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Chemotaxis, chemokine receptors and human disease

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Abstract

Cell migration is involved in diverse physiological processes including embryogenesis, immunity, and diseases such as cancer and chronic inflammatory disease. The movement of many cell types is directed by extracellular gradients of diffusible chemicals. This phenomenon, referred to as "chemotaxis", was first described in 1888 by Leber who observed the movement of leukocytes toward sites of inflammation. We now know that a large family of small proteins, chemokines, serves as the extracellular signals and a family of G-protein-coupled receptors (GPCRs), chemokine receptors, detects gradients of chemokines and guides cell movement in vivo. Currently, we still know little about the molecular machineries that control chemokine gradient sensing and migration of immune cells. Fortunately, the molecular mechanisms that control these fundamental aspects of chemotaxis appear to be evolutionarily conserved, and studies in lower eukaryotic model systems allowed us to form concepts, uncover molecular components, develop new techniques, and test models of chemotaxis. These studies have helped our current understanding of this complicated cell behavior. In this review, we wish to mention landmark discoveries in the chemotaxis research field that shaped our current understanding of this fundamental cell behavior and lay out key questions that remain to be addressed in the future.

Keywords

chemotaxis; chemokine; GPCR; actin; inflammation

1. Introduction

Chemotaxis is the phenomenon in which the direction of a cell's locomotion is determined by an extracellular gradient of chemicals. The first recorded observations of chemotaxis were made in 1888 by Leber, who found that leukocytes converge from all directions along straight paths to sites of rabbit corneal irritation [1,2]. This observation gave rise to the hypothesis that diffusible substances generated at sites of injury establish gradients that guide leukocyte migration. However, the extracellular signals responsible for guiding leukocytes to sites of

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inflammation would not be identified for decades to come. In 1947, Bonner discovered that the eukaryotic soil amoeba, *Dictyostelium discoideum*, also migrates robustly toward the source of diffusible chemical signals [3]. Since these early discoveries, tremendous progress has been made in understanding the fundamental biological process of eukaryotic cell chemotaxis [4]. Today, it is well known that chemotaxis plays a critical role in many diverse physiological processes, including the recruitment of leukocytes to sites of infection, trafficking of lymphocytes throughout the human body, and patterning of neuronal cells in the developing nervous system. Because of the essential roles of chemotaxis in development and physiology, improperly guided cell movements can cause diverse pathological conditions, including tumor growth, cancer metastasis and inflammatory diseases, such as asthma, arthritis and atherosclerosis [5,6,7].

Many molecular components involved in chemotaxis of eukaryotic cells have been discovered. In mammals, the extracellular signals that guide leukocyte migration are mainly a set of short peptides, called chemokines (chemoattractant cytokines), which exert their effects by binding to members of the large family of G-protein-coupled receptors (GPCRs). Chemokine GPCRs signal through heterotrimeric G-proteins consisting of $G_{\alpha i}$ and $G_{\beta\gamma}$ subunits, which in turn regulate a diversity of signal transduction pathways involved in chemotaxis (Fig. 1A). In general, cell migration is driven by the dynamic assemblies of the actin cytoskeleton. In chemotaxis, chemokine gradients strongly bias this actin assembly to the cell's leading edge and, hence, the direction of cell movement. In this article, we will review the landmark discoveries that have advanced our understanding of chemokines and their receptors, look back at the evolution of major concepts about the mechanism of chemotaxis, and provide examples of human diseases that are caused by mutations in chemokine receptors and inappropriate cell migrations. Finally, we will discuss the main challenges confronting the field of eukaryotic cell chemotaxis.

2. Chemoattractants, chemokines and their receptors

The idea that gradients established by diffusion of chemical substances can attract eukaryotic cells first took root in the early 1900s as discussed above. One of the first chemoattractants for eukaryotic cells to be reported was 3',5'-cyclic adenosine monophosphate (cAMP) for *D. discoideum* cells. It was demonstrated that cells of *D. discoideum* chemotax robustly along experimentally established gradients of cAMP [8]. Twenty years later, the first leukocyte chemokine, interleukin (IL)-8, was identified [9,10]. This landmark discovery stimulated a search for additional chemokines, the number of which now exceeds fifty. Typical chemokines can be found in genome sequences with relative ease due to their small size (~70–90 amino acids) and conserved N-terminal, cysteine motifs. Based on the number and spacing of cysteines in these motifs, chemokines have been classified into C, CC, CXC and CX3C subfamilies [11]. Most chemokines are produced under pathological conditions by infiltrating leukocytes or cells endogenous to the affected tissue. In addition, some chemokines are involved in normal housekeeping functions, including the maturation of leukocytes in the bone marrow, the trafficking and homing of lymphocytes, and the regeneration of circulating leukocytes. Reflecting these varied physiological roles, most chemokines can be alternatively classified as either “inflammatory” or “homeostatic” [12]. Inflammatory chemokines are specialized for the recruitment of immune cells to the inflamed regions, while homeostatic chemokines are present in various microenvironments in lymphoid or non-lymphoid tissues and support trafficking and positioning of cells belonging to the adaptive immune system [12,13].

The molecular identities of chemoattractant receptors for eukaryotic cells began to be revealed in late 1980s. The first breakthrough was the discovery of a *D. discoideum* chemoattractant receptor for cAMP and the realization that it belongs to the family of seven-transmembrane

G-protein-coupled receptors (GPCRs) [14]. A few years later, the human N-formylpeptide receptor and the IL-8 receptor of human leukocytes were discovered and also found to be GPCRs [15,16]. These landmark findings revealed that chemokine receptors represent a new subfamily of GPCRs. Binding of chemoattractants to the receptors activates heterotrimeric G-proteins, which in turn activate a signaling network that we are just beginning to understand, leading to chemotaxis (Fig. 1A). Currently, 18 chemokine receptors mediate the effects of the more than 50 known chemokines [11]. One interesting notion is that chemokines and their receptors, unlike other types of GPCRs, have overlapping specificities for each other. In addition, chemokine receptors are expressed in different leukocyte subtypes. Many chemokine-receptor-mediated signals appear to be redundant while some chemokine receptors are essential for life. For example, humans who lack functional CCR5 receptors are typically healthy [17, 18], but CXCR4 knockout mice are embryonic lethal [19,20]. Given the fact that chemokine receptors are expressed in almost all cell types and have central roles in many biological processes, ranging from immunosurveillance to inflammatory responses, it is essential to understand their significance in human diseases.

3. Chemokine receptors and human disease

The field of chemokine receptors and HIV virology surprisingly intersected in 1996 when CXCR4 and CCR5 were identified as co-receptors that together with CD4 provide docking sites for HIV viruses on the surface of host cells [21,22]. It is currently believed that 90% HIV strains use CCR5 (macrophage tropic) for initial infections, and the infected viruses undergo mutations that allow them to use CXCR4 (T cell tropic) as the infection progresses to AIDS. Entry of HIV into host cells requires the formation of an entry complex including the viral envelop glycoprotein gp120, CD4 and either CCR5 or CXCR4 receptors [23]. Extraordinary progress has been made in solving structures of gp120 and CD4 that lead to current understanding of the formation of the entry complex. The gp120 protein interacts with CD4 and the N-terminus of CCR5 or CXCR4 and these sequential interactions induce structural changes that lead the exposure of gp41 virus proteins that presumably form pores for the entry of HIV into host cells [17,24]. It is not clear whether CCR5- or CXCR4-mediated signal transduction is also involved in the entry of HIV in addition of being the structural components of the HIV entry complex. Earlier reports indicated that signaling by either chemokine receptors is not essential for HIV infection. Blocking chemokine receptor signaling through G α i by pertussis toxin did not affect HIV infection [25]. However, in order for the HIV large viral nuclear capsid to get through the membrane into the cytoplasm of the host cells, the membrane-associated actin cytoskeletons need to be rearranged. It is possible that the receptor-mediated signaling events contribute to the dynamic changes of the actin cytoskeleton during HIV infection just as they do in chemotaxis. The exact roles of chemokine receptor signaling in HIV infection require further investigation.

Chemokines and their receptors normally guide leukocytes to sites of infection and inflammation. However, excessive recruitment of these cells results in tissue damage and chronic inflammatory diseases. For example, chronic obstruction pulmonary disease (COPD) is an inflammatory disease characterized by irreversible progressive airflow limitation. The primary causes of COPD are significant inflammation and tissue destruction throughout the lung. Smokers who develop COPD have a chronic bronchopulmonary inflammation, characterized by an elevated recruitment of macrophages and CD8 T lymphocytes in the airway wall and of neutrophils in the airway lumen [26]. It has been shown that T cells infiltrating the airways of smokers with COPD display an increased expression of the chemokine receptor CXCR3 and its ligand CXCL10, suggesting that CXCL10-CXCR3 may be involved in the elevated recruitment of T cells in the inflammatory process underlying COPD [27]. An immunohistological study of patients with severe exacerbations of COPD demonstrated that the increase of neutrophil recruitment is associated with up-regulation of the chemokine

CXCL5 and its receptor CXCR2, indicating the roles of CXCL5-CXCR2 for initiating and maintaining the increased neutrophilia in acute exacerbations of COPD [28]. Chemokines and their receptors are also involved in the pathogenesis of several neurological diseases, including multiple sclerosis (MS) [29]. MS, the major cause of neurological disability among young adults, is an inflammatory disease of the central nervous system (CNS) white matter characterized by focal myelin destruction, axonal pathology and progressive neurological dysfunction. In typical cases of MS, a prominent histological feature of the lesions that develop in brain and spinal cord is perivascular infiltration of T cells, B cells and monocyte/macrophages. In demyelinating brain lesions of MS patients, chemokines, MIP-1 α and IP-10, were found to be expressed, and at the same time, CCR5⁺, (MIP-1 α receptor) and CXCR3⁺ (IP-10 receptor) T cells, a selective subgroup, were increased in the progressive MS patients, suggesting roles for MIP-1 α , acting on CCR5, and IP-10, acting on CXCR3, in the activation and recruitment of T cells to the CNS in MS [30]. In addition, chemokines and their receptors have also been implicated in many other inflammatory diseases, including atherosclerosis [6], inflammatory bowel disease [31], and endocrine autoimmune diseases [32].

Cancer cell migration, or metastasis, displays similarities to leukocyte trafficking, and recent results have shown that chemokines and chemokine receptors also play significant roles in cancer metastasis [5]. Metastasis is the transmission of cancerous cells from an original site to other selective organs elsewhere in the body. For example, in breast-cancer patients, secondary tumors often are found in the lungs and bone marrow, but rarely in the kidneys. A seminal finding by Muller et al. [33] indicated that chemokines and their receptors are important for this bias in breast cancer metastasis. In this study, the authors found that chemokine receptors CXCR4 was highly expressed in human breast cancer cells, and the respective ligand SDF-1 α was highly expressed in many other organs, such as lymph nodes, bone marrow and lungs, where breast cancer metastases are often seen. They also showed in vitro that SDF-1 α stimulated breast cancer cells to undergo directional migration and to successfully penetrate extracellular matrix for invasion. Additionally, in a mouse model, they successfully blocked metastasis of human breast cancer cells to SDF-1 α -rich lung tissue by treating the animals with an antibody that neutralizes CXCR4 activity. The study clearly establishes an association between chemokines, their receptors and cancer metastasis, and also suggests that antagonists of chemokine receptors may be useful in treating cancer patients in the future.

Given the crucial roles of chemokines and their receptors in a wide array of physiological functions and their association with many pathological conditions, the receptors have become important targets for treatment of the various human diseases. However, to successfully develop therapeutic strategies for modulating chemokine action, we need to gain a detailed understanding of the molecular mechanisms of chemotaxis mediated by chemokines and their receptors.

4. Chemotaxis of leukocytes and the social amoebas *D. discoideum*

Since the early 1900s, it was known that leukocytes migrate from the blood across the wall of micro-vessels and accumulate in inflamed tissues [34]. The mobility of leukocytes closely resembles that of the amoeboid cells of *D. discoideum*. In the 1970s, microscopic methods were developed to directly observe the behaviors of leukocytes and *D. discoideum* cells placed in a chemoattractant gradient [35,36]. Using time-lapse video-microscopy, it was observed that these chemotaxing cells have three characteristics: cell polarity, cell motility and the ability to detect and respond to gradients of chemoattractants [37]. In response to uniform stimulation with chemoattractants, the cells become elongated and polarized, with clear leading and trailing ends (Fig. 1B), and their random motility increases, a behavior termed chemokinesis. When confronted with a chemoattractant gradient, the cells undergo chemotaxis, migrating toward higher concentrations of chemoattractants (Fig. 1B). There are two key features of

chemoattractant-induced responses. First, responses to sustained, uniformly applied chemoattractants are rapidly terminated, a process called “adaptation”. Second, cells have the ability to detect and translate shallow, extracellular chemoattractant gradients into highly polarized intracellular responses, a process called “amplification” [38,39]. These features allow the cells to detect and respond to a wide range of concentrations of chemoattractants and concentration differences across the cell's length as small as 2% [37]. Dynamic assembly of actin at the leading edge drives the cell forward (Fig. 1B). The central questions are (i) how a cell adapts to the increasing concentration of a chemoattractants when it moves up to the gradient and (ii) how a cell amplifies small differences of extracellular stimuli into highly directional intracellular responses.

5. A GPCR-regulated signaling network generates directional responses

Over the years, many fundamental questions have been raised and concepts have been developed in the studies of chemotaxis using the model system *D. discoideum*. For example, to explain how a cell can persistently migrate in a gradient, one early hypothesis was that enrichment of chemokine receptors at the leading edge of cells is required for directional migration [40]. However, chemoattractant receptors were shown to be uniformly distributed in the membrane of chemotaxing *D. discoideum* cells [41], and this was subsequently demonstrated to be the case for chemokine receptors of human neutrophils [42]. Further studies demonstrated that even when receptors and their linked G-proteins are uniformly distributed on the membrane, the cells are still able to interpret gradients of chemoattractants to generate polarized responses [43]. Therefore, other signaling mechanisms instead of the simple localization of the receptors must play a critical role. The current view is that chemotaxing eukaryotic cells utilize an evolutionarily conserved GPCR-mediated signaling network that serves as a molecular “compass”, allowing the cells to sense and respond to small concentration differences over their lengths and thereby generate highly polarized responses.

Chemotaxing cells are morphologically polarized with a leading pseudopod and a trailing end (Fig. 2A). Many proteins and lipids involved in chemotactic responses are asymmetrically distributed in chemotaxing *D. discoideum* cells and neutrophils [44]. As a consequence, the front of a polarized cell shows greater responsiveness to chemoattractant than do the sides and the trailing end. Chemotaxis is a complicated cell behavior that conceptually consists of three inter-connected cellular processes: cell polarity, cell motility and the ability to detect chemoattractant gradients. It was discovered that the gradient sensing machinery (or molecular compass) could be uncoupled from cell polarity and motility by treatment of *D. discoideum* cells with Latrunculin, an inhibitor of actin polymerization [43,45]. When chemotaxing cells were treated with Latrunculin, they lost their polarity and became immobile but retained their ability to produce polarized biochemical responses to chemoattractant gradients (Fig. 2A and 2B). This approach allowed us to simplify the cell behavior to that of gradient sensing and thus focus specifically on understanding the chemoattractant gradient sensing machinery.

Many of the core components that constitute the molecular “compass” have been elucidated in *D. discoideum*. Chemoattractant, cAMP, GPCR-regulated signaling network carries out chemoattractant gradient sensing (Fig. 2C). The binding of chemoattractant to GPCR induces the dissociation of heterotrimeric G-proteins into $G\alpha$ and $G\beta\gamma$ subunits. Free $G\beta\gamma$, activates the small G-protein Ras predominantly at the cell's leading edge. Ras in turn directly activates PI3K, promoting conversion of the membrane phospholipid PIP₂ into PIP₃ [4,46,47]. Once generated, PIP₃ mediates intracellular polarization by recruiting proteins with Pleckstrin Homology (PH) domains to the plasma membrane at the leading edge. Among these proteins are CRAC (cytosolic regulator of adenylyl cyclase) and Akt/PKB [42,45,48], which play roles in regulating actin polymerization. Activation of receptors also regulates the membrane association of phosphatase PTEN, which dephosphorylates PIP₃ to PIP₂ [49,50,51].

Remarkably, the molecular compass of gradient sensing is evolutionarily conserved in human neutrophils. Activation of several GPCRs for chemokines, such as formylated peptides (fMLP), C5a and IL-8, stimulates class IB PI3K (known as PI3K γ) through actions of G $\beta\gamma$ and Ras, resulting in the production of PIP₃ in the plasma membrane [52]. Free G $\beta\gamma$ subunits generated as a consequence of interaction between the chemokine receptor and G α i subunits lead to activation of Ras. Activated Ras subsequently binds to PI3K γ , yielding a complex that begins the rapid conversion PIP₂ to PIP₃ in the plasma membrane [53]. PIP₃ then recruits PH-domain containing proteins such as Akt/PKB as well as small GTPase Rac and Cdc42 to the leading edge of the membrane [54,55]. Activated Rac and Cdc42 interact with the WASP/SCAR complex leading to actin polarization [54]. Activation of chemokine receptors also regulates PTEN localization and activity by small GTPase RhoA and Cdc42 to modulate PIP₃ levels around the cell membrane [56]. However, the conserved network appears to predominantly act at the front of migrating neutrophils, and another pathway appears to be oppositional at the back. It has been shown that the fMLP receptor activates trimeric G proteins G12 and G13, instead of G α i, at the back. G12 and G13 recruit the RhoA to the membrane to activate Rho-dependent kinase (ROCK) and myosin II, resulting in formation of contractile actomyosin complexes [57,58].

In order to comprehend how these components work together as a system to allow a cell to adapt to a uniformly applied stimulus and to sustain highly polarized biochemical responses when facing a gradient, it is essential to determine the spatiotemporal dynamics of localization and activation of these components in live cells and in real time.

6. Spatiotemporal dynamics of gradient sensing revealed by live cell imaging

Advances in live cell fluorescence microscopy permit direct visualization and quantitation of many signaling events in live cells that undergo adaptation or amplification. The first breakthrough was the discovery that spatiotemporal changes of PIP₃ levels around the cell membrane can be directly visualized by monitoring a green-fluorescent-protein (GFP) fused to CRAC (cytosolic regulator of adenylyl cyclase) or PKB protein kinase B in live *D. discoideum* cells [45,48]. Following this breakthrough, researchers have developed and applied state-of-the-art live cell imaging techniques to systematically measure spatiotemporal changes of the signaling components at various points along the GPCR-PIP₃ pathway in live cells in real time. In well controlled experiments, the spatiotemporal changes of chemoattractant concentrations around the cell surface can be measured by fluorescent dyes [59,60]. The levels of heterotrimeric G-protein activation in various regions of the cell membrane have been monitored by fluorescence resonance energy transfer (FRET) methods [60,61]. In addition, spatiotemporal activation of a small G protein Ras has been observed by tracing the membrane translocation of a fluorescence probe, RBD-GFP (active Ras-Binding Domain fused to GFP) [47]. Furthermore, the dynamic translocation of GFP-tagged versions of PI3K and PTEN to the plasma membrane has also been measured [47,49,50]. For the first time these experiments have allowed us to directly observe various spatiotemporal changes within the signaling network in response to defined perturbations of chemoattractant stimuli and, as a result, have provided quantitative insights into how dynamic molecular interactions within a GPCR-mediated signaling network give rise to cell behaviors [60].

Over the last three decades, models have been constructed to mathematically describe chemotactic behaviors, such as adaptation and amplification [46,62]. Evolution of these conceptual and computational models has been closely linked with an increasing understanding of the molecular mechanisms involved in the signaling network [51]. The rapid developments in live cell imaging experiments and modeling have significantly increased our understanding of the underlying relationships among individual molecular mechanisms in a signaling network. Recently, a spatially resolved computational model of chemosensing has been

reported [63]. This model, unlike previous ones that lack the detailed molecular components, is based on interactions of the known components and takes into account the concentrations of each component, numbers of binding sites, diffusion constants, and available kinetic constants [63]. The advantage of the model is the ability to simulate dynamic behaviors of each component in the processes leading to adaptation and amplification. Furthermore, these computer simulations can generate quantitative predictions, which can be experimentally tested in real cells with continuously improving tools [51]. An ongoing interplay between model refinement and experimental verification should ultimately lead to a deeper understanding of how components of the molecular compass work together as a system to produce complex cell behaviors.

7. Chemotaxis of lymphocytes in vivo

Chemokine receptor-mediated chemotaxis has been long implicated in the homing of leukocytes during immune surveillance and in the immune response. Until recently, migratory behaviors of individual lymphocytes had not been observed and it was previously thought that B and T cells were not as motile as neutrophils. In 2002, the application of laser-scanning microscopy, especially two-photon microscopy, enabled visualization of the motility of fluorescently labeled lymphocytes in tissues and lymph nodes in vivo [64]. More recently, the migrations of B cells and T cells in an intact lymph node were imaged in real time using two-photon microscopy [65]. A gradient of chemokine CCL21 was detected within 100 μm of the follicle edge within the lymph nodes. Furthermore, migration of antigen-engaged B cells toward the edge of these follicles was observed and these movements depended on CCR7, the CCL21 receptor [66]. These findings represent the first observation of lymphocyte chemotaxis in vivo. In a separate in vivo dynamic imaging study, Castellino et al. [67] demonstrated the CCR5-mediated movement of T cells toward dendritic cells, a critical prerequisite for the cell-cell interactions involved in antigen presentation and T cell activation. In vivo dynamic imaging of immune cells in tissues has only been developed in last several years. In this short period, imaging approaches have revealed that lymphocytes migrate vigorously in vivo. In tissues, migrating B cells and T cells displayed amoeboid chemotaxis behaviors that are similar to those observed in neutrophils and *D. discoideum* cells. B and T lymphocytes migrate through different tissues compartments during their development and differentiation. Although much is known about the paths taken by these cells and the chemokines and receptors they use, we are still at an early stage in the process toward of understanding how this complicated cellular trafficking is regulated.

8. Actin cytoskeleton in chemotaxing cells

Spatiotemporal organization of the actin cytoskeleton is essential for cells to undergo polarization and migration. One important function of the actin cytoskeleton is to drive locomotion by the extension of pseudopods. The force required for pseudopod extension is generated by the regulated growth of a branched network of actin filaments that underlie plasma membrane at the cell's leading edge (Fig. 3). In last several decades, actin cytoskeleton-driven cell motility has been extensively studied in model systems, especially *Acanthamoeba*, fish epidermal keratocytes and *D. discoideum* amoebae [68]. These cells undergo rapid, constitutive motility and assemble a large actin-filament network at the leading edge, and each of them is ideal for biochemical analysis, light and electron microscopy and (or) genetic manipulations. From extensive studies, the dendritic-nucleation/array-treadmilling model was proposed to explain how growing actin filaments push membranes forward [69,70]. Activation of cell surface receptors generates active Rho-family GTPases and PIP_2 . These signaling molecules in turn activate WASp/Scar proteins, which together with the Arp2/3 complex catalyze the formation of actin filaments that branch from preexisting ones [71]. Rapid growth of the new actin branch pushes the membrane forward. The formation of actin network at the leading edge

is mediated by other proteins that include CARML and myosin I [72], which in concert coordinate extension of the actin network.

Cell migration requires not only formation of dominant leading front but also suppression of errant pseudopod extension at the sides and retraction of the trailing end. Studies in *D. discoideum* and fish epithelial keratocytes indicated that myosin II interacts with actin filaments to form cell cortex to enhance cell cortical rigidity at the sides and rear of the cell to prevent lateral pseudopod extension, permit retraction of the trailing end, and ensure cell polarity for effective cell migration [73,74]. It appears that activation of GPCR sends different signals in regulating actin network in the front and actin/myosin filaments elsewhere in the cell [39,75]. For example in neutrophils, a chemokine receptor, the fMLP receptor, activates G_i heterotrimeric G-proteins, leading to Rac activation and actin network assembly in the front, while the same receptor activates G_{12}/G_{13} heterotrimeric G-proteins, resulting in activation of Rho-dependent kinases and myosin II [58]. However, the pathways from chemoattractant GPCR to the actin network at the leading edge and actin/myosin II assemblies elsewhere in the cell have not been fully mapped.

9. Pathways leading to cell migration

How a GPCR chemosensing network (or molecular compass) regulates assembly of the actin cytoskeleton required for directional force generation is not clear yet. Evidence indicates that local membrane PIP_3 levels provide intracellular cues for formation of the actin cytoskeleton. It is clear that PIP_3 accumulates at the leading front where actin filament polymerization leads to cell migration. In addition, cells lacking PTEN, which hydrolyzes PIP_3 to PIP_2 , are unable to sharply restrict PIP_3 to certain regions and, fittingly, extend actin-filled pseudopodia in all directions [50]. However, a recent study showed that *D. discoideum* cells lacking five PI3K genes were still able to chemotaxis in steep gradients of cAMP, indicating that PIP_3 signaling is not an essential link between the molecular compass and actin cytoskeleton in chemotaxis [76]. Two reports suggested that, in addition to the PIP_3 pathway, a parallel phospholipase A2 (PLA_2) pathway also links the compass to actin-driven cell migration of *D. discoideum* cells [77,78]. When either the PIP_3 or PLA_2 pathway was inactivated, cells were still capable of chemotaxis, while inactivation of both pathways blocked chemotaxis. Furthermore, a more recent study suggested that a soluble guanylyl cyclase provides additional signals to ensure chemotaxis [79]. While the role of PIP_3 in gradient sensing and cell polarization has been demonstrated in human neutrophils and fibroblasts [80,81], the functions of PLA_2 and guanylyl cyclase in the chemotaxis of these cells remain to be established. In addition, members of a protein family, Elmo (engulfment and cell motility), have been shown to regulate actin cytoskeleton and formation of membrane protrusions during cell migration in *C. elegans*. Together with Dock180, Elmo serves as an evolutionarily conserved mechanism for Rac activation leading to cell migration or neuronal outgrowth in *C. elegans*, *Drosophila* and human lymphocytes [82,83,84,85]. How Elmo proteins function in highly motile neutrophils or *D. discoideum* cell is still not clear.

How do these signaling pathways regulate actin assembly and pseudopod extension in the cell's front, the suppression of errant pseudopods elsewhere, and the myosin II-mediated retraction of the rear? Recent findings suggest that various small GTPases, some of them are mentioned previously, are involved in regulating these events. The superfamily of small GTPases comprises more than 150 human members with evolutionarily conserved orthologs in all eukaryotes including *D. discoideum* [86]. The superfamily can be divided into the following five subfamilies on the basis of sequence and functional and similarities: Ras, Rho, Rab, Ran and Arf. Small GTPases functions as GDP/GTP-regulated molecular switches, cycling between active GTP-bound and inactive GDP-bound forms. Two classes of proteins control the cycle, namely guanine-nucleotide-exchange factors (GEFs), which induce the exchange of

GTP for GDP, and GTPase-activating proteins (GAPs), which promote the formation of the inactive GDP-bound forms [86]. Accumulating data demonstrate that many small GTPases plays essential roles in chemotaxis. Rho subfamily GTPases, including Rho, Rac and Cdc42, are key regulators of actin reorganization. In the front of neutrophils, fMLP receptor-activated Rac1 and Rac2 regulate the generation of free barbed ends of actin filaments, which drive actin filament elongation and membrane protrusion. Rac1 and Rac2 work through two different pathways. While Rac1 induces generation of free barbed ends through uncapping of the existing actin filaments, Rac2 acts on cofilin activation and on the Arp2/3 complex [87]. At the sides and back, RhoA stimulates assembly of actomyosin contractile complexes [57]. In *D. discoideum*, several Rac proteins, such as Rac1A, Rac1B, Rac1C and RacG, have been implicated in regulating actin polymerization during chemotaxis [88]. Recently, RasGEF Q, an exchange factor for RasB, was shown to regulate processes requiring myosin II [84]. It has been proposed that active RasB regulates myosin II activity that ensures proper retraction for cell migration. The reader is referred to an excellent review article [88] on the roles of small GTPases in chemoattractant sensing, cell polarization and cell motility. Further studies will be required to dissect the functions of different small GTPases in the parallel signaling pathways that control chemotaxis.

10. Challenges in the research of eukaryotic cell chemotaxis

Despite the enormous progress we have made toward understanding the mechanisms of chemotaxis, many mysteries still remain to be solved. One of the most important challenges in the field is to define the molecular mechanisms that link chemokine receptor signaling to the reorganization of the actin cytoskeleton that drives chemotaxis. Recent progress in the field has resulted from the combined application of biochemical, genetic, cell biology, state-of-the-art microscopy and computational approaches and has permitted generation of dynamic models for the molecular mechanisms of chemosensing and pseudopod protrusion [46,62,63,71]. While, we have yet to understand the linkages between chemoattractant sensing machinery and actin-dependent cell polarization and motility, we expect to gather additional clues through the continued study of the model systems, which have so far shaped our understanding of all fundamental aspects of eukaryotic cell chemotaxis. We are beginning to apply the knowledge gained from these model systems to understand the role of chemotaxis in the in vivo trafficking of immune cells, including lymphocytes and neutrophils, as well as cancer cells undergoing metastasis. However, a number of technical hurdles stand in the way of acquiring an in-depth understanding of immune cell chemotaxis in vivo. For example, it is apparent that spatiotemporal patterns of diffusible chemokines throughout the body direct these immune cells to different target sites. Therefore, quantitative information about the spatial and temporal distribution of chemokines will be essential for understanding how they guide cell movement. However, direct visualization of chemokine gradients is still in its early stages, largely due to the technical difficulties in detecting extracellular diffusible molecules. In addition to understanding the complex biology of chemokines and immune cells, it will also be important to develop a more complete understanding of the molecular mechanisms of chemotaxis inherent in these cells so that we may exploit this knowledge for the treatment of immune system disorders. In this regard, full advantage should be taken of the mechanistic insights that have been and will continue to be gained from the study of model systems such as *D. discoideum*. Basic research in these areas will provide the foundation of knowledge needed to rationally select the targets for medical interventions and open therapeutic opportunities for inflammatory diseases, autoimmune diseases and immune deficiencies.

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Figure 1.

(A) A model of a chemokine GPCR-mediated signaling network. (B) In a chemoattractant gradient, the polarized cells migrate toward higher chemoattractant concentration (indicated by the intensity of red color). The actin cytoskeleton, which drives extension of leading pseudopods, is indicated by green. F, front; B, back.

Figure 2.

(A) The gradient sensing machinery can be studied in the absence of cell polarity and motility. When treated with latrunculin, a chemotaxing cell becomes non-polar and non-mobile. (B) The latrunculin-treated cells generate a directional biochemical response by forming PH-GFP (green) crescents toward the cAMP gradient (red). (C) A model of the cAMP receptor-mediated gradient sensing machinery that controls PIP₃ levels on the cell membrane.

Figure 3.

Actin and myosin assemblies at the leading and trailing ends of a chemotaxing cell. At the front, chemokine receptor coupled with heterotrimeric G-protein controls an actin network that includes Arp2/3, WASp/Scars, CARMIL, myosin I and ADF/cofilin. At the back and sides, the receptor and G-proteins regulate actin/myosin II complexes.