Current Topics in Biochemical Research
Vol. 16, 2014

Review

Cellular functions and mechanisms of GPCR-mediated chemotaxis

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ABSTRACT

Many types of eukaryotic cells have the ability to sense gradients of chemoattractants and to migrate toward the sources of these attractants, and this kind of chemical-gradient-guided cell migration is called chemotaxis. Chemotaxis is essential for development and immunity in mammals, and it also plays a key role in pathological events such as cancer metastasis and inflammation. The knowledge on cellular functions of chemotaxis has been enriched by the ability of monitoring dynamic behaviors of cells in diverse tissues and revealing chemoattractant gradients in these environments. The understanding of the molecular mechanisms has largely benefited from studies to identify components and mechanisms involved in chemotaxis. However, it is still not clear how these components work together to detect a gradient and to achieve directional cell migration. Here, we highlight recent progress in GPCR-mediated chemotaxis at the cellular and molecular levels.

KEYWORDS: G-protein-coupled-receptor, signal transduction, chemokine, chemotaxis

INTRODUCTION

Chemotaxis, the directional movement of cells toward chemoattractants, plays critical roles in diverse physiological processes, including the recruitment of leukocytes to sites of infection and trafficking of lymphocytes throughout human body [1-5]. Properly controlled movements of leukocytes and lymphocytes ensure immunity against pathogens, and prevent inflammatory diseases such as asthma, arthritis and atherosclerosis [6-9]. Chemotaxis is also implicated in metastasis of cancer cells from an original site to elsewhere in the human body [10-12]. For example, in breast cancer patients, secondary tumors are often formed in lungs, lymph nodes and bone marrows and rarely in other organs [10]. Chemotactic movements of leukocytes, lymphocytes and cancer cells are guided by extracellular signaling molecules, called chemokines, and mediated by chemokine G-protein-coupled receptors (GPCRs) expressed on the cell surface [9, 10, 13]. There are currently more than 50 known chemokines [14]. Typical chemokines are small proteins (~70 to 90 amino acids) that contain easily recognized cysteine motifs at N-termini. They are classified into C, CC, CXC and CX3C subfamilies, based on the number and arrangement of cysteines in the motif [14, 15]. There are 20 known chemokine receptors, each of which associates with heterotrimeric G-proteins to detect gradients of chemokines and to guide cell movement [14]. These G-protein-coupled receptors (GPCRs) detect spatiotemporal changes in chemoattractant levels and signal through an intracellular signaling network to control actin cytoskeleton, which drives cell migration [8]. In the last ten years, advances in fluorescence microscopy have allowed *in vivo* imaging of cell movement in living animals, which reveals chemotaxis behavior of the cells in their native environments [16]. Given the complexity of tissue environments, and the large number of chemokines

and chemokine receptors, dissecting the mechanism of chemotaxis in animals is an exciting yet challenging topic of research.

Dictyostelium discoideum, a social amoeba, is a key model organism for the study of eukaryotic chemotaxis [17-20]. Over the last twenty years, studies in this organism have contributed significantly to our current understanding of molecular mechanisms underlying chemoattractant gradient sensing, cell polarity and cell motility. Many (if not all) molecular mechanisms that control the fundamental aspects of chemotaxis are evolutionarily conserved. In this short review, we will highlight recent advances in GPCR-mediated chemotaxis made in mammalian systems and *Dictyostelium discoideum*.

Chemoattractants control neutrophil recruitment into inflammatory sites

Leukocyte recruitment into peripheral tissues is essential for the innate immune response, which forms the first line defense against different forms of pathogens [1, 2]. Neutrophils are important effector cells of innate immunity. Chemoattractants, such as lipids (LTB4, PAF), complement fragments (C5a, C3a) and chemokines, guide neutrophils to exit from bone marrow into the circulation, to move from blood into the tissue, and eventually to migrate to the sites of infection and inflammation [13]. These chemoattractants are detected by a family of GPCRs on the surface of neutrophils and activate heterotrimic G-proteins, Gi, to mediate a signaling network that controls cell movement [13]. Although rapid recruitment of neutrophils to infection sites is crucial for host defense, excessive recruitment of neutrophils into health tissues causes tissue damage and inflammatory diseases. Thus, neutrophil recruitment into tissue is tightly regulated by a multitude of chemoattractants and chemoattractant GPCRs *in vivo*.

Egress of neutrophils from bone marrow into blood is mainly controlled by two chemokine receptors, CXCR4 and CXCR2, and their chemokine ligands. CXCL12 (SDF-1α), a CXCR4 ligand, functions to keep neutrophils in the bone marrow, while, CXCL1 (KC) and CXCL2 (MIP-1), the CXCR2 ligands, promote neutrophil egress [21]. Several lines of evidence indicate that CXCL12/CXCR4 signaling plays a dominant role in retaining neutrophils in the bone marrow. First, CXCL12 is largely produced by stromal cells in the bone marrow [22]. Second, a deletion of CXCR4 gene in murine cells leads to an elevated neutrophil release from bone marrow [23, 24]. Third, blocking CXCR4 signaling by an antagonist or antibodies of the receptor leads to the mobilization of neutrophils in mice and human [25, 26]. Fourth, an elevated CXCL12/CXCR4 signaling causes a defect of releasing neutrophils from the bone marrow in patients with warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome [27, 28]. WHIM syndrome is characterized by neutropenia (an abnormally low number of neutrophils in bloodstream) despite normal or higher than normal number of neutrophils in the bone marrow [27]. Genetic studies have discovered that WHIM-associated mutations of CXCR4 result in the truncation of C-terminus of CXCR4 protein, which has impaired internalization and increased sensitivity to CXCL12 [29]. Therefore, an altered CXCL12/CXCR4 signaling leads to abnormal neutrophil retention in the bone marrow of these patients.

CXCR2 signaling is another chemokine guiding system that acts antagonistically with CXCR4 signaling to promote neutrophil release from the bone marrow [21, 30]. CXCR2 ligands, CXCL1 and CXCL2, are mainly produced by endothelial cells [31]. In mice, neutrophils lacking CXCR2 are selectively retained in the bone marrow, producing a myelokathexis phenotype [21]. Neutrophil mobilization in response to CXCR4 inhibition depends on CXCR2 signaling [21]. Interestingly, CXCR4 signaling is the dominant guiding system over CXCR2 signaling. In the absence of CXCR4, neutrophils lacking CXCR2 receptor can be mobilized [21]. It appears that the mobilization of neutrophils from the bone marrow can be achieved by shifting the balance of CXCL12-CXCR4 signaling and CXCL1 (CXCL2)-CXCR2 signaling in bone marrow. Under stress condition, expression of inflammatory cytokines, mainly G-CSF (Granulocyte colony-stimulating factor), is increased. G-CSF signaling reduces expression of CXCL12 in the bone marrow and increases the levels of CXCL1 and CXCL2 in the endothelial cells [31]. G-CSF promotes neutrophil mobilization from the bone marrow by decreasing

the CXCL12 gradient, which retains neutrophils, and simultaneously increases gradients of CXCL1 and CXCL2 [21, 31], which direct them into circulation.

Neutrophils leave circulation and transmigrate into peripheral tissues when there is an infection or damage. The recruitment of neutrophils involves a multistep adhesion cascade [2]. It starts with the contact of free-flowing neutrophils to the activated vascular endothelium, followed by slow rolling of the cells on the vessel wall. Neutrophil capture and rolling are mediated mainly by selectins [2, 32, 33]. During neutrophil rolling, chemokine receptors on the neutrophil surface bind to their endothelial-bound chemokines and trigger the activation of leukocyte-expressed β2 integrins. Activated integrins interact with endothelium-expressed ligand ICAM-1, which leads to slow rolling and eventually to firm leukocyte arrest on the endothelium [34, 35]. After firm adhesion, neutrophils undergo extravasation into tissues and transmigration across the endothelial cells [2].

Recruitment of neutrophils to an inflammatory site is guided by multiple chemoattractants [1]. In a mouse model of inflammatory arthritis, it has been recently discovered that a cascade of diverse signaling molecules, including the lipid LTB4, the cytokine IL-1β and chemokines CXCL2 and CCL3 (ligands of CXCR2 and CCR1, respectively), work together to control neutrophil trafficking into the joint and the development of arthritis [9]. After transfer of serum from K/BxN transgenic mice into recipient mice, immune complexes deposit in joints and initiate responses. Synovial leukocytes start to produce LTB4, which attracts a small number of neutrophils that express the GPCR BLT1, into the joint [36, 37]. Once transmigrating across endothelial cells, neutrophils produce the cytokine IL-1β that stimulates cells in the joint to generate several chemokines to attract more neutrophils into the joint [38]. They first release CCR1 ligands, CCL3, for attracting more neutrophils from the bloodstream, and then generate CXCR2 ligands, such as CXCL2, to further amplify the recruitment of neutrophils into the inflamed joint [38]. This temporal cascade of chemoattractants controls the neutrophil recruitment to arthritic joints. Recently, a spatial cascade of chemoattractants has also been reported to recruit neutrophils into damaged tissues. Using intravital microscopy, neutrophil recruitment to sites of focal hepatic necrosis has been imaged in a mouse model [39]. Damaged tissues first generate signaling molecules (DAMPs and ATP) that stimulate macrophages in the tissues to release IL-1β. IL-1β induces expression of adhesion molecules, ICAM1, to promote neutrophil adherence on the endothelium surrounding the damaged site. Neutrophil migration toward the lesion is guided by two zones of chemoattractants at different distances from the border of injury (Fig. 1). At a site distant from the injury site, a gradient of CXCL2 forms in the vasculature, directing neutrophils toward the site. Within the area immediately surrounding the injury site (a distance of about 150 μm), the gradient of CXCL2 drops, and neutrophils are guided into the site by a gradient of mitochondria-derived formylated peptides, which are released by dying cells and detected by FPRL1 receptors expressed on the neutrophils [39]. It has become increasingly clear that multiple gradients of chemoattractants are produced at the right space and time *in vivo* for neutrophil recruitment to inflammatory sites [1].

Chemokine-directed cell migration during metastasis

Metastasis is the process through which cancerous cells leave an original site and migrate to other selective organs [10]. In cancer patients, secondary cancers are common in certain organs, such as lung, liver, lymph nodes and bone marrow, and are rarely seen in other organs, such as kidneys and pancreas. A seminal study provided the first evidence that chemokines and chemokine receptors are important for organ-specific metastasis [40]. The expression of chemokine receptors is not random, and tumor cells only express selected chemokine receptors. Chemokine receptor CXCR4 is highly expressed in human breast cells, and its ligand CXCL12 is highly expressed in selected organs, such as lungs, lymph nodes and bone marrow. In a mouse model, blocking the CXCL12-CXCR4 signaling system significantly inhibits metastasis of the breast cancer cell line (MDA-MB-231) to the lung [40]. *In vitro*, CXCL12 stimulates breast cancer cells to undergo chemotaxis and to penetrate

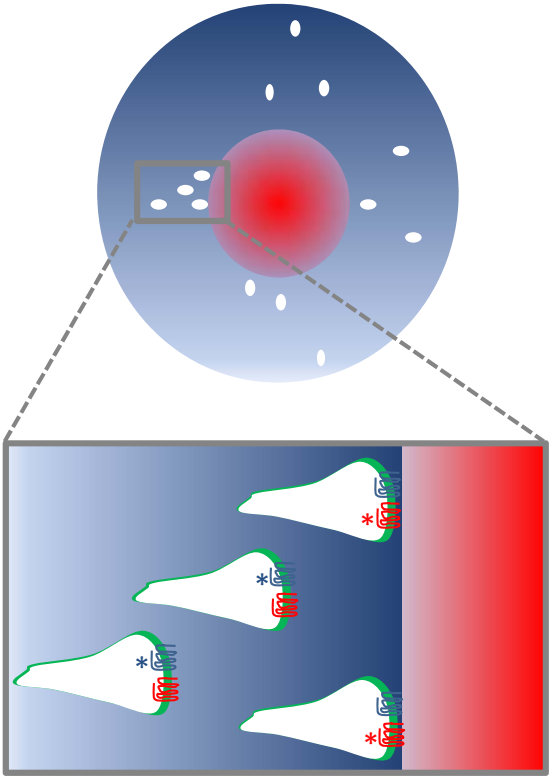

Fig. 1. Two spatial chemokine gradients guide neutrophils to migrate from tissues toward damaged sites. The formyl-peptide signals (red) are released from dead cells and form a chemoattractant gradient (red circle). Chemokine CXCL2 (blue) is produced in the tissue, which generates an intravascular chemokine gradient (blue zone) that guides chemotaxis of neutrophils with the vasculature toward the injury site (red zone). Red represents the chemoattractant of formyl-peptides, and blue represents the chemokine CXCL12.

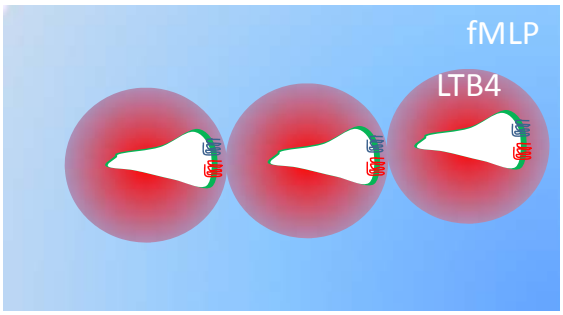

Fig. 2. Signal relay directs neutrophil chemotaxis. In an fMLP gradient (blue), three neutrophils respond and produce LTB4 (red) chemoattractant. Neutrophils respond to both fMLP and LTB4 and migrate toward the source of fMLP.

matrix for invasion. *In vivo*, metastasis of human breast cancer cells to lung is blocked by treating mice with an antibody that inhibits CXCL12-CXCR4 signaling [40].

The important role of CXCL12-CXCR4 signaling in organ-specific metastasis has been supported by studies in various cancers using two common approaches. First, using animal models, researchers have examined the effects of chemokine blocking antibodies or chemokine receptor antagonists on metastasis. They observed a dramatic inhibition of metastasis of a variety of cancer models by using CXCR4 antagonist or CXCL12-specific neutralizing antibodies [40]. Studies have shown that the CXCL12-CXCR4 signaling is required for metastasis to selected organs in many models of cancers, such as breast cancer [40], lung cancer [41, 42], colorectal cancer [43], gastric cancer [44, 45] and glioblastoma [46]. Second, using human patient samples, researchers have analyzed expression of chemokine receptors in tumor samples and correlated their expressions with tumor progression. Many of these studies have indicated that CXCR4 is commonly expressed in tumor cells and is responsible for metastasis to the lung, liver and bone marrow [10]. Interestingly, studies have indicated that alteration of the expression of chemokine receptors in tumor cells changed metastasis to targeted organs. For example, expression of CXCR4 by B16 melanoma cell line promotes metastasis of B16 cells to lung [47], while expression of CCR7 in B16 cells induces metastasis to the lymph nodes [48]. Furthermore, expression of CCR7 in a breast cancer cell line, which normally metastasizes to the lung, changes its metastasis route to lymph nodes instead [49].

Metastasis is a multistep process, which includes cancer cells escaping an original site, traveling throughout the body and reaching a secondary site [50, 51]. The first step of escape is the invasion of cancer cells through the basal membrane into a blood or lymphatic vessel, which is referred to as intravasation [11]. In a breast cancer model, intravasation has been investigated using multiphoton imaging in live mice [52]. A series of studies have demonstrated that migration and intravasation of breast cancer cells from solid tumors toward blood vessels require the reciprocal signaling between cancer cells and macrophages [11].

Breast cancer cells expressing EGF receptors (tyrosine kinase receptor) migrate toward EGF (epidermal growth factor) and produce CSF-1 (colony-stimulating factor), while macrophages express CSF-1 receptors (tyrosine kinase receptor), migrate to CSF-1 and release EGF. Two cell types migrate toward a blood vessel one after another. This relay-like collective chemotaxis results in a paracrine-dependent cancer cell streaming and transendothelial migration into the blood vessel [53]. Interestingly, the signal-relay mechanism of directional cell-cell communication has also been used for GPCR-mediated chemotaxis by social amoebae *Dictyostelium discoideum* cells and neutrophils [54, 55]. Chemokine signaling can promote relay of chemotaxis (Fig. 2). A recent study has found that secretion of CXCL12 by breast cancer cells can recruit macrophages to the primary tumor and enhance invasion of the cancer cells *in vivo* [56]. Another study has suggested that migration of cancer cells toward and into lymphatic vessel is guided by CCR7 signaling. The study has shown that tumor cells express functional CCR7 receptors, and tumor invasiveness toward lympatics is directed by a gradient of CCR7 ligands, CCL21/19 [57]. Together, these studies indicate that cancer cells, just like leukocytes and lymphocytes, use the guiding system of chemokine gradients to direct their journey.

Chemotaxis in the social amoeba *D. discoideum*

Many fundamental questions of eukaryotic chemotaxis have been raised and investigated using the lower eukaryotic organism, *D. discoideum* [17, 19, 20, 58]. In the 1970s, Gerisch applied video microscopy to record chemotaxis of *D. discoideum* cells toward a source of chemoattractant, cAMP, in real time. This groundbreaking experiment allowed us, for the first time, to monitor cell shape changes and cell migration in response to a controlled gradient of a chemoattractant [59]. In the 1980s, cAMP receptors in *D. discoideum* were identified as G-protein coupled receptors (GPCRs) [60]. A couple of years later, chemokine receptors, IL-8 (now known as CXCL8) receptor and N-formylpeptide receptor in human leukocytes, were also found to be members of the GPCR family [61, 62]. These findings established the paradigm that chemoattractants are detected by GPCRs in eukaryotic cells.

Eukaryotic chemotaxis is a cell behavior that conceptually consists of three inter-connected cellular processes, gradient sensing, cell polarization and cell motility [18]. Chemotaxing cells are morphologically polarized with a dominant leading front and a trailing end. At the front of the cell, actin-assembly drives pseudopod extension toward the source of a chemoattractant [63, 64]. At the sides and the trailing end, formations of lateral pseudopods are effectively suppressed to maintain one dominant pseudopod for efficient migration [65-67]. In the classic video microscopy experiment, it was found that a new pseudopod would form at the side of a cell when a strong cAMP stimulation was brought to the side, demonstrating that activation of GPCR can spatially regulate the formation of pseudopod in a chemotaxing cell [59]. To explain how a cell can directionally extend pseudopod in a gradient and form a new pseudopod in response to another stronger gradient, one early hypothesis was that the enrichment of GPCRs in a region determines the location of pseudopod formation [68]. However, cAMP receptors (cAR1) were found to be uniformly distributed in the membrane of chemotaxing *D. discoideum* cells [69], and chemokine receptors were subsequently found also to be localized around the cell surface [70]. Thus, the current model is that GPCRs detect concentrations of a chemoattractant around the cell surface and control a signaling network to translate differences of extracellular stimuli into highly directional intracellular responses that lead to chemotaxis [71].

The gradient sensing process of chemotaxis has two key features. First, cells can rapid terminate responses to uniformly applied chemoattractants, a process called “adaptation”. Second, cells are able to translate a shallow chemoattractant gradient into highly polarized intracellular responses, a process called “amplification” [18, 72]. These features allow the cells to respond to a wide range of concentrations of chemoattractants and migrate directionally in a gradient from a far distance. Advances in fluorescence microscopy have allowed us to visualize dynamics of GPCR and other signaling components in live *D. discoideum* cells since 1990s. One breakthrough was the discovery that the cAMP sensing machinery still operates in cells that lack the ability to polarize

and move. Specifically, upon treatment with latrunculin, an inhibitor of actin polymerization, cells lose their pre-existing polarity and motility. When these cells are stimulated with a uniform cAMP stimulation, a fluorescence probe of PHcrac-GFP, which binds to PIP_3, quickly translocates to the membrane and then returns to the cytosol, demonstrating the adaptation of PIP_3 response. When the cells are exposed to a cAMP gradient, PHcrac-GFP probes persistently accumulate in the membrane region facing the cAMP source, showing the amplification feature of gradient sensing [73-75]. Together, the cAR1/G-protein-controlled PIP_3 responses have key features of gradient sensing.

Over the last twenty years, many models have been proposed and modified to explain GPCR-mediated gradient sensing [72, 76-79]. In 1999, Parent and Devreotes first proposed the local excitation, global inhibition model [72], which was later explained mathematically and known as LEGI [80]. The model proposed a fast-acting local activator and a slow global inhibitor; both of them are activated in proportion to extracellular stimuli. By simply assuming that the response is determined by the difference in levels of activation and inhibition, the model can account for both adaptive responses to uniform stimuli and directional responses to gradients. However, the LEGI model does not sufficiently amplify the extracellular gradient into highly polarized responses, such as the cAR1/G-protein-controlled PIP_3 in *D. discoideum* cells [75, 81]. Therefore, additional mechanisms have been proposed in the LEGI-Biased Excitable Network (LEGI-BEN) hypothesis [17, 80]. The LEGI-BEN model can theoretically explain most of the behaviors of chemotaxing *D. discoideum* cells, including those observed in gradient sensing. Other models have also been proposed to explain chemotactic behaviors of eukaryotic cells [82-85]. These models, like LEGI-BEN, are abstract and have no clear connection to actual components in the GPCR-controlled signaling network. For example, the identities of activator and inhibitor in these models are still not clear. To construct a model that is closer to reality, Meier-Schellersheim *et al*. developed a computer-aided model of cAMP gradient sensing for *D. discoideum*, which includes known components as well as several assumed regulatory components [86]. The model is based on molecular interactions and provides specific molecular explanations for cAMP gradient sensing. To truly understand gradient sensing, it is necessary to identify regulatory components and to connect molecular mechanisms in a model with the complexity that reflects real cells.

Molecular components and signaling pathways involved in GPCR-mediated chemotaxis

Many components involved in chemotaxis have been identified in *D. discoideum* (Fig. 3). The binding of cAMP to cAR1 triggers dissociation of heterotrimeric G-proteins into $G\alpha 2$ and $G\beta\gamma$ subunits [87, 88], which activate downstream signaling components to control gradient sensing and to detect reorganization of actin cytoskeleton for chemotaxis. Free $G\beta\gamma$ activates the small G-protein Ras [89-91], which in turn activates PI3K to convert PIP_2 to PIP_3 [73]. PIP_3 recruits proteins with Pleckstrin Homology (PH) domains to the plasma membrane. The recruited proteins include CRAC (cytosolic regulator of adenylyl cyclase) and Akt/PKB [73, 92], which play roles in regulating actin cytoskeleton. Activation of cAR1 also induces transient membrane dissociation of the phosphatase PTEN, which dephosphorylates PIP_3 and converts it to PIP_2 [93, 94]. The cAR1/G-protein-controlled PIP_3 responses have been used as a system for in-depth studies of gradient sensing in cells lacking actin reorganization (i.e. latrunculin treated cells). How the gradient sensing machinery regulates the actin cytoskeleton required for directional cell migration is still not well understood.

Local membrane PIP_3 levels are believed to provide intracellular cues for actin polymerization. PIP_3 accumulates at the leading front where actin polymerization leads to cell migration. Cells lacking PTEN, which is critical in restricting PIP_3 to certain regions, do not have a dominant leading front, and instead, they extend actin-filled pseudopodia in all directions [93, 94]. Several PIP_3 binding proteins, including CRAC, Akt/PKB, PhdA, PhdB and PhdG have been identified in *D. discoideum* [95]. However, it is still not known how or whether these proteins function in PIP_3 signaling to actin polymerization. Recently, three myosin-I proteins, which are actin-based motors that function in membrane-actin cytoskeleton interaction, have

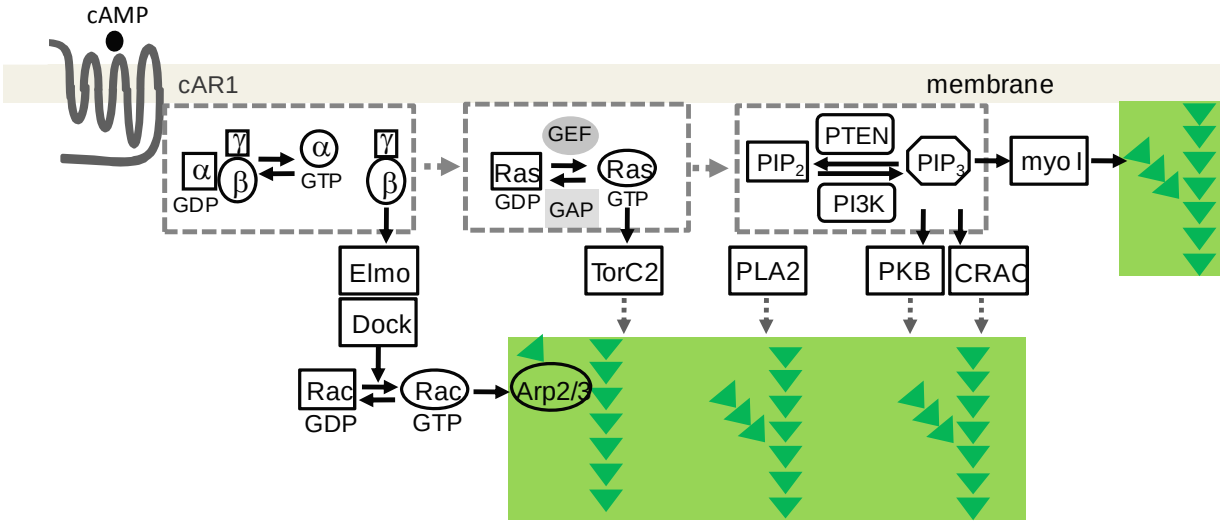

Fig. 3. Pathways involved in the GPCR-mediated migration of *D. discoideum* cells. Binding of cAMP to cAR1 receptor induces G-protein dissociation. Activation of heterotrimeric G-proteins triggers activation of Ras, leading to production of PIP_3. Free Gβγ associates with Elmo/dock complex to activate Rac, which signals to actin-cytoskeleton (green zone) via Arp2/3 complex. Myosin I binds to PIP_3 to regulate actin-cytoskeleton (green zone). Activation of cAR1 also regulates activities of TorC2, PLA2, PKB and CRAC, which contribute to the regulation of actin-cytoskeleton.

been found to bind PIP_3 in *D. discoideum*. The study suggests that the membrane recruitment of myosin-I proteins by PIP_3 promotes actin polymerization at the leading front of chemotaxing cells, which represents a molecular mechanism that links PIP_3 signaling to actin cytoskeleton [96].

Several PIP_3-independent pathways have also been implicated in chemotaxis of *D. discoideum*. A study shows that cells lacking PI3K proteins are still able to chemotaxis in steep cAMP gradients, indicating that PIP_3 signaling is not the only link between cAMP/G-proteins to the actin cytoskeleton. There appears to be several PIP_3-independent pathways involved in cAR1/G-proteins-controlled cell migration. One is the TorC2-PKB pathway, which includes Tor complex 2 and two PKB homologs (PKBA and PKBR1), and another is the PLA2 (phospholipase A2) pathway [89, 90, 97]. Furthermore, a soluble guanylyl cyclase (cGC) has been shown to influence chemotaxis [98]. However, it is not clear how these pathways are linked to the actin cytoskeleton, and thus, molecular components that link TorC2-PKB, PLA2 or cGC to the actin cytoskeleton need to be identified. A recent study reveals a new pathway, in which an ELMO/Dock complex functions to link the heterotrimeric G-protein Gβγ to Rac activation, leading to actin polymerization in chemotaxis of *D. discoideum* [99]. Interestingly, the pathway appears to be evolutionarily conserved, since a pathway consisting of CXCR4, Gαi2, ELMO1/Dock180, Rac proteins, regulates actin cytoskeleton for CXCL12-mediated chemotaxis and metastasis of breast cancer cells [100]. It will be interesting to learn the roles of PIP_3-independent pathways in GPCR-mediated chemotaxis of mammalian cells.

Chemokine GPCR-controlled signaling mechanisms for regulation of chemotaxis have also been extensively studied in neutrophils. Activation of chemokine GPCRs dissociates heterotrimeric Gi proteins into Gαi and Gβγ subunits, which in turn control several signaling pathways. In mouse neutrophils, chemoattractants, fMLP and CXCL2, stimulate PIP_3 production via activation of PI3Kγ [101, 102]. PIP_3 signaling localizes the activation of CDC42 (small G-protein) to direct actin polymerization at the leading front of a chemotaxing neutrophil. While PIP_3 and CDC42 localize formation of F-actin, they are not required for actin polymerization [103]. Chemoattractant-

mediated actin polymerization depends on the activation of small G-protein Rac. It appears that Gβγ and PIP_3 signaling regulate P-Rex1, a guanine nucleotide exchange factor (GEF), for Rac activation [104]. In addition, chemoattractant stimulation also regulates actin-depolymerizing factor (ADF)/cofillin, which plays a role in actin dynamics in cell migration. A recent study indicated that fMLP stimulation suppress GSK3 kinase activity through both PI3Kγ-AKT and PLCβ-PKC pathways. Both of the pathways regulate GSK3 phosphorylation and relieves GSK3-mediated inhibition of SSH2 (slingshot 2-a cofilin phosphatase), leading to cofillin dephosphorylation to control actin dynamics [105]. Chemotaxing neutrophils are polarized. GPCR-controlled pathways regulate pseudopod extension in the front and the suppression of errant pseudopods elsewhere. In neutrophils, it has been suggested that chemokine GPCRs couple to different heterotrimeric G proteins to generate "frontness" and "backness" signals. Heterotrimeric Gi proteins mediate "frontness" signaling to the actin cytoskeleton, while G12/13 produce "backness" signals to myosin II-mediated cell retraction [106]. A recent study suggested that chemoarractant-induced activation of TorC2 and PKC promotes the activation of AC9 (adenylyl cyclase 9) and production of cAMP at the front of a cell. cAMP molecules diffuse and enrich at the back of the cell, where cAMP signals through PKA and RhoA or MLCK, to regulate myosin II assembly [107]. Over the years, researchers have identified many components and pathways involved in chemotaxis. How to link these molecular mechanisms together into a signaling network is a major challenge.

CONCLUSION

GPCR-mediated chemotaxis of eukaryotic cells is highly dynamic and complex *in vivo*. Advances in fluorescent microscopy have allowed direct observation of chemotaxis behaviors of leukocytes, lymphocytes and cancer cells in live animals. While appreciating their migration to target regions in real time, we still know little about spatiotemporal dynamics of chemoattractants, the guiding system, *in vivo*. Furthermore, eukaryotic cells have devised sophisticated signaling networks to transduce signals from GPCR to actin network. While many components involved in GPCR-mediated chemotaxis have been discovered, and will continue to be discovered, the future goal for the field is to understand how these components work together mechanistically by constructing and testing models that have clear biological relevance.

ACKNOWLEDGEMENT

Xu and Jin are supported by NIAID/NIH intramural funds.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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