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Review

Molecular basis of localized responses during chemotaxis in amoebae and leukocytes

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Abstract. Free-living amoebae as well as mammalian leukocytes sense chemoattractants with seven helix receptors linked to G-proteins. The cells respond by extending pseudopods and moving in the direction of the highest concentration. Recent studies using GFP-tagged proteins in *Dictyostelium* have shown that the directional response becomes sharply localized downstream of the receptors and G-proteins but upstream of the actin cytoskeleton. These studies together with the isolation novel genes by insertional mutagenesis in *Dictyostelium* are leading to a new understanding of chemotaxis in eucaryotic cells.

Key words. Chemotaxis; *Dictyostelium*; leukocytes; chemoattractant receptors; G-proteins; signal transduction.

Chemotaxis and phagocytosis in *Dictyostelium discoideum* and leukocytes

Chemotaxis and phagocytosis are fundamental responses shared by many eukaryotic cells. In chemotaxis, motile cells sense and respond directionally to chemical gradients; in phagocytosis, they bind and engulf foreign organisms and debris. Both processes are central to the inflammatory responses mounted by leukocytes and are displayed by cells of the primitive immune systems of sponges, nematodes and flies. Moreover, free-living amoebae display both chemotaxis and phagocytosis. One such amoeba, *Dictyostelium discoideum*, provides a powerful system for molecular genetic analysis of these processes (see fig. 1). In this review, we will describe the general features of chemotaxis shared by amoebae and leukocytes. We will not focus on motility per se, since this subject has been extensively reviewed [1–3] and, although motility and chemotaxis are related, these phenomena can be separated. We will outline the genetic and biochemical tools available in *D. discoideum* to study chemotaxis and update our current understanding. Finally, we will discuss the relationship between phagocytosis and chemotaxis.

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Similar chemotactic behavior in amoebae and leukocytes

D. discoideum amoebae and leukocytes sense a wide variety of chemoattractants. Adenosine 3′-5′ cyclic monophosphate (cAMP) is the major chemoattractant controlling cell movements during the developmental program of *D. discoideum* when cells aggregate to form a multicellular organism. However, cells are also responsive to components found in bacterial extracts such as folic acid, platelet-activating factor (PAF) and lysophosphatidic acid (LPA) [4, 5]. Leukocytes respond to PAF and LPA and, in addition, to N-formylated peptides (fMLPs), leukotrienes, complement factors and chemokines [6, 7]. All of these attractants in both amoebae and leukocytes interact with specific seven

Box 1. Adaptation and gradient sensing.

- Chemotactic cells sense the direction of the gradient regardless of its mean concentration. This requires a mechanism of adaptation to ambient levels of attractant.
- Mechanisms of desensitization have been described for rhodopsin and the β-adrenergic receptor. In these cases, agonist-induced receptor phosphorylation leads to arrestin binding and uncouples the receptor from its G-protein, terminating the response [9, 10].
- However, for chemoattractant receptors, adaptation to persistent chemoattractant stimulation does not require phosphorylation. Removing or substituting all of the serines in the C-terminal domain of cAR1 showed this [11]. Cells expressing such mutated receptors were able to carry out chemotaxis and to terminate responses appropriately. Similar data have been obtained for the CCR2B and FMLP receptors [12, 13].
- Therefore, the adaptation process required for gradient sensing likely occurs downstream of the receptor by modification of the activity of the G-proteins or effectors.

transmembrane domain surface receptors that transduce signals by coupling to heterotrimeric G-proteins (fig. 2). This suggests that despite their evolutionary distance, both amoebae and immune cells and perhaps all chemotactic, phagocytic cells sense chemoattractants by similar mechanisms.

Both *D. discoideum* and leukocytes display similar behavior as they orient chemotactically and appear to sense gradients by similar mechanisms. Both cell types move by regulating pseudopod extension and substrate adhesion. Neutrophils, the best-characterized leukocytes, display a rigid head-to-tail polarization and greater persistence, whereas amoebae are more irregular and switch fronts more often. In the absence of chemoattractant amoebae continue to move, albeit randomly, suggesting that motility can be separated from chemotaxis [8]. Static chemoattractant gradients elicit chemotactic responses, but cells can also sense moving gradients. During *D. discoideum* development, for example, moving gradients are formed by cell-to-cell relay of secreted cAMP. Waves of extracellular cAMP are propagated through the cell monolayer. As a wave approaches, the cell experiences a gradient that is increasing temporally. The back of the cell continually experiences the same cAMP concentration that was seen by the front a few seconds earlier. Yet only the front will form a pseudopod. It is likely that, as they traffic between the vascular and lymphatic systems, leukocytes must also encounter both static and moving gradients.

Upon stimulation with an abrupt uniform increase in chemoattractant, both cell types undergo a similar series of events. They immediately 'freeze' then round up or 'cringe'. After about 20 s, they begin to adopt a flat

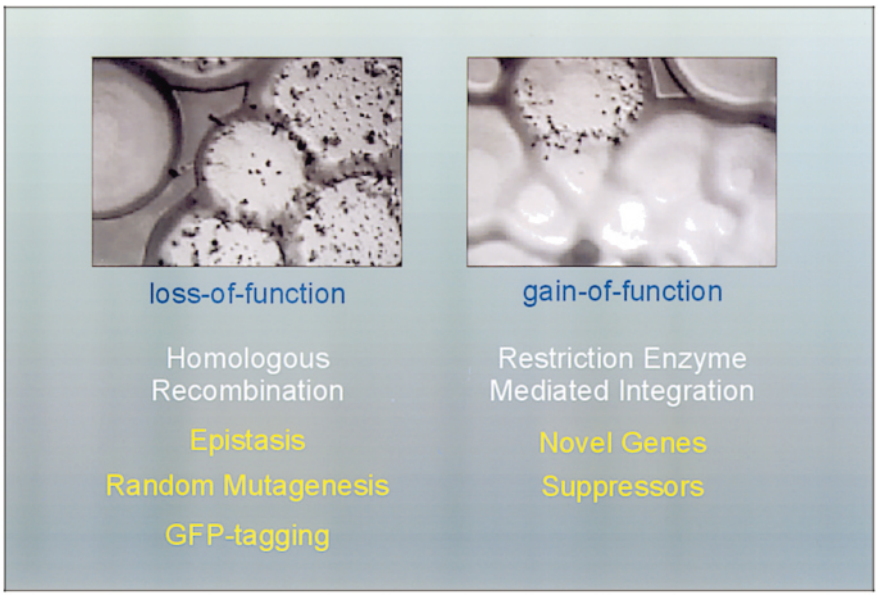


Figure 1. Features of *D. discoideum* that facilitate genetic analysis of chemotaxis and phagocytosis. Individual cells plated on bacterial lawns grow within a few days to form $\sim$1-cm plaques. Mutants with defects in phagocytosis can form plaques smaller than 2 mm (not shown). The genetically identical cells within the plaque aggregate and differentiate to form multicellular structures. If the original cell bears a genetic defect that impairs the developmental program, the plaque forms, but the cells remain as a monolayer. Rough versus smooth plaque phenotypes can thus easily distinguish mutant and wild-type cells (left). Gain-of-function mutations can be identified by reversion of the phenotype (right). Genetic tools include homologous recombination for gene deletion. Extrachromosomal vectors facilitate random mutagenesis and functional assessment of GFP-fusion proteins. REMI allows genome-wide random insertional mutagenesis for identification of novel genes associated with a given phenotype.

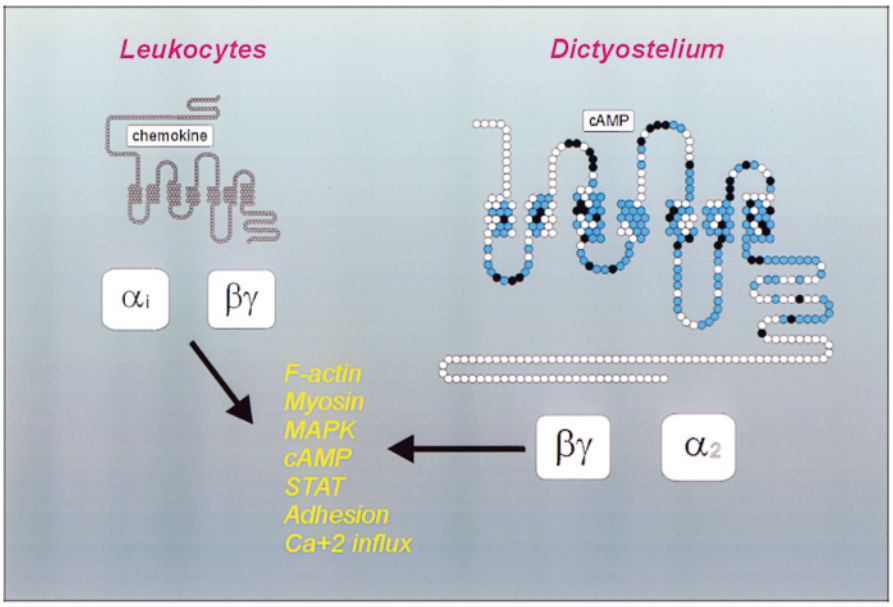


Figure 2. Evolutionarily conserved signal transduction events mediate chemotaxis in *D. discoideum* and leukocytes. In *D. discoideum*, a family of seven transmembrane domain cAMP receptors that couple to G2 senses extracellular cAMP. In leukocytes, a family of seven transmembrane domain receptors that couple to Gi senses chemotactic cytokines. In each cell type, increments in the concentration of chemoattractant elicit transient changes in actin polymerization, phosphorylation of myosins, cyclic nucleotides, activation kinases and transcription factors, ion fluxes and cell shape. Most of these responses subside within a few minutes of persistent stimulation, although some, such as phosphorylation of surface receptors, persist.

morphology by spreading on the substrate and extending pseudopods in multiple directions. Following several minutes of persistent stimulation, they resume random motility. Not only shape changes but also most chemoattractant-elicited responses adapt within seconds or minutes to continuous, steady chemoattractant concentrations (box 1). This adaptation to the ambient concentration of chemoattractant is relative: the series of shape changes and parallel biochemical changes can be repeatedly elicited by very small increments in stimulus.

Adaptation is thought to be critical to the process by which cells can sense shallow gradients regardless of the mean concentration. Cells stop responding after a few minutes of constant stimulation but can still respond to higher concentrations of chemoattractant. Such temporal increases in stimulus concentration might be achieved by the extension of pilot filopods or pseudopods. However, cells can also persistently sense stable gradients, but it is unclear by what mechanism this occurs. An attractive model is that activation signals are local, providing a stronger signal at the front than in the back. Adaptation signals, however, diffuse and redistribute in the cell, thereby providing a global indication of the average level of the stimulus. Subtraction of the average adaptation signal from the local level of excitation would result in specific excitation only at the front.

Another key question about chemotaxis concerns the point in the signal transduction pathway at which the chemotactic signal becomes localized to the front of the cell. Signal transduction components may become redistributed upon stimulation with chemoattractants. For example, receptors may cluster at the leading edge as they become occupied with chemoattractant. Alternatively, the signal may become localized further downstream in the pathway. In this review we will assess the role of different signal transduction components in mediating and localizing the cellular response.

Function and localization of chemoattractant receptors

Genetic analyses have demonstrated that chemoattractant receptors are essential for chemotaxis (box 2). *D. discoideum* amoebae express four cAMP receptors (cAR1–cAR4), which control chemotaxis as well as a variety of additional cellular responses [14, 15]. cAR1 and cAR3 are sequentially expressed during the early stages of development [15]. The two receptors functionally overlap, and cAR3 can substitute for cAR1 at

10-fold higher cAMP concentrations [16, 17]. *car1/car3* double *null* mutants are completely unresponsive to cAMP. The other two receptors, cAR2 and cAR4, are expressed in specific cells and are required for morphogenesis and gene expression later in development [18, 19]. The essential role of cAR1 in *D. discoideum* chemotaxis has been useful for functional analyses of this receptor [20]. Random mutagenesis of cAR1 and screening for loss of function mutants has led to the discovery of classes of point mutations that specifically alter cAMP binding, that prevent receptor activation without altering binding or permit receptor phosphorylation but do not couple to G-proteins (see box 3).

The study of chemokine receptors in leukocytes is complex because cells contain multiple different chemokine receptors, and each receptor can bind more than one chemokine. For example, the chemokine receptor CCR5 (CC-chemokine receptor 5) can bind RANTES (Regulated on Activation, Normal T Expressed and Secreted), as well as macrophage inflammatory proteins MIP1α and MIP1β [21]. Nevertheless, there are numerous examples where heterologous expression of a single chemokine receptor confers sensitivity to an appropriate set of chemokines. In vitro migration studies have shown that the neutrophils isolated from interleukin-8 (IL-8) receptor knockout mice are unresponsive to IL-8 but can still respond to other attractants [22]. Thus, multiple receptors can independently couple to a common signaling pathway for chemotaxis. It is believed that differential expression of chemokine receptors in a cell population has an important role in targeting subpopulations of leukocytes to their appropriate destinations.

Are chemotactic signals localized at the level of receptor occupancy? It has been observed in several laboratories that the surface receptors for chemokines are polarized on migrating leukocytes or that the presence of a chemoattractant gradient localizes receptors to the front of the cell [23–25]. In contrast, recent observations in living cells indicate that receptors can be uniformly distributed over the cell surface: a cAR1-GFP (green fluorescent protein) fusion protein was used to restore chemotactic responsiveness of the car1/car3 *null* mutants and the distribution of GFP-tagged receptors on the plasma membrane was observed during chemotaxis. Before and during chemotaxis, cAR1 was evenly distributed along the entire plasma membrane, including the smallest filopods and pseudopods. This uniform distribution persisted during repositioning of the pipette or increases or decreases in the gradient [26]. A similar result has recently been obtained for the C5a receptor, ectopically expressed in PLB985 cells [27]. Again, the GFP-tagged C5a receptor remained evenly distributed when these neutrophil-like cells were subjected to a variety of C5a gradients. These results demonstrate that the sensing of a gradient does not require an asymmetric localization or redistribution of the receptors. Therefore, localization of the signal, caused by differences in receptor occupancy between the front and the back of the cell, must occur at some point downstream in the transduction pathway. These differences in receptor occupancy can be quite small, since a cell can sense gradients that vary by less than 10% across the cell. Yet these differences are sufficient to generate highly polarized responses.

Function and localization of G-proteins in chemotaxis

Chemoattractant receptors couple to heterotrimeric G-proteins, consisting of α- and $\beta\gamma$-subunits. In *D. discoideum*, the genes encoding 11 different Gα subunits have been cloned [[28–32], H. Kuwayama and P. van Haastert, personal communication]. One Gα-subunit, Gα2, couples to cAR1 and mediates its role in chemotaxis. Gα2 *null* mutants do not aggregate and lack nearly all cAMP-induced responses (box 2). Chemotaxis to folic acid is mediated by another Gα subunit, Gα4. Mutants lacking Gα4 cannot sense folic acid, indicating that chemotaxis to folic acid and cAMP are mediated by different G-protein α-subunits [33]. Leukocyte chemotactic responses are sensitive to pertussis toxin, indicating that chemokine receptors are coupled to a Gα_i [34, 35], and a large number of observations support this view [6]. In addition, some of these receptors also couple to α-subunits from the Gq class [36]. Gα_i2, the major Gα_i in leukocytes, has recently been inactivated in mice [37]. Thus, it is now possible to establish the role of this Gα-subunit in leukocyte chemotaxis.

Are chemotactic responses localized at the level of the G-proteins? While the chemoattractant receptors remain evenly distributed when cells are in a gradient, it is possible that localized receptor activation leads to the redistribution of Gα or G$\beta\gamma$ subunits. The distribution of Gβ subunits has been analyzed by rescue of Gβ *null* cells with a Gβ-GFP fusion protein in *D. discoideum*. This tagged protein is evenly distributed along the plasma membrane before and after stimulation with chemoattractant and resembles the distribution of the chemoattractant receptor (T. Jin et al., unpublished results). Thus, cell polarization must be mediated by localized differences in activation as opposed to distribution of receptors or G-protein subunits.

Further evidence that G-proteins are essential for sig-

Box 2.

Gene	Protein	Phenotype of mutant	Role in chemotaxis
caR1/car3	Receptor	no development	Essential for chemotaxis to cAMP
cAR2/cAR4	Receptor	arrest in late development	Mediates chemotaxis at high cAMP
Gα2	G-protein	no development	Essential for chemotaxis to cAMP
Gα4	G-protein	wild-type, small plaques	Needed for chemotaxis to folic acid
Gβ	G-protein	no development, small plaque	Essential for chemotaxis to cAMP and folic acid
YakA	Protein kinase	no development, small plaque	Essential for chemotaxis to cAMP and folic acid
PLCδ	Phospholipase C	wild-type	None
ACA	Adenylyl cyclase	no cAMP production	No role in chemotaxis
Crac	Cytosolic regulator	no cAMP production	Minor role in chemotaxis to cAMP
PiaA	Cytosolic regulator	no cAMP production	Minor role in chemotaxis to cAMP
Erk2	MAP kinase	low cAMP production	Minor role in chemotaxis to cAMP
AleA	Ras Gef	low cAMP production	Minor role in chemotaxis to cAMP
Mek1	Map kinase kinase	small step to cAMP wave	Major role in chemotaxis to cAMP
TorA	Unknown	small step, small plaque	Major role in chemotaxis to cAMP
TsuA	Unknown	small step	Major role in chemotaxis to cAMP
Myosin II	Myosin heavy chain	defective in cytokinesis	Minor role in motility
MHCK	Protein kinase C	no aggregation	Essential for chemotaxis to cAMP
StmF	cGMP phosphodiesterase	large streams	Required for repolarization
Coronin	Actin-binding protein	wild-type	Required for normal psuedopodia and motility
Filopodin	Actin-binding protein	wild-type	Required for optimal substrate adhesion
Severin	Actin-binding protein	wild-type	Required for normal motility
Profilin	Actin-binding protein	wild-type	Required for normal pseudopodia and motility

naling and chemotaxis has been provided by analysis of the $\beta\gamma$ complex (box 2). Single Gβ and Gγ subunits have been cloned in *D. discoideum*, and the unique Gβ subunit is highly homologous to its mammalian counterparts. Its disruption leads to the inactivation of all G-protein-dependent signaling cascades and cells that are completely defective in chemotaxis. Not only are the cells unresponsive to cAMP, they also fail to carry out chemotaxis to folic acid and other chemoattractants. Interestingly, these cells are also very defective in phagocytosis. This suggests that G-proteins are involved in this process. Biochemical analyses of the Gβ *null* mutants show that they are deficient in most cAMP- and folic acid-induced responses, such as cGMP and cAMP accumulation and actin polymerization [38, 39]. Random mutagenesis of the Gβ-subunit has yielded alleles of Gβ that are defective in specific pathways. Such mutants are useful for dissecting pathways leading to chemotaxis from those that are not involved in chemotaxis, such as the activation of adenylyl cyclase. Other Gβ point mutants are defective in both chemotaxis and adenylyl cyclase activation but can still express developmentally regulated genes [40].

Many observations indicate that both in *D. discoideum* and mammalian cells, $\beta\gamma$ complexes released through activation by chemoattractants can directly regulate effectors, but it is not yet clear which of these events is essential for chemotaxis. Phospholipase Cβ2 (PLCβ_2) is activated after stimulation with C5a, fMLP or IL-8. This activation requires the pertussis toxin-sensitive release of G$\beta\gamma$ from the Gi class of Gα subunits, and PLCβ_2 is activated by $\beta\gamma$-subunits in vitro. PLCβ_2 is the major PLC isoform, and a mouse line lacking PLCβ_2 lacks 80% of the chemokine-stimulated PLC activity. Surprisingly, neutrophils of PLCβ_2-deficient mice show enhanced chemotaxis to a variety of chemokines [41]. This suggests that PLCβ_2 is a negative regulator in the signaling pathway leading to chemotaxis. In *D. discoideum*, PLC activity is transiently activated by cAMP, but this activation is also not required for chemotaxis, since a PLC *null* mutant shows normal chemotaxis to cAMP [42]. The role of G$\beta\gamma$ in the activation of adenylyl cyclase in *D. discoideum* has been studied in detail, and all of the evidence suggests that the enzyme is activated via a pathway that depends on the $\beta\gamma$ complex. In leukocytes, both fMLP and C5a cause a rise in intracellular cAMP levels via pertussis toxin-sensitive Gα subunits. This effect may be due to release of $\beta\gamma$ since, in vitro, the activation of the mammalian type II adenylyl cyclase by Gαs is enhanced by $\beta\gamma$ subunits [43–45]. In *D. discoideum*, some of the

Gβ-alleles that lack activation of adenylyl cyclase are able to perform chemotaxis, suggesting that production of cAMP is not essential for chemotaxis [46]. It is not clear whether the activation of adenylyl cyclase by chemokines has a role in leukocyte chemotaxis.

Localization of Crac

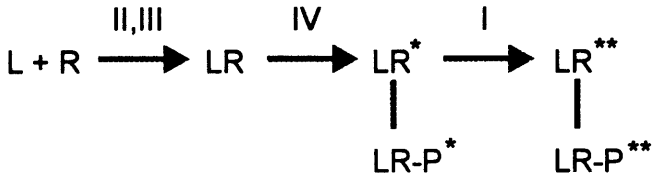
A novel signal transduction component was isolated in *D. discoideum* that becomes localized to the leading edge of the cell during chemotaxis (box 2). This protein, cytosolic regulator of adenylyl cyclase (Crac) is essential for the activation of adenylyl cyclase (ACA) by chemoattractants. [47, 48]. Crac contains an N-terminal pleckstrin homology (PH) domain [49]. PH domains may have a role in binding membrane lipids or free G$\beta\gamma$ subunits [50]. Indeed, Crac translocates from the cytosol to the membrane within seconds after chemoattractant stimulation. When the stimulus is applied uniformly, Crac translocates to the entire cell perimeter. When the stimulus is applied from one direction, Crac translocates to the side in the higher concentration (fig. 3) [51]. It is thought that the localized exposure of as yet unidentified Crac binding sites at the inner face of the membrane causes the translocation of this cytosolic protein. It will be interesting to find homologues of Crac or other PH domain-containing proteins that specifically localize to the front of a leukocyte during chemotaxis.

The nature of the Crac binding site on the inner face of the membrane is unknown. Possible candidates are the G$\beta\gamma$ subunits, which have been shown to bind PH domains or membrane-associated phosphoinositides. If the binding site were a phosphoinositide, it would imply that the receptor-G-protein complex would control the activity of enzymes involved in phospholipid remodeling. Phosphoinositides have been considered to play a major role in neutrophil chemotaxis, since a major response triggered by chemokines is the activation of phospholipase C. However, as noted above, PLC activation is not essential for chemotaxis. Activation of PI kinases can also alter the distribution of membrane phosphoinositides. Moreover, PI3Kγ can be directly activated by G$\beta\gamma$ subunits [52]. A speculative mechanism for orientation is that coordinated regulation of PI kinases, phospholipases and lipid phosphatases leads to the redistribution of phospholipids in the membrane. The binding of PH domains of Crac and other proteins at the cell's leading edge may reflect a high concentration of these lipids.

The cGMP/myosin heavy chain connection

Another response triggered by chemoattractants is the activation of guanylyl cyclase. In *D. discoideum*, guanylyl cyclase activation by cAMP is rapid and transient and requires G-proteins. Several nonchemotactic mutants have been isolated with specific defects in either total or activated guanylyl cyclase activity, suggesting that activation of this enzyme is required for chemotaxis [53]. In the cGMP-deficient mutants, chemoattractants still elicit other responses such as the activation of adenylyl cyclase and actin polymerization. A key role of guanosine 3′–5′ cyclic monophosphate (cGMP) in chemotaxis is also suggested from the behavior of the mutant streamer F (box 2). This mutant lacks cGMP phosphodiesterase activity, and cAMP induces a prolonged elevation of cGMP levels [54]. This leads to a persistent polarization of cells after the chemoattractant gradient is removed. The proposed role of cGMP in chemotaxis is the activation of myosin heavy chain

Box 3. Activation of G-protein coupled receptors by chemoattractant receptors.
The results of random mutagenesis of cAR1 suggest that binding of agonist causes a series of conformational changes in the receptor during the activation process. A model depicting these steps is illustrated below.



cAMP binding to cAR1 leads to an activated state of the receptor, LR*, which is able to interact with a receptor kinase. Additional conformational change(s) lead to the formation of LR**, enabling the receptor to interact with G-proteins. Mutants of class II limit access of cAMP, while class III mutants influence interactions of the agonist with the binding site. These mutations do not prevent the formation of active receptor intermediates, at saturating concentrations of cAMP. In contrast, the general activation mutants of class IV effectively bind cAMP, but show markedly reduced or absent responses. These defects are not overcome by high concentrations of cAMP, suggesting that the receptors are unable to undergo conformational changes required for the generation or stabilization of any active intermediates. In class I mutants, cAMP elicits essentially wild-type phosphorylation responses yet activates poorly G protein-dependent events. These data suggest that there may be a hierarchy among signaling functions; generation of the LR* intermediate is sufficient for receptor phosphorylation, while LR** is required for coupling to G-proteins.

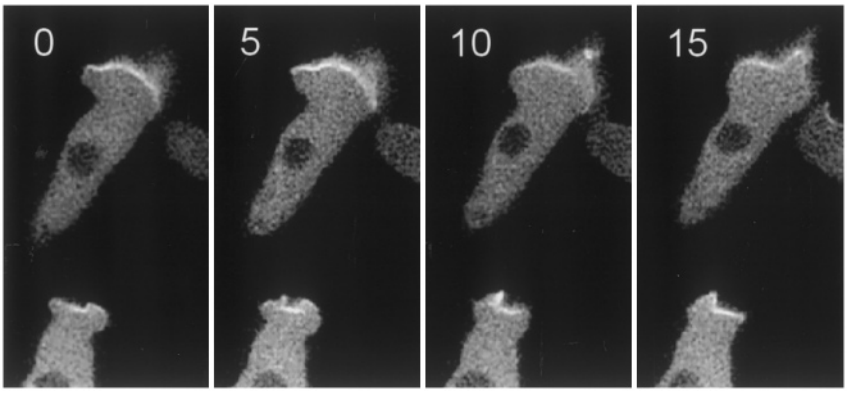


Figure 3. Crac-GFP translocates to the leading edge of the cell during chemotaxis. Confocal image of Crac-GFP expressing amoebae. The Crac-GFP fusion protein translocates to the leading edge of the cell while moving towards a micropipette containing 1 μM cAMP. Frames are taken every 5 s.

kinase (MHCK). When cells are exposed to chemoattractant, the heavy chain of myosin II and MHCK translocates to the cell cortex [55]. Phosphorylation of the heavy chain then causes its release and return to the cytosol. In MHCK *null* mutants, excessive heavy chain is associated with the cortex and, in MHCK overexpressors, the heavy chain is shifted to the cytosol [56]. Both situations lead to strong defects in chemotaxis. Surprisingly, cells lacking the heavy chain itself are still motile and able to perform chemotaxis, although they crawl more slowly than wild-type cells. This may be caused by the decreased ability of the mutant to retract its pseudopods from the substratum. Consistently, a myosin heavy chain GFP fusion protein is localized in retracting pseudopods [57].

Function and localization of F-actin and actin-binding proteins during chemotaxis

Continuous reorganization of the actin cytoskeleton is required for efficient chemotaxis. Actin is repeatedly polymerized and depolymerized in a moving cell. In unstimulated, randomly moving cells, F-actin is localized in newly extended pseudopods. The localization of new actin filaments to the front of the cell is essential for chemotaxis. It is generally thought that chemotactic stimulation creates new filament ends, and therefore regulation of actin-binding proteins that have a role in exposing filament ends may have an important role in chemotaxis. This event is generally thought to provide the force required for pseudopod extension. Within seconds after chemoattractant stimulation, F-actin localizes to the cortex of the extended pseudopods. Biochemical assays show that the F-actin concentration peaks twice after stimulation with chemoattractant. The first peak occurs within 5 s after stimulation and coincides with the 'freeze' response. Levels fall rapidly by 15–30 s and then rise to peak again after 1 min of stimulation, and corresponding to the extension of pseudopods. Both peaks in F-actin may be required for efficient chemotaxis.

Previous observations suggest that most regulators of actin assembly are involved in the basal organization of the cytoskeleton, and their disruptions affect motility rather than sensing of the gradient (box 2). Several actin-binding proteins are colocalized with F-actin to the cell's leading edge during chemotaxis. Actin-binding proteins probably have a function in the regulation of the amount of F-actin and the reorganization of filaments. One actin-binding protein, coronin, is localized to extended pseudopods in randomly moving cells and to the leading edge of cells moving in a gradient [58]. Chemotaxis in coronin *null* mutants is somewhat impaired, but this could be explained by the more general defect in basal motility [59]. Another actin-binding protein that becomes localized during chemotaxis is filopodin. Filopodin is a homologue of talin, a protein that triggers actin nucleation, leading to its assembly. Filopodin is localized to the tips of filopods, both those that touch the substratum and those that are free. Disruption of the filopodin gene strongly impairs the interaction of the cell with its substrate during movement [60]. Null mutants in other actin-binding proteins, such as the actin cross-linking protein severin show alterations in cell motility, but still show chemotaxis to cAMP [61]. The question arises by what mechanisms these proteins, such as F-actin, coronin and filopodin, become localized to the leading edge when cells are exposed to a gradient. As for the sites that bind the PH

domain of Crac, the chemotactic signal for actin polymerization may become localized upstream of these cytoskeletal components, and they may target to a predesignated site.

Isolation of chemotaxis and phagocytosis mutants in *D. discoideum*

Genetic analysis in *D. discoideum* has proved to be very useful in understanding signal transduction and cell motility and can potentially provide important insights to eukaryotic chemotaxis. Gene disruptions of known signal transduction components such as receptors or G-proteins do block chemotaxis but also impair many other responses. The components in signaling pathways leading to specific chemotactic responses, such as actin polymerization, remain largely unknown. The development of restriction enzyme-mediated integration (REMI) has opened the possibility to screen directly for appropriate mutants. Many of the initial signal transduction mutants isolated thus far are defective in the production of the cAMP and weakly defective in chemotaxis (see box 2) [62, 63].

The inability to identify more, specific chemotaxis mutants by REMI suggests that (i) the number of genes that are essential for chemotaxis is very limited, so the actual number of mutants is very low; (ii) there is redundancy between several proteins involved in chemotaxis, so individual mutations in chemotaxis genes may not result in obvious phenotypes; (iii) The isolation of chemotaxis mutants was based on screening for the 'wrong phenotype'. In our view, the last problem has affected previous searches for chemotaxis mutants. As noted above Gβ *null* mutants, which are defective in chemotactic responses to all chemoattractants, show an additional defect: the rate of uptake of bacteria is severely impaired, suggesting that chemotaxis and phagocytosis are linked. Since selection for mutants is typically performed by seeding individual clones on bacterial lawns (fig. 1), mutants with defects in phagocytosis that grow slowly under these conditions have been overlooked. To circumvent this problem, we devised a strategy to select mutants with defects in both processes.

We generated 20,000–30,000 REMI transformants and isolated colonies that showed no or weak aggregation or grew slowly on bacterial lawns. Ten of these mutants appeared to have no or an aberrant cAMP-induced actin polymerization response. In one of these mutants the disrupted gene was YakA, a homologue of the yeast Yak1 protein. In yeast, Yak1 was obtained as a suppressor of the lethal phenotype of a PKA_{cat} *null* mutant [64]. Overexpression of Yak1 in yeast causes a growth arrest. Kuspa and co-workers have shown that overexpression of YakA causes a growth arrest in both *D. discoideum* and yeast, demonstrating that the proteins are functional homologues [65]. Mammalian homologues of Yak1 and YakA are members of the minibrain (Mnb) or Dyrk protein kinases that were believed to have a role in brain development. A *Drosophila* Mnb mutant has severe defects in brain development, and human Mnb maps to the Down syndrome critical region on chromosome 21 [66, 67]. The recently isolated Mnb homologues Dyrk2 and 3 are expressed in tissues other than brain, indicating that members of the Yak/Mnb family have a more ubiquitous expression pattern and suggesting more diverse roles for these proteins [68]. With regard to the role of YakA in signal transduction, the phenotypes of the YakA *null* mutant strongly resemble that of the Gβ *null* mutant (box 2). Both mutants fail to enter development and have slow rates of plaque expansion on bacterial lawns. This observation suggests that YakA and G$\beta\gamma$ operate in the same signal transduction pathway. Preliminary experiments suggest that YakA mutants lack G-protein-mediated responses.

Another class of mutants are impaired in chemotaxis but can still aggregate to form small structures (box 2). These mutants have in common that they produce normal cAMP waves representing cell-to-cell relay of cAMP signals, but they are defective in chemotaxis towards the gradient presented by the cAMP wave. One of these mutants lacks a functional Map kinase kinase (DdMek1) gene [69]. The mutant does not properly accumulate cGMP after chemoattractant stimulation. This biochemical lesion may be the reason for its chemotaxis defect. Two other genes that were isolated by REMI, TorA (tortoise) and TsuA (tsunami), also have a strong influence on chemotaxis (S. van Es, M. Y. Chen and P. N. Devreotes, unpublished results). TsuA *null* cells initially do not move towards the cAMP waves, but acquire a weak chemotactic response after longer periods of pulsing with cAMP. Cells of TorA have a similar defect; they do respond to cAMP waves but take much smaller chemotactic steps than wild-type cells. This chemotaxis defect, and perhaps the similar defects in MEK and TsuA, are not due to the inability to sense a gradient, since the GFP-tagged Crac protein localizes to the leading edge when TorA *null* cells are in a gradient (S. van Es and P. N. Devreotes, unpublished results). As in Gβ *null* and YakA *null*, TorA *null* cells show a slow rate of plaque expansion on bacterial lawns, suggesting a role for the gene in phagocytosis. The complementary DNA (cDNA) sequences of TsuA and TorA do not show homology to known genes, suggesting that these are novel genes with specific functions in chemotaxis.

Summary and future directions

In chemotaxis and phagocytosis external stimuli trigger localized activation of cellular signal transduction systems. *D. discoideum* and leukocytes respond to chemoattractant stimulation via a G-protein-linked signal transduction pathway. Increments in attractant concentration elicit changes in actin polymerization, myosin phosphorylation, adenylyl and guanylyl cyclase and PLC activity, ion fluxes, adhesion and gene expression. All of these responses subside within a few minutes as cells adapt to the ambient concentration. When cells are placed in gradients of chemoattractant, the surface receptors and G-protein subunits do not redistribute. Instead, the signal transduction pathway appears to be locally activated at the leading edge of chemotaxing cells. This is readily visualized by the redistribution of a PH domain containing protein, Crac, as well as numerous cytoskeletal components, to the anterior of the cell. The genetic tools that allow us to visualize the localization of signal transduction and cytoskeletal proteins in living cells and the isolation of novel genes from mutants with defects in chemotaxis will contribute to the understanding of the mechanisms of chemotaxis.

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