasG pathway is essential for the activation of PI3K and initiating PIP3 signaling required for phosphorylation of PKBA [56]. Note that RasC-mediated activation of PKBR1 and phosphorylation of PKBA substrates provide a PIP3-independent pathway from chemoattractant to cytoskeleton activation.

In *Dictyostelium* the mechanisms of PKBA and PKBR1 activation appear to be more complex than the phosphorylation of the consensus sites in the activation loop and hydrophobic motif required for mammalian Akt activation. While phosphomimetic substitutions in the activation loop and hydrophobic motif of mammalian Akt induce constitutive activation of the kinases, the same mutations in PKBR1 do not [55]. There is also evidence for reciprocal regulation of phosphorylation between the activation loop and hydrophobic motif sites, specifically with the hydrophobic motif phosphorylation by TORC2 being required for optimal phosphorylation of the activation loop site by PDKs [55, 97, 154]. *Dictyostelium* possesses two orthologous PDKs, PDKA and PDKB, and, surprisingly, their PH domains are not essential for their activity [154]. PDKA translocates to the membrane upon stimulation and phosphorylates both PKBA and PKBR1 on their activation loop sites, while PDKB is always in the cytosol and phosphorylates only PKBR1 on its activation loop site. Interestingly, in *pdkA⁻/pdkB⁻* cells residual activation loop site phosphorylation remains on both PKBA and PKBR1 suggesting additional kinase(s) play a role in regulating these proteins [154].

Leukocytes The role that Akt and mTORC2 play in leukocyte chemotaxis is only beginning to be defined. In neutrophils phosphorylation of Akt occurs after fMLP stimulation and is lost in PI3K-null and wortmannin-treated cells (Fig. 6) [107]. GFP-tagged PH domain of Akt shows "front" protein localization dynamics in response to fMLP stimulation, and a pertussis toxin sensitivity for this localization demonstrates $G_{\alpha i}$ dependency [104, 156]. Chemotactic behavior elicited by activating the chemokine receptor CXCR4 in mature dendritic cells also utilizes Akt [157]. As appropriate chemotaxis responses require Akt activity in leukocytes, it is not surprising that the knock-down of PDK1 leads to decreased chemotaxis [158]. The mTORC2 complex has been shown to regulate F-actin production in a Rac/CDC42-dependent manner in neutrophils [159]. Knock-down of Rictor leads to decreased chemotaxis migration speed and F-actin production. In addition, mTORC2 regulates myosin II activity via the protein kinase A (PKA)/RhoA pathway, as will be described in the RhoA/myosin II module [122]. There are some major differences in the observed TORC2 signaling between neutrophils and *Dictyostelium*. First, Rictor in neutrophils localizes to the leading edge, whereas PiaA in *Dictyostelium* does not. Second, in neutrophils the kinase activity of mTORC2 is reportedly not required for chemotaxis [159].

PLA$_2$ module

Dictyostelium The ability of cells to migrate in the absence of PIP3 generation prompted the search for parallel pathways. Phospholipase A_2 (PLA_2A) was identified as a chemotaxis mediator using a screen for mutants that show increased migration defects in the presence of a PI3K inhibitor LY294002 [160, 161]. Exogenous arachidonic acid can rescue the increased sensitivity of PLA_2A-null cells to PI3K inhibition. Interestingly, the PLA_2A pathway appears to be regulated by intracellular Ca^{2+}, although this effect is indirect, since similarly to its mammalian group VI PLA_2 ($iPLA_2$) homologs, PLA_2A is Ca^{2+}-independent.

Leukocytes Leukocytes have five types of PLA_2, of which secretory, cytosolic group IV ($cPLA_2$), and Ca^{2+}-independent PLA_2 ($iPLA_2$) have been analyzed with respect to chemotaxis. In neutrophils, both $cPLA_2$ and $iPLA_2$ are implicated in chemotaxis to fMLP [140]. Furthermore, inhibition of $cPLA_2$ reduces IL-8 induced polarization and migration in neutrophils; however, in this situation it is unlikely that $cPLA_2$ is part of a parallel pathway since it lies downstream of PI3K, ERK1/2, and p38 MAPK when regulating integrin-based adhesion [162]. In monocytes, both $cPLA_2\alpha$ and $iPLA_2\beta$ regulate MCP-1 induced cell migration by producing arachidonic acid and lysophosphatidic acid, respectively [163–165]. Interestingly, while downregulation of either enzyme reduces cell speed, only $iPLA_2\beta$, which localizes to the leading edge with stimulation, affects directionality and actin polymerization. Parallel inhibition of PLA_2 with other chemotactic pathways has not been tested in leukocytes thus far.

Ca^{2+} *module*

Dictyostelium Although chemoattractants trigger Ca^{2+} influx and a rise in intracellular Ca^{2+}, the importance of this process for chemotaxis is controversial. Deletion of the homolog of the IP3 receptor (IplA) alone, which abolishes chemoattractant-triggered Ca^{2+} increase without perturbing resting Ca^{2+} concentration in the cytosol, does not affect any of the chemotactic responses [166]. However, *iplA*-null cells might still have very low Ca^{2+} influx that complicates the conclusion of Ca^{2+} independence [167]. Furthermore, Ca^{2+} chelation reduces cell spreading and lowers speed during chemotaxis, and extracellular Ca^{2+} can improve cAMP-mediated migration at certain concentrations [168–171]. Interestingly, similar concentrations of Ca^{2+} can induce improvements in cell migration in the absence of cAMP, suggesting that most of the effects on chemotaxis are due to changes in migration. Overall, it is possible that cAMP-mediated chemotaxis, as well as random migration, require low resting levels of Ca^{2+}, for example, for maintenance of proper adhesion; however, Ca^{2+} flux is likely not essential. Few studies have assessed a role for Ca^{2+} when other pathways are co-inhibited.

Leukocytes Similarly to *Dictyostelium* the role of Ca^{2+} in leukocyte chemotaxis has been the subject of controversy. Chemoattractants trigger a transient increase in intracellular Ca^{2+} that depends on PLCβ-mediated generation of IP_3 [115]. However, inhibition of the IP3 receptor does not affect T lymphocyte chemotaxis [137]. In contrast, although disruption of Ca^{2+} transients has no effect on pseudopod extension, it can prevent uropod release and thereby reduce migration of neutrophils on adhesive surfaces (reviewed in [172]). In addition, intracellular Ca^{2+} forms a gradient, with the highest concentration in the uropod of basophils [173]. It appears that transient elevation of Ca^{2+} likely has two roles in leukocytes: disruption of specific integrin-based adhesions and activation of Ca^{2+}/calmodulin-dependent MLCK [174, 175]. Together, this allows for myosin II-mediated uropod retraction. Thus, although Ca^{2+} is not required for pseudopod extension, it is important for overall cell motility.

MAPK Module

Dictyostelium *Dictyostelium* cells express homologs of mitogen-activated protein kinases (MAPKs)/extracellular signal-regulated kinases (ERKs) that in many other eukaryotic cells are induced by extracellular receptors to modify gene expression and behavior. Kinomic analysis of the *Dictyostelium* genome revealed that they have a simplified collection of the MAPK/ERK family of kinases: two MAPKs (ERK1 and ERK2), one MAPKK (MEK1), and one MAPKKK (MEKK) (compared to 12, 7, and about 14, respectively, in mammals) [176, 177]. This makes *Dictyostelium* an exceptional simplified model for the study of the MAPK signaling cascades and their role in chemotaxis. *Dictyostelium* lack homologs for parts of the JNK and p38 pathways, but do possess an ERK7 homolog [176]. Two MEKK-like proteins have also been identified in *Dictyostelium*: MEKKa and stress-activated protein kinase α (SAPKα). MEKKa localizes to the cortex and is important for developmental morphogenesis [178]. SAPKα is localized to actin-based protrusions, positively regulates F-actin, and when knocked out or overexpressed causes decreased chemotaxis [179]. Neither of these MEKK-like proteins couple to the downstream ERK1 or ERK2 pathways.

The MEK1/ERK1 pathway is essential for proper chemotaxis to cAMP and folic acid in *Dictyostelium*. A null mutation in the MEK1 gene significantly decreases chemotaxis and leads to lower cAMP-mediated stimulation of both guanylyl cyclase and adenylyl cyclase [180]. MEK1 requires transient stimulated SUMOylation for appropriate localization to the cortex and the leading edge of migrating cells. MEK1 is upstream of ERK1 as overexpression of a constitutively active form of MEK1 does not alter the loss of directed migration phenotype in e*rk1*$^-$ cells and there is no cAMP-stimulated activation of ERK1 in MEK1-null cells [181]. A MEK1/protein phosphatase 4 complex (PP4C)/SMKA (SMEK) pathway has also been shown to be important for chemotaxis and is independent of, but may interact with, the cAMP-activated MEK1/ERK1 pathway [182, 183]. The e*rk1*$^-$ cells exhibited similar defects in development and chemotaxis as MEK1-null cells [181]. ERK1 has leading edge localization and has maximal cAMP-stimulated activity at 15 s. A negative regulatory interaction exists between phosphotyrosine phosphatase 2 (PTP2) and ERK1 activity, at least at the genetic level [184].

The ERK2 MAPK pathway is important in chemotaxis relay and the formation of developmental aggregates.

ERK2 was originally discovered in a mutagenesis screen for mutants defective in aggregation. The developmental defect in the *erk2*$^{-}$ cells is due to a lack of adenylyl cyclase A (ACA) activity [185, 186]. The cAMP-stimulated activation of ERK2 requires cARs, but there are conflicting reports on its requirement for G_α and G_β heterotrimeric G proteins [187, 188]. Unlike many chemotaxis ligand-activated pathways, ERK2 appears to be non-adaptive under continuous stimulation by cAMP, since dephosporylation does not occur in the presence of the stimulus [189]. Folic acid also stimulates ERK2 phosphorylation leading to ACA activity [190]. ERK2 has been shown to regulate intracellular cAMP by inhibition of the phosphodiesterase RegA [191]. It is postulated that PKA negatively regulates ERK2 and thus establishes an oscillatory circuit containing ERK2, RegA, ACA, and PKA [191, 192]. RegA is also reported to be important during chemotaxis for the suppression of lateral pseudopods [193].

Leukocytes Like *Dictyostelium*, leukocytes utilize MAPK pathways to regulate chemotaxis induced by different GPCRs with cell type specificity. In neutrophils, stimulation of FPR1 with fMLP induces transient phosphorylation and activation of MEK/ERK with a peak ~ 2-5 min, and this activation is reduced by chelation of Ca^{2+} [194]. MAPK stimulation by fMLP is greater than by other chemoattractants, such as C5a, LTB4, platelet-activating factor (PAF), and IL8, although the dynamics of activation are similar [70, 194–196]. Chemoattractants stimulate activation of the upstream kinase of MEK, Raf (MEKK), in a $G_{\alpha i}$- and PI3K-dependent manner [69, 197, 198]. The p38 MAPK pathway is also activated by fMLP/PAF, and this activation is also PI3K-dependent as it is inhibited by wortmannin [199]. Whether p38 MAPK activation is dependent on $G_{\alpha i}$ heterotrimeric G protein is not clear since there are mixed reports as to the effect of pertussis toxin treatment [197, 199].

Chemotaxis to IL8 utilizes both MEK/ERK and p38 signaling as inhibition of these pathways with PD098059 and SKF86002, respectively, lowers chemotaxis parameters in neutrophils [111, 200]. The p38 MAPK pathway appears to be more important for neutrophil chemotaxis to fMLP than the MEK/ERK pathway since inhibition of p38 with SB20358 has a much stronger effect on chemotaxis than inhibition of MEK/ERK with PD098059 [201]. Moreover, phosphorylated p38 is localized to the leading edge of neutrophils migrating towards fMLP [202]. In eosinophils the effects of the PD098059 inhibitor show that the response to their chemokine eotaxin requires MEK/ERK signaling to increase F-actin levels in vitro [203]. T-lymphocytes require MEK/ERK signaling to chemotax to SDF-1α only when infiltrating interstitial tissue, or, by analogy, in a 3D extracellular matrix culture [204]. A Ras/MEK/LIM domain kinase (LIMK)/Cofilin pathway is believed to be responsible for allowing the lymphocytes to increase actin kinetics to "slide" through a 3D matrix [205]. Cofilin activity is inhibited by LIMK, and LIMK is inhibited by MEK signaling. Therefore, MEK activity enables cofilin to sever and depolymerize actin, generating free barbed ends and increasing G-actin levels, which leads to elevated actin turnover rates [206]. Another potential mechanism for the regulation of chemotaxis by p38 and ERK is activation of map kinase activated protein kinase-2 (MK2) as neutrophils from MK2$^{-/-}$ mice display decreased directionality in fMLP gradients [200, 207].

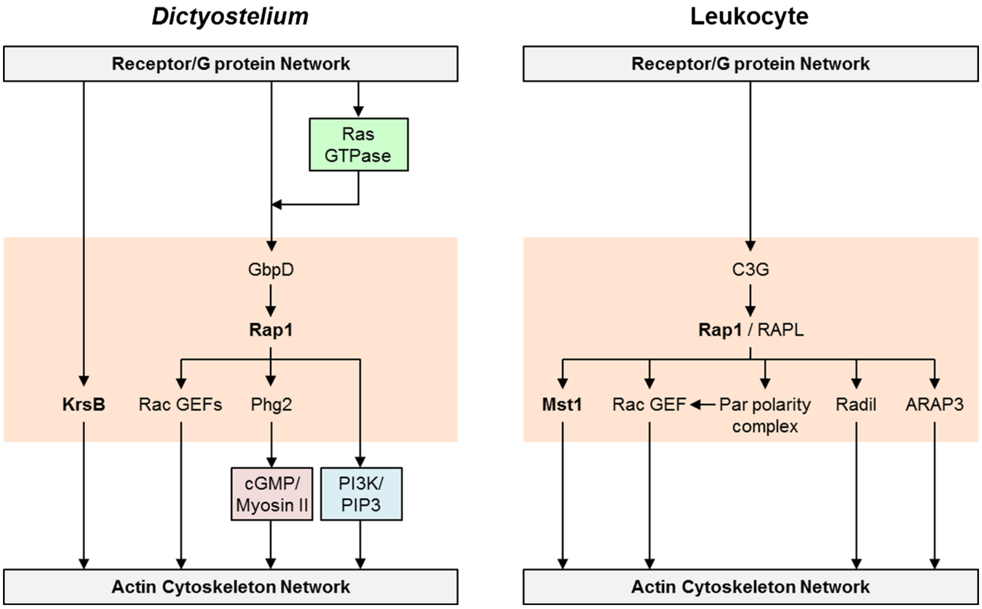
The importance of the p38 pathway for specific chemoattractants can be clarified when examining its functions in leukocyte migration to a site of infection through hierarchical gradients of chemoattractants. As previously mentioned, end-target chemoattractants, such as fMLP, require p38 signaling, but intermediary chemoattractants, such as IL8, do not. In addition, p38 MAPK signaling from fMLP can inhibit the PI3K/Akt signaling required for intermediary chemoattractants [147]. This creates a hierarchy that allows neutrophils to gradually lose preference for the intermediary chemoattractants, and accumulate at the site of infection.

Signaling at the level of the chemoattractant GPCRs provides additional degrees of regulation in MAPK signaling pathways. MEK/ERK and p38 pathways have opposing roles in the regulation of the FPR1 receptor in neutrophils. The ERK pathway potentiates the activity of the GPCR kinase (GRK) activity, thereby inhibiting neutrophil migration, while p38 phosphorylates the receptor to counteract GRK activity [202]. These two opposing signals most likely lead to an increase in the sensitivity of the system and ensure optimal neutrophil chemotaxis. In T-lymphocytes homo- and heterodimers of CXCR4 and CXCR7 receptors can form to detect SDF-1α. The CXCR4 receptors alone utilize $G_{\alpha i}$-induced signaling to activate ERK1/2, but when they are in a complex with CXCR7, the ERK1/2 and p38 pathways are activated through β-arrestin [208]. The CXCR4/CXCR7 signaling through β-arrestin can persist even after receptor internalization, which provides prolonged signaling compared to the more transient heterotrimeric G protein-dependent signaling [209]. The signaling through either the CXCR4/CXCR4 or CXCR4/CXCR7 complexes depends on the expression of each receptor and therefore provides a mechanism to fine tune different MAPK signaling pathways through receptor levels.

Rap1 and KrsB/Mst1 Module

Dictyostelium *Dictyostelium* Rap1 belongs to the Ras family of small GTPases and is very highly conserved with human Rap1 isoforms. Activation of the Rap1 pathway, either by using a constitutively active Rap1 mutant (G12V)

Fig. 7 Rap1 and KrsB/Mst1 pathways in *Dictyostelium* and mammalian leukocytes. The arrow and bar lines represent positive and inhibitory links, respectively, and the lines shown depict interactions that are strongly supported in the literature



or by overexpression of the Rap1 GEF, GbpD, leads to flattened cells that have increased adhesion to the substrate [210–212]. In addition, these cells have many lateral pseudopods, reduced polarity, and consequently poor chemotaxis. Conversely, knockout of *gbpD* or expression of the dominant-negative Rap1 mutant (S17N) reduces cell-substrate attachment, improves polarity, and enhances chemotaxis [210, 213].

Chemoattractants trigger rapid activation of Rap1 in a heterotrimeric G protein-dependent manner with kinetics only marginally slower than the activation of Ras (Fig. 7) [213, 214]. Consistent with this, the Rap1-GTP binding domain from human RalGDS, which is used as a probe for activated Rap1, transiently translocates to the plasma membrane with uniform chemoattractant stimulation, and localizes to the leading edge and weakly to the sides of cells migrating in a gradient [213]. The localization pattern of Rap1-GTP is broader than for Ras-GTP. RasG may regulate Rap1, since no activation is observed in *rasG*⁻ cells in pulldown assays [215]. Rap1 GTPase activating protein RapGAP1 appears to be a negative regulator of Rap1 function, and also translocates to the cell cortex with chemoattractant stimulation, albeit with slower kinetics than the appearance of Rap1-GTP [216]. The recruitment of a GAP to the front of the cell likely limits the ability of Rap1 to stabilize pseudopods allowing for detachment and forward projection necessary for efficient chemotaxis. Interestingly, the role of Rap1 in regulating chemotaxis might not entirely depend on its function in adhesion, since a second Rap1 GAP (RapGAPB) also contributes to the negative regulation of Rap1 function in adhesion, but not chemotaxis under certain conditions; however, the localization of this GAP has not been examined [217].

Activated Rap1 interacts genetically or biochemically with a number of proteins; however, it is not yet clear how all of these various interactions are integrated to implement the functions of Rap1. First, a Ser/Thr kinase Phg2, which was originally discovered in a screen for phagocytosis mutants, has a Ras-binding domain that interacts with Rap1-GTP. Phg2 co-localizes with Rap1-GTP and is important for myosin II disassembly at the leading edge [213, 218]. Details of this pathway will be further discussed in the cGMP/Myosin II module. Although there is some controversy in the literature, the most recent study suggests that Phg2-null have increased adhesion and defective chemotaxis [213]. Interestingly, Phg2 appears to mediate the effects of Rap1 on adhesion but not on cell polarity, further highlighting that the role of Rap1 in chemotaxis is not limited to its effects on adhesion [211]. Second, Rap1 interacts with Rac GEFs in vitro, potentially explaining the increased F-actin accumulation observed in cells expressing constitutively active Rap1G12V [219, 220]; however, further studies on the direct involvement of the Rap1 pathway in the cytoskeletal dynamics are warranted. Third, activated Rap1 was shown to directly interact with the RBD of PI3K [221]. Furthermore, overexpression of GbpD leads to increased PIP3 levels both basally and following cAMP stimulation and also fails to enhance adhesion in the absence of PI3K1/2.

Another mediator of *Dictyostelium* spreading and adhesion that is regulated by chemoattractants is Kinase Responsive to Stress B (KrsB) [222]. KrsB is a Ser/Thr kinase that is homologous to tumor suppressors Hippo and Mst1/2 in *Drosophila* and mammals, respectively. Chemoattractants positively regulate KrsB function by inducing a transient G protein-dependent increase, like that seen for other "front" responses, in the phosphorylation of a conserved Thr residue (T176) in the activation loop. KrsB-null cells, as well as cells expressing KrsB that cannot be phosphorylated on T176 or is catalytically inactive are very adherent to the substrate, which results in poor chemotaxis. Given the similarity between the phenotypes of KrsB-null and GbpD-overexpressing cells, it is possible that KrsB participates in the Rap1 pathway, although this remains to be tested.

Leukocytes As in *Dictyostelium*, Rap1 is an important mediator of leukocyte adhesion, polarity, and migration. In mammalian cells, there are two Rap1 isoforms, Rap1a and Rap1b, although most studies to date do not distinguish between the two [223]. Rap1 is important for random and directed migration of B lymphocytes to SDF-1 [224]. Overexpression of a constitutively active Rap1V12 construct induces polarization and migration of T cells, even in the absence of a chemoattractant [225, 226]. Furthermore, expression of a Rap1-specific GAP, Spa1, completely abrogates chemokine-stimulated adhesion, polarization, and transmigration under shear flow. In neutrophil-like PLB-985 cells, expression of a constitutively active Rap1aQ63E construct causes increased cell adhesion and reduced tail retraction, leading to poor chemotaxis [227]. It should be noted that Rap2, which shares ~ 60 % homology with Rap1, also appears to regulate migration, although this effect might be cell-type or context-dependent [224, 225, 228].

Chemoattractants trigger transient Rap1 activation with the kinetics varying depending on the cell type (Fig. 7). Similarly to the kinetics in *Dictyostelium,* Rap1-GTP accumulation in fMLP-treated human neutrophils and SDF-1 or CCL21-treated T lymphocytes peaks within seconds [225, 229]. In contrast, in B cell lines the peak activation occurs after several minutes [224]. In T cells, Rap1 activation is sensitive to pertussis toxin suggesting it is mediated by G_i-type heterotrimeric G protein [225]. How the signal is transmitted from the G protein to Rap1 is not known, although several candidates have emerged. In HL-60 cells, fMLP activates a non-RTK Lyn in a G_i protein-dependent manner [230]. Lyn recruits and activates the adaptor protein CrkL, which constitutively associates with a Rap1 GEF C3G. Consistently, depletion of Lyn results in reduced activation of Rap1 and β2 integrin at the leading edge. Furthermore, either Lyn or CrkL knockdown leads to defects in tail retraction and the ability to form stable protrusions, which results in poor chemotaxis. Tyrosine kinases might also be involved in Rap1 activation in T cells, where Abl family kinases Abl and Arg mediate chemokine-stimulated phosphorylation of the adaptor protein human enhancer of filamentation 1 (HEF1), which is required for Rap1 activation and chemotaxis [231].

Two key Rap1 effectors are the regulator for cell adhesion and polarization enriched in lymphoid tissues (RAPL) and Rap1 interacting adapter molecule (RIAM). Overexpression of RIAM increases Jurkat T cell adhesion and spreading, and reduction in RIAM counteracts enhanced integrin-dependent adhesion caused by constitutively active Rap1E63 [232]. Importantly, RIAM appears to be necessary for the localization of Rap1 to the plasma membrane. In addition, RIAM might provide a link between Rap1-GTP and the cytoskeleton since it constitutively associates with VASP and profilin and is necessary for the formation of Rap1-profilin complexes. Although the above studies did not examine chemokine-induced responses, CCL21 stimulates the association of Rap1 with a RIAM-containing complex in human primary T cells [233]. RAPL is essential for integrin-mediated adhesion and migration of T and B lymphocytes and dendritic cells both in vitro and in vivo [234, 235]. Overexpression of RAPL leads to T cell polarization similarly to the overexpression of constitutively active Rap1V12 [234]. In addition, chemoattractant-stimulated Rap1 activation leads to the association of RAPL with LFA-1, which is necessary for RAPL-mediated redistribution of LFA-1 to the leading edge [234].

The effects of Rap1 in lymphocytes also depend on the mammalian Ste20 family kinase Mst1, which is the homolog of *D. discoideum* KrsB [236]. In T cells CCL21 triggers a rapid accumulation of Mst1 at the leading edge about 30 s after stimulation, as well as a transient increase in Mst1 phosphorylation with a peak at 10 min in a RAPL-dependent manner. Reduction in Mst1 levels by siRNA prevents chemokine or Rap1V12-induced adhesion and polarization. Consistently, lymphocytes from Mst1$^{-/-}$ mice show reduced chemotaxis and interstitial migration in vivo [237, 238].

Other Rap1 effectors that mediate its effects on integrin-dependent adhesion and chemotaxis include Radil, ARAP3, partitioning defective (Par) polarity complex and T lymphoma invasion, and metastasis 1 (Tiam1) [226, 227, 239]. Treatment with fMLP triggers a rapid translocation of the adapter protein Radil to the plasma membrane of neutrophils. Similarly to Rap1E63, overexpression of Radil leads to integrin activation, increased cell-substrate adhesion, and defects in tail retraction [227]. On the other hand, reduction of Radil by siRNA leads to reduced adhesion and chemotaxis. In neutrophils Rap1-GTP also activates ARAP3, a GAP for Arf6 and RhoA, following PIP3-dependent recruitment of ARAP3 to the plasma membrane [239]. ARAP3-deficient cells have elevated RhoA-GTP and LFA-1 clustering, resulting in increased attachment to the substrate and reduced chemotaxis. T cell polarization downstream of Rap1 depends on the activation of the Par polarity complex and a Rac GEF Tiam1, which act together to induce Rac1 activation [226]. Reduction in Tiam1 levels leads to impaired chemokine- or Rap1V12-induced polarization, as well as SDF-1α-mediated chemotaxis. Since all of the effectors have only been examined in one particular cell type thus far, it remains unclear whether the same pathways are conserved between different leukocytes.

cGMP/Myosin II Module

Dictyostelium Tail retraction during amoeboid migration is thought to be mediated by conventional non-muscle myosins. *Dictyostelium* possesses a single conventional non-muscle myosin isoform (myosin II), which is a hexamer composed of two heavy chains (MHC), two essential light chains (ELC), and two regulatory light chains (RLC) [240]. *Dictyostelium* cells with disrupted myosin II are motile, although their speed and the ability to polarize are reduced to various extents depending on the approach used to perturb myosin II. Disruption of MHC results in the most severe phenotype, including reduction in overall speed, as well as the ability to polarize and move directionally to folic acid or cAMP [241–244]. In contrast, ELC appears to be dispensable for the ability of cells to move directionally, although it affects speed during cAMP, but not folic acid-mediated chemotaxis [244, 245]. Expression of myosin II that lacks RLC or has RLC with a mutation in a key regulatory residue (S13A) does not impair chemotaxis to cAMP, although it affects the ability to track natural cAMP waves due to defects in cell depolarization [246, 247].

Chemoattractants regulate myosin II both spatially and temporally. Chemoattractants stimulate phosphorylation of three Thr residues (T1823, T1833, T2029) in the coiled-coiled tail of MHC by a family of MHC kinases (MHCK) [248, 249]. This phosphorylation favors the monomeric form of myosin II, which is typically found in the cytosol [250, 251]. Mutant myosin that cannot be phosphorylated because the 3 Thr residues have been substituted with Ala (3XAla) constitutively assembles into filaments and localizes at the cortex, whereas the 3XAsp substitutions prevents filament assembly and cortical localization [251]. Cortical localization of myosin II also depends on its association with actin, since Latrunculin A treatment, which disrupts the actin network, prevents myosin II recruitment [252]. Global stimulation with chemoattractants leads to two phases of myosin II regulation. The initial phase, which occurs on the same time scale as cAMP-stimulated actin polymerization, is a rapid MHC phosphorylation, filament disassembly and loss of myosin II at the cortex [253, 254]. The rise in MHC phosphorylation is followed by a broader peak of MHC dephosphorylation, filament overassembly, and localization to the cortex [253, 254].

Transient reduction of myosin II localization at the membrane is similar to the behavior of PTEN and other “back” proteins and, consistently, myosin II, like PTEN, accumulates at the lateral edges and the tail of a moving cell, as well as on retracting pseudopods [244, 246, 255]. Such spatial restriction is thought to be achieved by the activation of MHCK specifically at the front of the cell [256]. This leads to localized myosin II disassembly at the front. Regulation of MHC phosphorylation is critical for myosin II function in chemotaxis, since 3XAla mutant shows reduced speed and polarization in response to cAMP [246, 257]. Interestingly, 3XAla myosin II still localizes to the tail of a moving cell, suggesting an additional mechanism must be involved in restricting myosin II to the back of a cell [246].

Dictyostelium has four MHCK isoforms (A to D). While all MHCKs are capable of phosphorylating MHC, only MHCKA localizes to the front of migrating cells, consistent with its role in restricting myosin II localization to the back [256, 258]. Two independent pathways regulate MHCK A activation (Fig. 8). The first involves activation of Rap1 and its effector Phg2 [213]. Phg2 is a Ser/Thr kinase; whether it directly phosphorylates MHCK A is unclear. Both Rap1 and Phg2 localize to the leading edge of a migrating cell. Cells without Phg2 exhibit increased basal accumulation of cortical myosin II and lack cAMP-stimulated MHC phosphorylation. On the other hand, following cAMP stimulation, cells expressing constitutively active Rap1 (G12V) show a prolonged MHC phosphorylation and a reduction in myosin II overassembly at the cortex. The second pathway participating in MHCK A recruitment to the cortex in response to cAMP stimulation involves RasB and its GEF (RasGEFQ) [45]. Myosin II phosphorylation is reduced leading to its overassembly in *gefQ*-null cells. Interestingly, although *gefQ*-null cells have aberrant polarity with more random pseudopods and increased frequency of turning compared to wild-type cells, other chemotactic parameters, such as speed and persistence, are not affected. This might point to the redundancy between the two pathways regulating MHCK A function. MHC phosphorylation is reversible, and dephosphorylation is mediated by the phosphatase PP2A [259]. The importance of this regulation is highlighted in cells that lack the huntingtin protein [260]. These cells have reduced PP2A activity, reduced myosin II disassembly, and aberrant chemotaxis.

Another regulator of myosin II function is cGMP. Chemoattractants trigger a transient increase in cGMP levels via the action of two guanylyl cyclases (sGC and GCA) and two cGMP-specific phosphodiesterases (DdPDE3 and DdPDE5) [261–264]. The association of cGMP with its high-affinity binding protein GbpC (cGMP binding protein C) is important for cAMP-stimulated myosin II accumulation at the cortex, and loss of GbpC or the two GCs leads to reduced chemotaxis [210, 265]. Interestingly, sGC appears to have a dual role in chemotaxing cells: sGC-mediated cGMP production is important for suppressing pseudopod formation at the back, whereas sGC protein, which is localized at the front, helps with reducing the frequency of turning behavior [266]. In addition, cGMP enhances cAMP-stimulated activation of MLC kinase A (MLCK-A), which phosphorylates

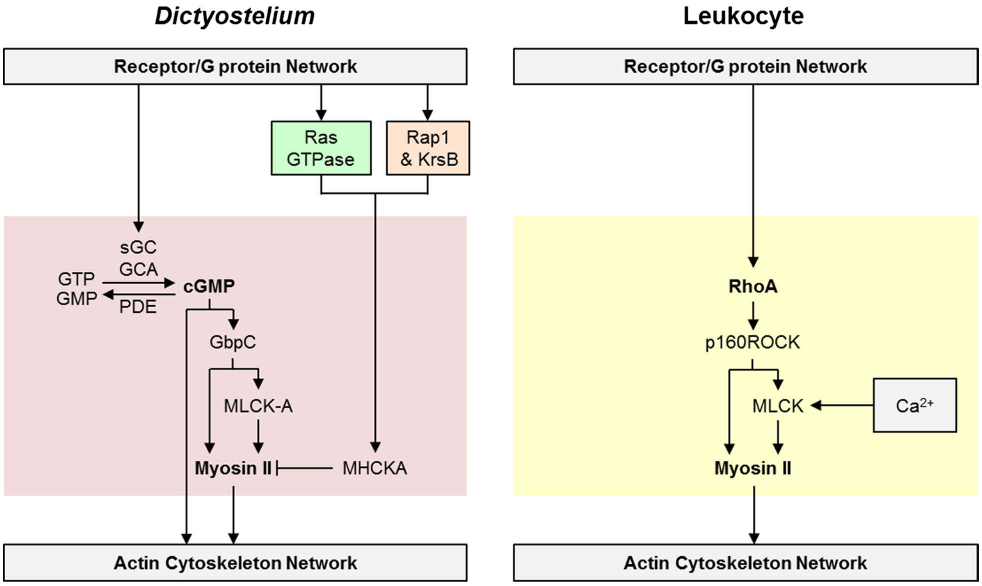


Fig. 8 *Dictyostelium* cGMP/Myosin II and leukocyte RhoA/Myosin II pathways. The arrow and bar lines represent positive and inhibitory links, respectively, and the lines shown depict interactions that are strongly supported in the literature

RLC on S13 and leads to increased ATPase activity of myosin II [267, 268]. Although this regulation appears to be dispensable for myosin II function in growth and development, it is involved in cell depolarization during chemotaxis as mentioned above [247, 268]. A recent report suggests that in addition to its role in myosin II regulation, cGMP can also stimulate actin polymerization [269].

Another protein thought to regulate myosin II dynamics is PakA. This Ste20 family kinase co-localizes with myosin II in the back of a migrating cell and appears to be required for myosin II assembly [88]. *pakA*-null cells have reduced cortical myosin II and exhibit defects in chemotaxis. PakA likely mediates its effects by negatively regulating MHCK.

RhoA/Myosin II Module

Leukocytes Assembly of non-muscle myosin II plays multiple roles in leukocyte migration, including the generation of actomyosin contractility and the release of integrin-mediated adhesions involved in tail retraction, as well as maintenance of microtubule stability, which is important for establishing polarity [270–273]. Mammalian non-muscle myosin II is a hexamer composed of 2 MHCs, 2 ELCs and 2 RLCs; however, unlike *Dictyostelium* myosin II it is primarily regulated by light chain phosphorylation (reviewed in [274]). There are three isoforms of MHC, which define three types of non-muscle myosin II (A-C). Of these, IIA is the only isoform expressed in T lymphocytes [275]. Chemoattractants promote RLC phosphorylation by activating RhoA, which activates Rho-associated, coiled coil-containing kinase (p160ROCK) (Fig. 8). P160ROCK either phosphorylates MLC directly, or activates another kinase MLC kinase (MLCK), while also inhibiting MLC phosphatase (Protein Phosphatase 1) (reviewed in [274]).

Pathways leading to RhoA activation during chemotaxis are not well defined, although several possibilities have emerged. For example, LFA-1-mediated migration of T cells to low SDF-1 concentrations depends on pertussis-insensitive G_q, which activates RhoA and mediates contraction [276]. Using the same system Tan et al. demonstrated that activation of the RhoA/p160ROCK/myosin II cascade is independent of $G_{\alpha i}$ and instead depends on $G_{\alpha 12/13}$ [277]. Using wild-type and Rac1-null primary neutrophils, Pestonjamasp et al. have demonstrated that Rac1 is required for both activation of RhoA/myosin II pathways in the back and local inhibition of the RhoA pathway at the front of a cell [278].

Myosin II localization is not clear in leukocytes, with some reports of myosin II both at the leading edge and the uropod, whereas others in the uropod only [174, 279, 280]. Interestingly, the two kinases regulating myosin II dynamics have opposing localization in T cells: p160 ROCK is in the uropod and is important for tail detachment, whereas MLCK is at the leading edge and participates in front attachment [280]. How this differential localization leads to specific myosin II function is not clear. One possible pathway that restricts myosin II activity to the back of a migrating neutrophil was described by Liu et al. In this system, mTORC2 activation at the front of a cell leads to the generation of cAMP and consequent activation of PKA, which can inhibit RhoA and MLCK locally [122].

Actin Cytoskeleton network

Dictyostelium

Cell migration in amoeboid cells is largely dependent on the attachments, modifications, and regulatory mechanisms of the actin cytoskeleton. Biochemical analyses have shown that uniform cAMP stimulus in developed cells induces rapid F-actin polymerization followed by disassembly within about 20 s, similarly to the dynamics of typical "front" protein activation and localization [31]. The fluorescently tagged LimE$_{\Delta\text{coil}}$ molecular probe binds newly formed F-actin at the front of protrusions and has thus allowed for the direct monitoring of in vivo spatiotemporal regulation of actin polymerization [281, 282].

At the interphase between the signal transduction and actin cytoskeleton networks are the members of the Rho family of small GTPases, which play essential roles in transmitting upstream signaling to the modulation and reorganization of cortical actin cytoskeleton. In *Dictyostelium* Rac1b, RacA, RacB, and RacC play a role in directed cell migration by regulating actin polymerization, but the precise role for each of these Rac proteins is still difficult to describe [283–286]. These small G proteins are activated by a number of GEFs that receive different upstream signaling inputs and thus provide a convergence point for several pathways. The Rac GEF GacG has been shown to interact with PKBs in *Dictyostelium* and thus provides a putative direct connection from the upstream receptor-mediated signaling events to actin cytoskeletal regulation [86]. Small G proteins can also share signaling elements as the GEF GxcC potentially relays Rap1 signaling to the Rac cytoskeletal signaling [287]. The ElmoE and Dock180 proteins (described in the PI3K/PIP3 module) also provide Rac GEF activity from the receptor/heterotrimeric G protein module [96, 288].

Several downstream targets of Rac GTPases act directly on actin to influence its polymerization and dynamics. The SCAR/WAVE complex (containing WAVE, Abi, Nap125, Sra-1, and HSPC-300) is important in the activation of Arp2/3 nucleation and elongation in protrusions [289, 290]. HSPC-300 is of particular importance as it is essential for most of SCAR functions and when labeled with GFP can track localized activation of the SCAR complex [291, 292]. Mutations and knockout cell lines in the SCAR complex itself demonstrate that it is important in *Dictyostelium* directed motility [293, 294]. The protein PIR121 is a putative relay point from the Rac G proteins to the SCAR complex [293]. *Dictyostelium* also possess a homolog of the mammalian F-actin-assembling Wiskott-Aldrich syndrome proteins (WASP) [295]. *Dictyostelium* WASP has been shown to interact with RacC [285]. Although the SCAR/WAVE complex appears to play a predominant role in directing cell migration in *Dictyostelium*, WASP can complement its loss to regulate pseudopod formation [291]. In addition, a WASP-related protein, WASP-B, has been shown to negativity regulate RacC activity that controls pseudopod extension during chemotaxis to cAMP [296].

Many posttranslational modifications, actin-binding proteins, and signaling pathways alter localized actin polymerization and stabilization. Phosphorylation of actin at Tyr-53 during the later stages of development inhibits F-actin elongation and destabilizes filaments [297]. Arp2 phosphorylation is required for normal chemotaxis towards cAMP as mutations in the threonine and tyrosine phosphorylation sites lead to decreased directionality and speed [298]. The PIP3-dependent, actin-binding proteins myosin ID/E/F, of the Myosin I class, provide spatial regulation of actin polymerization at the leading edge of migrating cells [95]. The actin-binding and nucleating formins, such as *Dictyostelium* dDia2, are also required for the formation of protrusions [299]. Certain actin-crosslinking proteins, such as filamin and α-actinin, are required for the stabilization of actin polymers during motility [300, 301]. The actin-crosslinking proteins cortexillin I and cortexillin II are also required for directed migration [302, 303]. The IQGAP (DGAP1 in *Dictyostelium*) and the cortexillin I/II proteins may form a functional quaternary complex with Rac1 GTPase at the trailing edges of migrating cells independent of the integrity of the actin cytoskeleton [286]. This complex is different than the one which contains Rac1 and Pak1, which localizes to the leading edge. Stabilization of actin filaments is specifically important for the development of adhesions required to establish the traction needed to transmit the motility forces [304]. The cross linker α-actinin is known to help form adhesion complexes, but *Dictyostelium* do not form canonical focal adhesions (see Fig. 9) [305]. Paxillins are also known to localize to focal adhesions in mammalian cells and act as a complex adaptor proteins at the plasma membrane, leading to the reorganization of the actin cytoskeleton [306, 307]. PaxB in *Dictyostelium* has been shown to be important for maintaining directionality during chemotaxis and localize to punctate regions on the basal surface of *Dictyostelium* cells (see Fig. 9) [308].

The negative regulation of actin polymerization and F-actin disassembly is equally important in establishing appropriate actin dynamics for directed cell migration. A coronin protein displays an inhibitory effect on the steady state of F-actin and is partially localized to sites of actin polymerization, specifically the decaying ends of actin tails [309]. The F-actin filament severing protein cofilin helps F-actin form appropriate bundles by increasing turnover rates and localizes at the leading edge of a protrusion within 30–60 s after the 20-s peak [310, 311]. Profilin proteins sequester monomeric actin (G-actin) regulating its polymerization and are required for chemotaxis, as *Dictyostelium* cells lacking profilin III display defects in directed migration [312, 313].

Leukocytes

The majority of spatiotemporally regulated actin dynamics in chemotaxing leukocytes share great similarity to that

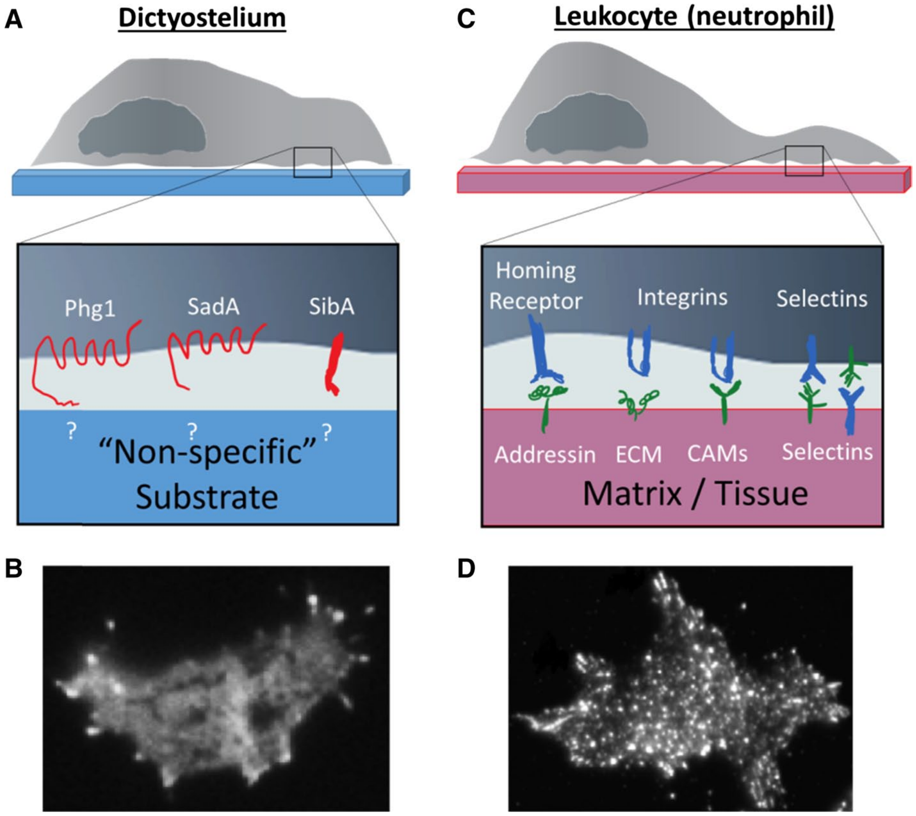


Fig. 9 Non-specific and specific modes of cell adhesion during migration. To be able to migrate cells must generate traction, which is most commonly achieved by cell adhesion to the substrate. **A.** *Dictyostelium* cells possess the ability to navigate over numerous different substrates found in their environment. Their migration is independent of canonical integrin-mediated focal adhesions. Integrins are transmembrane adhesion receptors that comprise a family of 18 α and 8 β subunits in mammals a can associate to form 24 different heterodimers that bind to a variety of ligands [398]. A transmembrane protein similar to integrin beta (SibA) is found in *Dictyostelium* and has some similarity to integrin β-chains, including the ability to bind talin, but they do not possess the homologous genes for focal adhesion kinase (FAK) or integrin-α chains [305]. The interaction between SibA and talinA is required for proper substrate adhesion and motility in vegetative *Dictyostelium* cells [399–401]. Although cells lacking SibA have impaired adhesion, they have no defects in cell migration possibly due to the presence of redundant family members [400, 402]. In contrast, lack of talin A/B results in reduced adhesion, as well as migration of vegetative cells; however, it remains unclear which receptor, if any, is necessary for these effects [399]. Two nine transmembrane domain-containing proteins, Phg1 and SadA, positively regulate adhesion to non-specific substrates in *Dictyostelium* [403, 404]. Recent evidence indicates that an important mechanism for cell-substrate attachment in *Dictyostelium* is via van der Waals interactions, which allow the cells to adhere to a variety of surfaces within minutes [401]. Although the molecular mechanism of *Dictyostelium* attachment to the substrate is largely unknown, several signaling pathways that alter cell spreading and adhesion have been identified and are discussed in this review. **B.** There are focal adhesion-associated proteins, including talin, paxillin, and vinculin, that make transient foci on the basal surface of migrating cells, which may be homologous to mammalian focal adhesions. TIRF microscopy analyzing the basal layer of the cells shows paxillin localizing to these foci [405–407]. **C.** In 2D environments, leukocytes specifically attach to the tissues via selectins, addressins, and to the extracellular matrix via integrins. L-selectin and P-selectin are cell–cell adhesion molecules that bind glycoproteins, and are utilized by leukocytes and endothelial cells, respectively, to regulate adhesion of leukocytes to specific tissues [408, 409]. Selectins are associated with numerous intracellular signaling proteins to modify cell behavior. Endothelial target localized addressins (such as MAdCAM-1) are bound by leukocyte homing receptors (for example, CD34) to help leukocytes adhere to their targets [410]. Although leukocytes express a number of different integrins, α4β1 (very late antigen-4, VLA-4) and β2-containing integrins αLβ2 (lymphocyte function-associated antigen 1, LFA-1, CD11a/CD18) and αMβ2 (Mac-1, macrophage antigen-1, CD11b/CD18) are particularly important for leukocyte chemotaxis [411, 412]. **D.** Unlike integrins found in mesenchymal cells, leukocyte integrins do not form well-defined focal adhesions, even though they associate with many proteins typically found in these structures, including talin, paxillin, and focal adhesion kinase (FAK). Paxillin localization in foci at the bottom of a leukocyte under TIRF microscopy is shown [407]. The reason for the absence of well-defined focal adhesions in leukocytes is likely the requirement for very rapid turnover of the cell attachments to the extracellular matrix to allow for the fast migration rates of these cells. Integrins also bind cell adhesion molecules (CAMs), such as VCAM, to aid in targeting appropriate vasculature adhesion [413]. Interestingly, while important for migration on 2D surfaces, in 3D environments, leukocytes switch to integrin-independent migration, where confinement by the extracellular matrix generates enough traction to allow forward propulsion [8]

previously described in *Dictyostelium* [314]. Using biochemical dyes and in vivo probes it has been observed that the chemoattractant-induced changes in F-actin polymerization and its localized distribution display “front” protein behavior in leukocytes [315]. In neutrophils, a maximal F-actin response in a gradient of fMLP is observed within ~45–60 s using phalloidin staining and fluorescent TMR-actin. This activity shares sequential leading edge co-localization with the Arp2/3 complex that nucleates branching daughter filaments in the direction of fMLP during neutrophil chemotaxis [315, 316]. Using several pharmacological inhibitors, Chodniewicz et al. demonstrated in human neutrophils that two pathways, PI3K/AKT/PKC and Rho/ROCK/Src, were critical for F-actin production in pseudopod extension [124]. T and B cells show peak SDF-1-stimulated F-actin production at about 15 s in flow cytometry experiments with fluorescently tagged phalloidin [317]. The small 17 amino-acid bioprobe Lifeact binds F-actin and does not interfere with actin dynamics. When fluorescently tagged, this probe has helped demonstrate that stable F-actin resides at the tail, whereas dynamic F-actin is at the leading edge of migrating neutrophils in vivo [318, 319].

Like *Dictyostelium,* Rac small GTPases are critical for activation of the actin nucleating SCAR/WAVE and WASP complexes in mammalian leukocytes [320, 321]. In P-Rex1$^{-/-}$ neutrophils the chemotaxis speed in response to fMLP is significantly decreased since P-Rex1 is the primary GEF for the Rac1/2 proteins [322]. P-Rex1 is activated by both $G_{\beta\gamma}$ and PIP3 to stimulate Rac activation [131, 322]. The Vav proteins are members of the Dbl family of GEFs, possess tandem PH domains, and predominantly provide GEF activity for Rac [323, 324]. Vav1 expression is restricted to hematopoietic cells, while Vav2 and Vav3 have broad expression and possess some, but not completely redundant activity to Vav1 [325, 326]. Vav1 activity regulates adhesion during chemotaxis by increasing attachment of integrins in T lymphocytes in response to CXCL12 [327]. The Zap-70 kinase is a direct upstream regulator of Vav1, and its kinase activity is stimulated when T-cells are exposed to SDF-1 [327]. Lawson et al. have demonstrated that both P-Rex1 and Vav1 synergistically control adhesion and chemotaxis in fMLP-stimulated neutrophil responses [328]. Other RacGEFs, such as Dock2 and Dock180/Elmo, and their regulation by inositol signaling have been previously discussed in the PI3K/PIP3 module. Reduced chemotaxis in T-cells deficient in the Tiam1 RacGEF suggests that the PKC/Tiam1/Rac signaling pathway is necessary for polarization and crawling on endothelial cells [329]. The protein ArhGAP15 is a negative regulator of Rac in both neutrophil and macrophage behavior, but only neutrophils from ArchGAP15 deficient mice show increased chemotaxis [330]. Unlike *Dictyostelium,* leukocytes possess Cdc42, which is a commonly reported strong inducer of directed actin dynamics [321, 331]. Cdc42 has been shown to have specificity for the N-WASP actin pathway in regulating directed migration of neutrophils towards a gradient of fMLP [321]. In addition to regulating LIMK, Pak1 is also an adaptor protein for the activation of Cdc42 in neutrophils [126].

In addition to the many signaling pathways that feed into actin regulatory mechanisms, this major cytoskeleton component in leukocyte chemotaxis can also modulate upstream pathways to establish feedback loops. If actin polymerization is inhibited by the addition of latrunculin, polarity of the WAVE complex is lost in chemotaxing neutrophils [320]. In addition, there is a significant decrease in the amplification of internal PIP3 gradients in neutrophil cells in a gradient [332]. Moreover, when actin dynamics are stabilized using several pharmacological inhibitors, the actin cytoskeleton positively regulates receptor desensitization in neutrophil fMLP responses [333].

There are several additional proteins that interact with actin and play important regulatory roles on F-actin growth and depolymerization in leukocyte chemotaxis. Like in *Dictyostelium,* Myosin I class protein Myo1f regulates cell motility in immune cells [334]. The initial steps of filamentous actin formation are regulated by the direct nucleation of actin monomers from the profilin-sequestered pool. First, as in *Dictyostelium,* many chemotaxis signaling cascades feed into SCAR/WAVE and WASP complexes, which are activators of Arp2/3 induced actin filament branching. In contrast, formin proteins nucleate the production of linear unbranched actin filaments [335, 336]. The formin mDia1 is a RhoA effector and localizes to the leading edge of migrating T cells to control filamentous actin production during chemotaxis to SDF-1 [337]. Moreover, mDia1 interacts with WASP at the leading edge of migrating neutrophils, and neutrophils from mDia1$^{-/-}$ mice show strong defects in chemotaxis to both fMLP and MIP2 [338]. Second, profilin, which helps actin polymerization by catalyzing the exchange for ATP and delivering G-actin to barbed ends of a growing filament, has been shown to enhance Cdc42-induced actin polymerization in neutrophils [339]. Cofilin/ADF can be induced by Rac2 and, as in *Dictyostelium*, is necessary for the amplification of barbed F-actin ends, which is critical for fMLP-directed migration in neutrophils [340, 341]. Chemoattractant-mediated activation of cofilin also depends on the inhibition of the phosphatase slingshot2 downstream of Akt/GSK3, as mentioned previously [125]. Third, coronin-1 is another actin-associated protein that accumulates at the leading edge of migrating neutrophils, and most likely enhances barbed-end production via its association with Arp2/3 [342]. Fourth, the actin crosslinker FilaminA is important for the negative regulation of integrin activity, as well as uropod retraction through RhoA during neutrophil chemotaxis [343, 344]. Last, the actin fiber associated α-actinin is a cortex-localized protein that works to reduce adhesion in pseudopods and establish the structure of the retraction fiber network in neutrophils [345].

Polarity network

Amoeboid cells, whether randomly migrating or in a chemoattractant gradient, form and maintain a dominant leading edge. This polarized active front can be established with chemoattractant stimuli or spontaneously arise to varying extents in numerous amoeboid cell types and conditions. Cells display both temporary and intrinsic polarity. Temporary polarity is characterized by the presence of more dynamic and easily outcompeted “front” and “back” restricted localizations of specific biomarkers and is expanded upon in Fig. 2. Intrinsic polarity can occur without external stimuli, but can be maintained even in the face of an opposing chemoattractant gradient. *Dictyostelium* cells that have progressed through their developmental cycle to the social stages have increased intrinsic polarity. These fully differentiated *Dictyostelium* cells will maintain the leading edge and when exposed to a shallow gradient of chemoattractant will make a U-turn rather than create a new leading edge facing the chemoattractant source. Neutrophils also possess intrinsic polarity as after they are exposed to uniform chemoattractant they will create and maintain a single leading edge.

There are numerous molecular components that have been implicated in the intrinsic polarity state of amoeboid cells. In *Dictyostelium* cells the microtubule network is involved in polarity, as depolarization with benomyl or genetic manipulations of Lissencephaly protein I (LisI), Dynein, or Tsunami (TsuA) all cause defects in polarity [346, 347]. Surprisingly, there appears to be an opposite trend in neutrophils, where disruption of the microtubule network with nocodazole induces polarity [348]. In addition to the role of microtubules, genetic disruptions of MEK1, Tortoise (TorA), and a NA–H exchanger (Nhe1) all lead to decreased polarity in *Dictyostelium* [182, 349, 350]. Not surprisingly, the actin cytoskeleton is also required for intrinsic polarity as treatment with Latrunculin abolishes the spatial localization of leading edge proteins in these amoeboid cells [332, 351].

Most models for polarity balance positive feedback elements at the front with some global inhibitor that prevents the formation of other fronts. Some have suggested that localized recruitment of cytosolic components at the protrusion inhibits additional protrusions at other regions due to depletion of these components [352, 353]. Another model suggests that membrane tension plays a role as the inhibitor. Houk et al. demonstrated in neutrophils that a leading edge can be abolished if tension of another region of the membrane is increased through pipette aspiration [354]. Furthermore, cell-severing experiments and uniform reduction of membrane tension can establish new regions of actin assembly, suggesting diffusion mechanisms are not necessary. It is likely all of these models play a role in the complex processes of maintaining polarity in migrating amoeboid cells.

Computational models explaining chemotaxis

A series of computational models have been proposed to explain various aspects of chemotaxis. Some of the models seek to explain single aspects of chemotaxis such as directional sensing, motility, or polarity (see Fig. 1). Some of these models are conceptual, while others describe the interactions of specific molecules. Some of the conceptual models are able to describe the overall behavior of chemotaxing cells but the parameters represent the aggregate behavior of groups of components that change dynamically. Most of the molecular models focus on a particular set of results and describe the temporal and spatial changes in the components involved but do not take into account the full complexity of the networks mediating chemotaxis. Ultimately, models are needed that simulate all aspects of chemotaxis with accurate molecular detail.

Whether specific or conceptual, the most promising models are those that focus on biochemical excitability. Clearly there is evidence for biochemical excitability in chemotactic signaling systems. In the past decade, there have been increasing observations of propagating waves of cytoskeletal components. For example, oscillations and propagating waves of subunits of the SCAR/WAVE complex and actin-binding proteins have been reported on the basal surface of *Dictyostelium* amoebae, human neutrophils, and mouse fibroblasts [281, 355–362]. Furthermore, oscillations and propagating waves of signal transduction events, such as PIP3 accumulation and Ras activation, have been observed in *Dictyostelium* [358, 359, 363–369]. Further evidence for excitability has come from stimulation experiments where short and longer stimuli produce an “all-or-none” response of Ras and PI3K activation, which displays absolute and relative refractory periods [366]. This may suggest that the entire signal transduction network we are describing here is excitable.

Several models have incorporated excitability to explain the stochastic nature of pseudopodia production, the observations of propagating waves, or both. Predating all of the observations, Meinhardt proposed a local activator–inhibitor system that provided excitability and stochastic response together with a global inhibitor that confined the activity to a region [370]. Similar excitable systems have been proposed to explain the stochastic nature of pseudopodia [368, 371]. The Meinhardt scheme can be guided by providing input to the activator, constituting what was later termed a “biased excitable network” (BEN). This system was later shown to simulate chemotactic behavior [372]. Arai et al. proposed a molecular model involving reciprocal feedback loops to explain oscillations of PI3K and PTEN that were observed in immobilized *Dictyostelium* cells [363]. Xiong et al. employed an upstream local-excitation, global-inhibition (LEGI) module to bias a downstream excitable network (LEGI-BEN) to account for the adaptive spatiotemporal behavior of the signal transduction network [368]. Shi et al. extended this model using level set methods to simulate chemotactic behavior and added a polarity module that derived from and fed back to the excitable network [17].

The networks regulating chemotaxis in *Dictyostelium* and leukocytes share many similarities

Having examined the details of the networks regulating chemotaxis we see that the overall topologies are remarkably similar between *Dictyostelium* and mammalian leukocytes. In both systems the receptor/G protein network provides an input to various modules in the signal transduction network, ultimately all of which lead to changes in the cytoskeletal dynamics. Feedback from the cytoskeleton network further amplifies the responses of the signal transduction module, and contributes to the polarity network. A direct comparison of the major molecular events within each network, which is presented in Table 1, reveals remarkable conservation of the core components, either by sequence or functional homology, between the two cell types.

As outlined above the receptor/G protein networks are very similar in *Dictyostelium* and leukocytes, except that the leukocytes are known to sense a wider variety of chemoattractants. Accordingly, the known chemoattractant receptors in *Dictyostelium* comprise a small family of cAMP receptors, whereas in leukocytes a large family of chemokine receptors mediates their chemotactic responses. Most signaling downstream of chemoattractant receptors is transduced by heterotrimeric G proteins in both *Dictyostelium* and leukocytes, although the specific subunits and complexity differ between the two systems.

There is symmetry in the overall organization of the signal transduction networks in *Dictyostelium* and leukocytes, although in *Dictyostelium* the Ras GTPase module relays the input from the receptor/G protein network to several downstream modules, including PI3K/PIP3, TORC2/PKB, Rap1 and KrsB, and cGMP/Myosin II, whereas in leukocytes studies to date have demonstrated Ras GTPase involvement upstream of PI3K/PIP3 and MAPK modules only.

The similarities between *Dictyostelium* and leukocytes continue beyond the overall topology of their signal transduction networks and are clearly evident within the individual modules as well. For example, Ras or Rap1 GTPases are highly conserved between *Dictyostelium* and mammalian cells; however, the GEF and GAP proteins that regulate these GTPases, while present in both systems, are often not direct orthologs. Many protein kinases, including PKB, PDK, Pak, TOR (within the TORC2 complex), ERK, MEK, KrsB/Mst1, as well as lipid-modifying enzymes, including PI3K, PLC, and PLA_2, are implicated in directed migration for both *Dictyostelium* and leukocytes. Perhaps, one interesting exception is in the roles that PIP3-degrading enzymes play in the two cell types. In *Dictyostelium* PTEN degrades PIP3 to PI(4,5)P2, and deleting this enzyme leads to excessive spreading and the inability to restrict PIP3 signal to the side facing the gradient, resulting in defects in chemotaxis. In contrast, in leukocytes degradation of PIP3 to PI(3,4)P2 by SHIP1 appears to play a more dominant role, with SHIP1-null cells showing excessive adhesion and spreading, as well as impaired motility. The reason for why *Dictyostelium* and leukocytes preferentially utilize PTEN vs. SHIP1, respectively, is not known. It is possible that 5-phosphatases are involved in *Dictyostelium* chemotaxis, since all four putative 5-phosphatases have not been deleted together.

One notable difference between *Dictyostelium* and leukocytes is the organization of the modules regulating myosin II dynamics. First, in *Dictyostelium*, the main regulatory mechanism for myosin II function is its phosphorylation by MHCK, whereas in leukocytes it is phosphorylation by MLCK. Interestingly, unlike *Dictyostelium* MLCK-A, MLCK activity in neutrophils is Ca^{2+}-dependent [174]. Second, a cGMP signaling pathway plays a prominent role in myosin II dynamics and localization in *Dictyostelium*, whereas RhoA/p160ROCK is the major regulator of myosin II in leukocytes. Interestingly, it has been reported that cGMP levels increase following chemoattractant treatment and that cGMP can modulate chemotaxis in neutrophils and monocytes [373–376]. However, the mechanism of cGMP action in leukocytes remains very ambiguous. First, cGMP has been reported to both enhance and inhibit chemotaxis, possibly reflecting concentration dependence of this process [376]. Second, although the direct target of cGMP appears to be cGMP-dependent protein kinase, downstream effectors are not known, although some candidates include cytoskeletal and focal adhesion proteins, including vimentin, vasodilator-stimulated phosphoprotein, Rap1 and Rap2 [374, 377, 378]. Although it is not known if cGMP affects myosin II dynamics in leukocytes as it does in *Dictyostelium*, cGMP has been shown to inhibit myosin II via the RhoA/ROCK pathway in other mammalian cells, for example, vascular smooth muscle cells [379].

A curious observation is that cell-substrate attachment is regulated by the Rap1 and KrsB/Mst1 module in both

Table 1 Molecular components in *Dictyostelium* and leukocyte chemotaxis

	Molecular Component		References	
	Dictyostelium	Leukocytes	*Dictyostelium*	Leukocytes
Receptor/G protein Network				
Receptors	GPCRs(cAR1-4)	GPCRs(FPR1, C5aR, PAFR, chemokine receptors)	[383]	[34, 39, 384]
Heterotrimeric G proteins	$G_{\alpha}(2,4)$, $G_{\beta\gamma}$	$G\alpha(I, 12/13, q)$, $G_{\beta\gamma}$'s	[26]	[24, 76, 259, 385]
Signaling Network				
Ras Module	RasS,RasD,RasB, RasC, RasG	H-Ras, N-Ras, KA-Ras, and KB-Ras	[43, 44, 47, 55] [41, 42]	[66, 72, 73] [69–71]
	Sca1 complex: AleA/GEFA,Sca1, GEFH, PHR, PP2A GEFR	SOS RASGRP4	[60–63] [54]	[74] [76]
	DdNF1	NF1	[48] [54]	[78]
	–	Gap120	–	[77]
PI3K/PIP3 Module	PI3K1-5	PI3Kγ, δ	[56, 98, 101–103]	[106–109, 117–121]
	PTEN	PTEN	[81]	[138, 140–144]
	SHK1	*MAP3K7**	[84]	–
	5-phosphatase (Dd5P1-4)	SHIP1	[80]	[116, 145, 146, 386]
	PLC	$PLC\beta\text{-}_{2/3}$	[83]	[115, 136, 137]
	GpbB	RACK1	–	[135]
	PKBA, PKBR1	Akt	[55, 85, 86, 148]	[122, 123]
	PakA	Pak	[86, 88]	[126–128]
	CRAC	–	[91]	–
	PhdA PhdB (RapGAP3) PhdG	–	[92–94]	–
	Myosin ID, IE, IF	Myosin IF	[95]	[95]
	Rac GEFs (DockA, DockD)	Rac GEFs (DOCK2, P-Rex1)	[96]	[131, 132]
TORC2/PKB Module	TORC2 complex: TOR, RIP3, PiaA, LST8	mTORC2 complex: mTOR, mSIN1, Rictor, LST8	[47, 150, 151, 154, 155]	[153, 159, 387]
	PDKA, PDKB	PDK1	[55, 97, 154]	[158]
PLA_2 Module	PLA_2A (via AA)	$iPLA_{2}\text{-}\beta$ (via LPA)	[160]	[140, 163, 164]
	–	$cPLA_2\alpha$ (via AA)	–	[140, 163–165]
Ca^{2+} Module	Ca^{2+}	Ca^{2+}	[168–171]	[172, 174, 175]
MAPK Module	ERK1/2	ERK	[181] [185, 186, 189]	[194, 195].
	MEK1	MEK	[180, 182, 183]	[194, 195].
	MEKK	MEKK/Raf	[177]	[69, 197]
	–	p38	–	[147] [201] [202]
Rap1 and KrsB/Mst1 Module	–	Tyrosine kinases (Lyn, Abl, Arg)	–	[230, 231]
	–	Adaptor proteins (CrkL, HEF1)	–	[230, 231]
	Rap1 GEF (GbpD)	Rap1 GEF (C3G)	[210, 211]	[230]
	Rap1 GAP (RapGAP1, RapGAPB)	Rap1 GAP (Spa1)	[216, 217]	[225, 226]

Table 1 continued

	Molecular Component		References	
	Dictyostelium	Leukocytes	*Dictyostelium*	Leukocytes
	Rap1	Rap1	[213]	[224–227]
	Phg2	–	[211, 213]	–
	–	RAPL	–	[234, 235]
	KrsB	Mst1	[222]	[236–238]
	–	Radil	–	[227]
	–	ARAP3	–	[239]
	Rac GEFs (RacGEF1, GxcC)	Rac GEF (Tiam1)	[219, 220]	[226]
	–	Par polarity complex	–	[226]
cGMP/Myosin II and RhoA/Myosin II Modules	guanylyl cyclase (sGC, GCA)	guanylyl cyclase (sGC)	[261, 265, 266]	[373, 376]
	cGMP phosphodiesterase (DdPDE3, DdPDE5)	–	[262, 263]	–
	GbpC	–	[210]	–
	MHCKA	–	[246, 256, 257]	–
	MHC phosphatase (PP2A)	–	[260, 388]	–
	PakA	Pak	[88]	[126–128]
	–	RhoA	–	[271, 272, 280, 389]
	–	P160ROCK	–	[271, 272, 280, 389, 390]
	–	Ca^{2+}/calmodulin	–	[174, 280]
	MLCK-A	MLCK	[247, 267]	[174, 272, 280, 391]
	Myosin II	Myosin II	[241–244, 246]	[174, 270, 272]
Actin Cytoskeleton Network	G/F-Actin (Act8)	G/F-Actin (ActB)	[380, 381]	[392, 393]
	Rac GEFs (GacQ, GxcC)	Rac GEFs (P-Rex1,Vav1-3, Tiam1)	[86, 287]	[130, 131, 322–324, 326, 327, 329]
	Rac1, A, B, C	Rac1/2	[283–286]	[322]
	SCAR/WAVE	SCAR/WAVE	[289–294]	[320]
	N-Wasp	N-Wasp	[285, 295]	[321, 338]
	–	RhoA	–	[337]
	–	Cdc42	–	[321]
	Filamin	Filamin	[301]	[343]
	α-actinin	α-actinin	[300]	[345]
	Paxillin	Paxillin	[308]	[307]
	Coronin	Coronin	[309]	[342]
	Cofilin	Cofilin	[310, 311]	[125, 340, 341]
	Profilin	Profilin	[312, 313]	[339]
	Cortexillin I/II	–	[302, 303]	–
	IQGAP	*IQGAP*	[394]	–
	PakA	Pak	[286]	[126–128]
	Myosin ID, IE, IF	Myosin IF	[95]	[334]
	dDia1	mDia1	[395]	[337, 338]
	dDia2	*mDia2*	[299]	–
	Arp2/3	Arp2/3	[298]	[315, 316]

* The proteins in italics are direct homologues, although their function in chemotaxis has not been studied

systems, even though the mechanism of adhesion itself appears to be different between *Dictyostelium* and leukocytes (see Fig. 9). In addition to the same core components (Rap1, Rap1 GEFs and GAPs), the two systems also share similarities in some of the downstream targets, for example, Rac GEFs. However, in leukocytes, but not *Dictyostelium,* several Rap1 effectors, including ARAP3, Radil and Mst1, modify adhesion by specifically affecting integrins. Interestingly, a *Dictyostelium* homolog of Mst1, KrsB, also alters cell-substrate attachment, although the mechanism of this process, as well as whether KrsB is an effector of Rap1, is not known. The effects of Rap1 itself on *Dictyostelium* adhesion appear to be mediated by Phg2, which affects myosin II dynamics. Whether an analogous mechanism exists in leukocytes is not known.

The overall organization of the actin cytoskeleton network, as well as the mechanisms of regulation of actin dynamics, is highly conserved between *Dictyostelium* and leukocytes. In the *Dictyostelium* genome, duplication events have created 17 separate actin genes, but the act8 proteins comprise 95 % of total actin in the cell [380]. Out of the numerous cell type-specific actin genes in humans, act8 in *Dictyostelium* has the closest homology to ACTB, ACTG, ACTA, and ACTC in mammalian cells [381]. In both systems Arp2/3 proteins are utilized to nucleate actin polymerization. Both *Dictyostelium* and leukocytes utilize SCAR/WAVE and WASP actin nucleating complexes activated by Rac-type Rho GTPases. In contrast, *Dictyostelium* does not possess Cdc42 or RhoA GTPases that are known to regulate WASP and mDia, respectively, in leukocytes [382]. Leukocytes do not possess the actin-binding cortexillins, but like *Dictyostelium* they possess IQGAP actin crosslinkers [286, 302]. The function of IQGAPs in leukocytes has not been explored.

The molecular networks examined in this review do not include the numerous feedback loops that add to the overall complexity of the signaling events involved in chemotaxis. Future studies will likely reveal further parallels between the mechanisms of chemotactic signaling not only in *Dictyostelium* and leukocytes, but also in other cells undergoing amoeboid migration, including metastatic tumor cells.

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