The molecular genetics of chemotaxis: sensing and responding to chemoattractant gradients

Richard A. Firtel* and Chang Y. Chung

Summary

Chemotaxis plays a central role in various biological processes, such as the movement of neutrophils and macrophage during wound healing and in the aggregation of *Dictyostelium* cells. During the past few years, new understanding of the mechanisms controlling chemotaxis has been obtained through molecular genetic and biochemical studies of *Dictyostelium* and other experimental systems. This review outlines our present understanding of the signaling pathways that allow a cell to sense and respond to a chemoattractant gradient. In response to chemoattractants, cells either become polarized in the direction of the chemoattractant source, which results in the formation of a leading edge, or they reorient their polarity in the direction of the chemoattractant gradient and move with a stronger persistence up the gradient. Models are presented here to explain such directional responses. They include a localized activation of pathways at the leading edge and an ''inhibition'' of these pathways along the lateral edges of the cell. One of the primary pathways that may be responsible for such localized responses is the activation of phosphatidyl inositol-3 kinase (PI3K). Evidence suggests that a localized formation of binding sites for PH (pleckstrin homology) domain-containing proteins produced by PI3K leads to the formation of ''activation domains'' at the leading edge, producing a localized response. *BioEssays* 22:603–615, 2000.

Introduction

Chemotaxis, cell movement up a chemical gradient, is vital to many biological processes in eukaryotic cells, including migration of macrophage and neutrophils during wound healing, homing of thymocytes, migration of neural crest cells, and the aggregation of *Dictyostelium* cells to form a multicellular organism.[1–6] All of these responses require the ability of the cell to sense and respond to slight differences in the concentration of a chemoattractant, normally a small molecule, between the front or leading edge and the posterior part of the cell body. Chemoattractants serve as ligands for a variety of cell-surface receptors. Ligand binding, through the activation of signal transduction pathways, leads to the rearrangement of the actin and actino-myosin cytoskeletons. Actin polymerization at the leading edge results in the protrusion of pseudopodia or lamellipodia, whereas myosin contraction at the back of the cell causes retraction of the posterior of the cell (Fig. 1). This two-step process results in the directed migration of the cell up the chemoattractant gradient. Chemoattractants stimulate the rate and regulate the direction of the basic cellular machinery that controls cell motility.

In this essay, we review signaling pathways regulating chemotaxis, and describe models of mechanisms by which cells sense small concentration differences in a chemoattractant that lead to a change in the direction and speed of cell movement. The review focuses on insights derived from analyses of vertebrate cells and the social amoebae *Dictyostelium discoideum* which, in the last few years, have provided new understanding of the regulation and complexities of these pathways.

Section of Cell and Developmental Biology, Division of Biology, Center for Molecular Genetics, University of California, San Diego, La Jolla, California.
Funding agencies: Research grants from the USPHS to RAF.
*Correspondence to: Dr Richard A. Firtel, Center for Molecular Genetics, Room 225, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0634. E-mail: rafirtel@ucsd.edu

Chemoattractants cause a change in the direction of movement of motile cells

In contrast to fibroblasts and other predominantly non-motile cells, amoeboid motile cells such as neutrophils, macrophage, *Dictyostelium*, and *Acanthamoeba* are highly migratory. They lack stress fibers but have an extremely dynamic actin cytoskeleton.[2] During amoeboid movement in the absence of external cues, cells appear to maintain an inherent polarity, the basis of which is not fully understood (as discussed by Verkhovsky et al.),[7] but they do not move persistently in any one direction. They move for a short time in one direction and then in another, such changes in direction appearing to occur randomly through the protrusion of pseudopodia in multiple directions and further extension of one of these. When exposed to chemoattractant gradients,

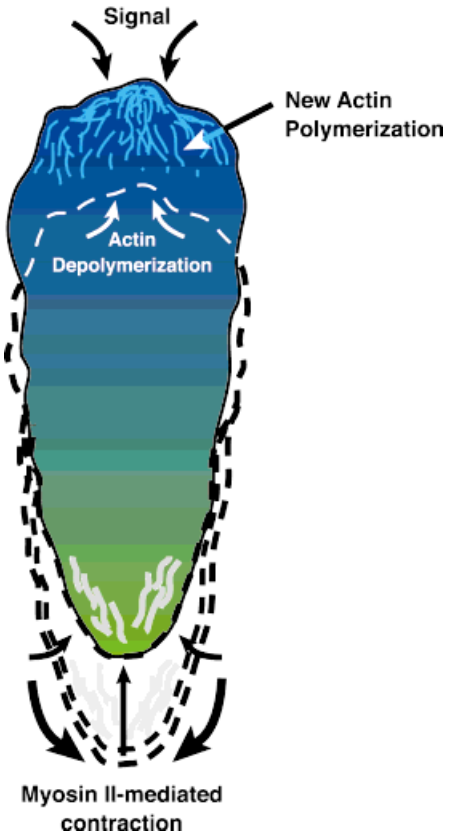

Figure 1. The cartoon shows a polarized, chemotaxing cell with an actin-enriched leading edge and a posterior containing a cytoskeleton enriched in assembled myosin II. A cell moves forward in a two-step process in response to a chemoattractant signal. New F-actin is polymerized in the front or leading edge, resulting in a forward-moving force to produce a pseudopod (lamellipod). The unpolymerized G-actin presumably comes, in part, from the depolymerization of F-actin at the old leading edge. A retraction of the posterior follows, which results in the contraction of the myosin II containing cytoskeleton, causing a lifting of the posterior of the cell from the substratum and a moving forward of the posterior of the cell. See text for references and further discussion.

however, their movement becomes highly biased in the direction of the chemoattractant source. Cells that had been moving at random rapidly change direction towards the chemoattractant source and increase their speed. Once cell polarity is stabilized in the direction of the gradient, extension of pseudopodia in the direction of chemoattractant is heavily favored over extension in other directions. A change in the direction of the chemoattractant, however, can result in the rapid formation of a new leading edge and a change in the direction of cell movement toward the new chemoattractant source, as illustrated in Figure 2. As discussed below, such changes in cell movement are thought to result from a localized activation of receptor-mediated signaling pathways at the edge of the cell that faces the chemoattractant source. In contrast to responses to a directional signal, however, flooding cells with a level of chemoattractant that is sufficient to saturate the chemoattractant receptors causes cells to arrest movement and often to round up. This probably results from a non-directional activation of chemotactic responses over the entire cell surface, leading to a uniform accumulation of F-actin around the cell.

Signaling pathways controlling chemotaxis

Our understanding of the integrated pathways that regulate chemotaxis has been advanced considerably through genetic and molecular genetic analyses in *Dictyostelium*. Chemotaxis pathways are better understood in *Dictyostelium* than they are in mammalian cells and will be the focus of the present discussion. In *Dictyostelium*, chemotaxis plays key roles during the life cycle of the organism. The initial step in *Dictyostelium* multicellular development is the chemoattractant-mediated aggregation of up to 10^5 cells to form a multicellular organism by chemotaxis to cAMP.[6,8] Aggregation requires the coordination of several signaling pathways, including those required for the synthesis of cAMP (activation of adenylyl cyclase) and relay of the extracellular cAMP signals, chemotaxis, and aggregation-stage gene expression. The requirement of chemotaxis for multicellular development in *Dictyostelium* provides a phenotype to screen for mutations affecting chemotaxis and a biological process in which to study the function of these identified genes. Strains defective in chemotaxis or the activation of adenylyl cyclase either do not aggregate or aggregate very poorly. They are very easy to identify in mutant screens. Secondary screens and analyses enable one to determine not only which pathways are defective but the cellular and molecular bases of the various defects. From such approaches, many signaling components that are important in the regulation of chemotaxis have been identified in *Dictyostelium*. Figure 3 shows a schematic diagram of the signaling pathways known to be involved in the regulation of chemotaxis and which are discussed in this review.

Chemotaxis in leukocytes and *Dictyostelium* is regulated by ligands that interact with serpentine receptors.[6,8–10] In leukocytes, chemokine receptors are coupled to heterotrimeric G proteins that contain the Gαi subunit and which mediate chemotaxis through the release of Gβγ.[11] In *Dictyostelium*, cAMP mediates chemotaxis through G protein-coupled/serpentine cAMP receptors (cARs) which are coupled to the heterotrimeric G protein containing the Gα2 subunit. In contrast, folate mediates chemotaxis through a distinct receptor and the G protein containing the Gα4 subunit.[6,8,12] There are four developmentally regulated cAMP receptors in *Dictyostelium*, each with a specific

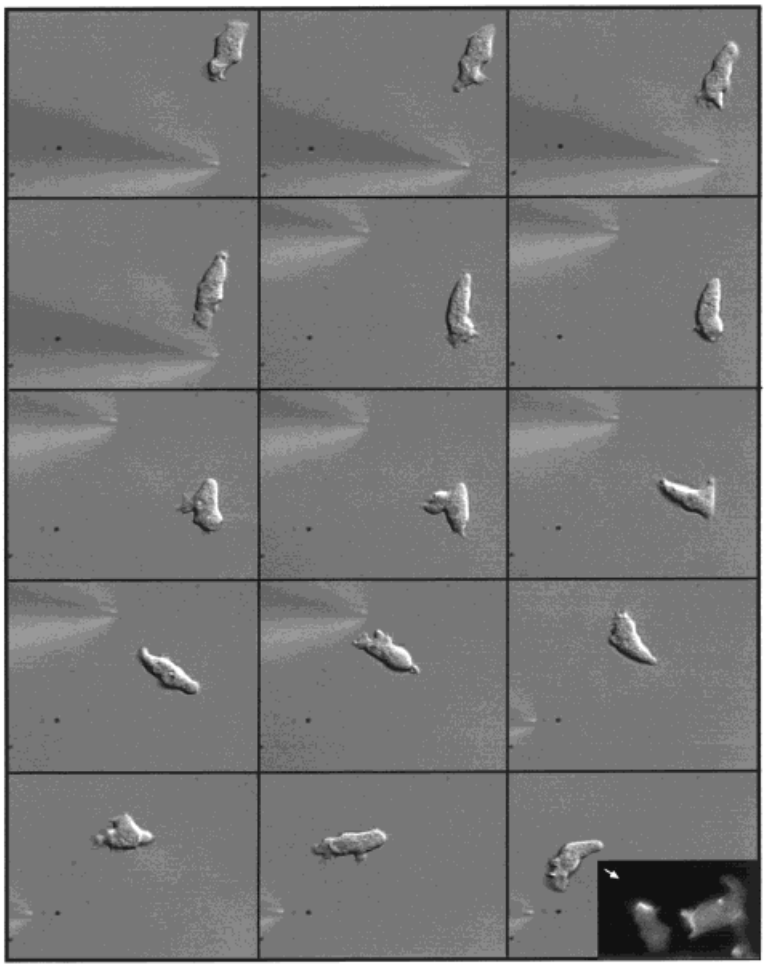

Figure 2. Chemotaxis of a *Dictyostelium* cell to the chemoattractant cAMP. The figure shows the movement of a *Dictyostelium* cell (aggregation-stage) toward a micropipette emitting the chemoattractant cAMP. The cell extends a pseudopod in the direction of the micropipette (chemoattractant source). When the position of the micropipette changes, the cell first retracts its present pseudopod and then puts out a new pseudopod in the new direction of the micropipette and produces a new leading edge. Several changes in the position of the micropipette are shown. Arrows point to the extended pseudopod. A star identifies the opening of the micropipette. The frames are from a time-lapse video with images taken approximately every 15 sec using a Nikon DIC microscope. The inset in the last frame shows the localization of a GFP fusion with the PH domain of *Dictyostelium* Akt/PKB to the leading edge of chemotaxing cells. The direction of the micropipette is shown by the arrowhead. Results from Meili et al.[24]

in vivo function in controlling aggregation and cell-type differentiation; cAR1 is the major receptor during aggregation.[6,8] Ligand binding activates a series of signaling pathways that control different aspects of the chemotaxis response.

A central pathway required for chemotaxis in *Dictyostelium* involves the activation of guanylyl cyclase and the production of the second messenger cGMP.[13] Guanylyl cyclase is rapidly and transiently activated in response to chemoattractants via a cAR1/G protein-dependent pathway, with cGMP levels peaking at 10 sec after the onset of stimulation. cGMP functions, in part, through cGMP-dependent protein kinase, which independently regulates myosin II heavy and light chain kinases.[14] In addition to regulating the function of myosins, cGMP is probably required for aspects of the regulation of the actin cytoskeleton. Mutations that severely affect guanylyl cyclase activity are unable to chemotax and lack chemoattractant-mediated changes in the actin cytoskeleton.[13,15] Since myosin II null cells can chemotax,[16] although they do so poorly compared to wild-type cells, cGMP must have another function in addition to the regulation of myosin II. Null mutants of the MAP kinase kinase (MEK1) are unable to chemotax[17] and exhibit defects in the rearrangement of the actin cytoskeleton in response to cAMP (H. Ma and RAF, unpub. obser.). These cells are defective in chemoattractant-mediated guanylyl cyclase activation. The genetic results, although indirect, suggest that cGMP is a second messenger required for changes in the actin cytoskeleton.

Role of phosphatidyl inositol-3 kinase and downstream effectors in chemotaxis

There is growing evidence that a key step in mediating responses to chemoattractants is the activation of PI3 kinase (PI3K) and the downstream effector Akt/PKB, which is a homologue of mammalian Akt/PKB. Both proteins are important regulators of cell polarization and motility in *Dictyostelium*. *Dictyostelium* contains three PI3 kinases (PI3Ks) related to mammalian type I PI3Ks.[18] Two (PI3K1

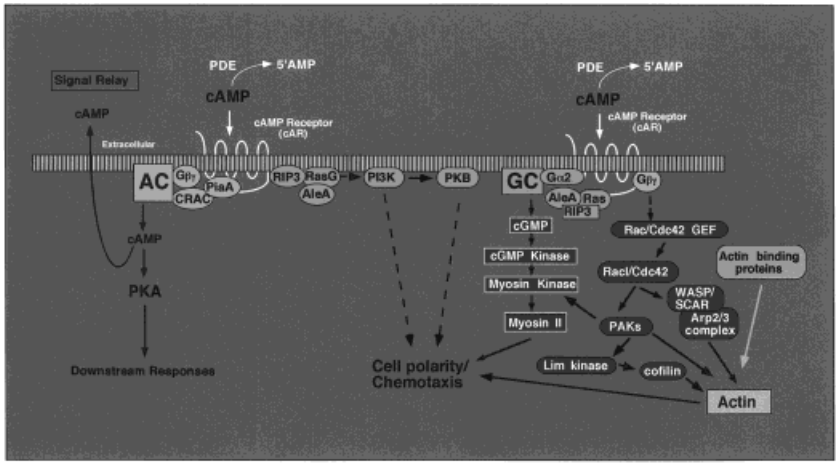

Figure 3. Chemotaxis pathways controlling chemotaxis during aggregation in *Dictyostelium* cells. For purposes of illustration only, some of the pathways are shown on the right and some on the left. In *Dictyostelium*, during aggregation, they are all activated by the same cAMP receptors coupled to the same G protein. Aspects of this pathway are conserved between man and *Dictyostelium*, which includes the pathways that lie downstream from Rac/Cdc42 GEF (note that no Cdc42 has yet been found in *Dictyostelium*). In mammalian cells, Rac and Cdc42 have some overlapping as well as distinct downstream effectors, which may depend upon the cell type (see text). PI3K plays an important, yet not fully defined, role in both *Dictyostelium* and mammalian cells and includes the activation of Akt/PKB, which is important for cell polarity and chemotaxis in *Dictyostelium*. The right side outlines the pathway and some of the components required for the activation of adenylyl cyclase (AC), which is tightly coupled to the activation of pathways that control chemotaxis, including the activation of guanylyl cyclase (GC). The Ras components including a Ras GEF AleA and Ras interacting protein RIP3 are required for both chemotaxis and the activation of adenylyl cyclase.[79,80] The Ras protein RasG may regulate PI3K activation in *Dictyostelium* and is required for proper cell movement.[6,8,70] AC, adenylyl cyclase; GC, guanylyl cyclase; RIP3, Ras interacting protein; PiaA, a gene required for receptor activation of adenylyl cyclase; CRAC, cytosolic activator of adenylyl cyclase (see text); cGMP kinase, cGMP-dependent protein kinase; myosin kinase, myosin II heavy chain kinase; Rac/Cdc42 GEF, exchange factors for Rac and Cdc42; PKB, Akt/PKB; PI3K, phosphatidyl inositol 3 kinase. See text for details.

and PI3K2) are most closely related to p110α (type Ia), which is activated by tyrosine kinases and Ras, whereas *Dictyostelium* PI3K3 is most closely related to Gβγ-activated forms (type Ib).[19] PI3K1 and PI3K2 are genetically redundant. While single mutants show little abnormality, a double knockout of the two genes produces growth defects, an abnormal actin cytoskeleton, and defects in pinocytosis. Furthermore, the double knockout cells do not effectively polarize and show poor chemotaxis.[18,20,21] In human macrophages and eosinophils, inhibition of PI3K activity, produced either by microinjecting antibody against the gamma isoform of PI3K or by treating the cells with Wortmannin, causes a significant decrease of migration, which suggests that PI3K also plays an important role in regulating migration, via the cytoskeleton, in at least some mammalian cells.[22,23] The importance of Akt/PKB in chemotaxis is evident from the phenotype of *Dictyostelium akt/pkb* null cells. These cells do not become polarized in cAMP gradients but produce pseudopodia randomly along the entire perimeter of the cell, irrespective of the direction of the cAMP gradient, and move very slowly and inefficiently toward the cAMP source.[24] As Akt/PKB lies downstream from PI3K (see below), at least some of the phenotypes of the PI3K double knockout cells are presumed to be a result of an inability to activate Akt/PKB.

In *Dictyostelium*, Akt/PKB, like guanylyl cyclase, is rapidly and transiently stimulated in response to the chemoattractant cAMP, with activity peaking at 10-15 sec after cAMP addition. Evidence indicates that receptor activation of *Dictyostelium* Akt/PKB is controlled by a regulatory pathway that is similar to the one that activates mammalian Akt/PKB. Akt/PKB contains an N-terminal PH domain, a kinase domain, and a C-terminal domain. Activation of Akt/PKB requires its phosphorylation on a conserved site in the activation loop of the kinase domain by an upstream kinase, designated PDK1, and a second site in the C-terminus.[25] $PI(3,4,5)P_3$ is thought to regulate Akt/PKB activation in two ways: by binding to the PH domain, resulting in its translocation to the plasma membrane, and through the activation of PDK1.[19,25,26]

In *Dictyostelium* and mammalian cells, Akt/PKB lies downstream from the lipid kinase phosphatidyl inositol-3 kinase (PI3K),[24,25,27] which phosphorylates phosphatidyl inositol-(4,5)-bisphosphate [$PI(4,5)P_2$] to produce $PI(3,4,5)P_3$.[28] In *Dictyostelium*, Akt/PKB kinase activity is not stimulated by cAMP in *pi3k1/pi3k2* nulls cells or when wild-type cells are preincubated with an inhibitor of PI3 kinase, LY294002. The results confirm that PI3 kinase is required for Akt/PKB activation.[24] cAMP activation of Akt/PKB requires the cAR1 receptor and the coupled heterotrimeric G protein containing the Gα2 subunit, as shown with strains that lack the respective signaling components. These observations suggest that a cAMP receptor-mediated pathway, involving heterotrimeric G protein- and PI3 kinase-

dependent activation of Akt/PKB, is essential for efficient chemotaxis. As the defects of *pi3k1/pi3k2* null cells are more severe than those of *akt/pkb* null cells, PI3 kinase presumably has downstream effectors in addition to Akt/PKB that are required for chemotaxis.

In mammalian cells, PI3K regulates the actin cytoskeleton too, although the details of this pathway are not known.[28,29] It is also not known whether Akt/PKB is involved in regulating chemotaxis in mammalian cells as it is in *Dictyostelium*, but evidence suggests that this may be the case. In neutrophils, Akt/PKB is activated by chemoattractants via a PI3K-dependent pathway.[30] The PH domain of mammalian Akt/PKB also localizes to the leading edge in neutrophils that chemotax toward fMet-Leu-Phe, a chemoattractant that functions via G protein-coupled receptors.[31] These parallels between the mammalian and *Dictyostelium* responses to chemoattractants suggest that the pathway of response, from PI3K to Akt/PKB, is highly conserved in eukaryotes.

Altogether, these results suggest that the translocation of PH domain-containing proteins in response to an activation of PI3 kinase is a crucial step in initiating the chemotaxis response. A localized activation of PI3 kinase would generate phospholipids that function as binding sites for PH domain-containing proteins, including Akt/PKB, on the plasma membrane, which leads to the translocation of the PH domain-containing proteins to the plasma membrane and their subsequent activation (Fig. 4). This idea has also been proposed by Parent and Devreotes.[5] Such "activation domains" could focus the responses of multiple, co-regulated pathways needed for pseudopod extension, cell polarization, and chemotaxis to be activated in a highly localized area of the cell (see below).

Phospholipids also appear to be involved in regulating other key components required for cytoskeletal regulation during chemotactic movement. Phospholipids can differentially stimulate or inhibit activities of myosin I heavy chain kinase, actin binding, and capping proteins,[1,32–34] which can differentially activate or inhibit their activity and thus regulate the actin cytoskeleton. For example, the activity of severin, a major actin filament severing protein, is inhibited by PIP_2 and other negatively charged phospholipids, whereas the function of profilin, an actin capping protein, is stimulated by PIP_2.[32]

Role of small G proteins

Chemotaxis requires the coordinated regulation of changes in the actin and myosin cytoskeletons. Most of our understanding of the role of small G proteins comes from analyses of the phenotypes exhibited by dominant negative and constitutively active forms of small G proteins microinjected into mammalian fibroblasts. These studies revealed that the small G proteins Rho, Cdc42, and Rac differentially regulate the formation of stress fibers, filopodia, and pseudopodia/lamellipodia, respectively.[35]

Experiments using dominant active and negative forms of Rac and Cdc42 in macrophage and *Dictyostelium* point to essential roles for these small G proteins in mediating chemotaxis and in fibroblasts to control cell motility.[36–39] Constitutively active (GTP-bound) Rac or disruption of a gene encoding a Rac GAP (DdRacGAP1) leads to an increased number of pseudopodia and an up-regulation of the actin cytoskeleton.[36] In contrast, expression of dominant negative Rac prevents pseudopod projection and cell movement. Such cells have reduced cortical actin, consistent with a role for Rac in mediating actin polymerization in

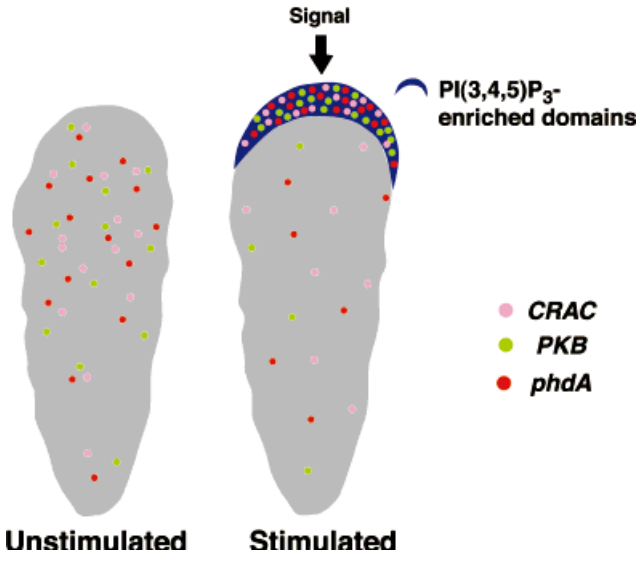

Figure 4. Model for the formation of activation domains at the leading edge. In unstimulated cells, the PH domain-containing proteins CRAC, Akt/PKB, and phdA are cytosolic. In a chemotaxing cell, these proteins become localized to the leading edge. Growing evidence suggests that this localization is in response to the activation of PI3K due to the fact that these PH domain-containing proteins are thought to bind to $PI(3,4,5)P_3$ and the translocations do not occur in *pi3k1/2* double knockout cells. It is expected that this activation is localized at the leading edge, presumably from the formation of domains enriched in $PI(3,4,5)P_3$. The localization of multiple signaling proteins at the leading edge would result in the formation of "activation domains," resulting in the production of a pseudopod at this site and cell movement. See text for references and discussion.

in vitro cell extracts from neutrophils and *Dictyostelium* cells.

Similarly, a dominant negative Cdc42 predominantly reduces the ability of macrophage to chemotax toward the signal and affects cell polarity.[38,39] Such cells move randomly in terms of the direction of the chemoattractant gradient, suggesting that Cdc42 is required for sensing the direction of the signal, whereas Rac, on the other hand, regulates the actin cytoskeleton directly. Recent evidence indicates that a directed, non-receptor-mediated translocation of activated Cdc42 to the plasma membrane leads to the formation of actin-enriched filopodia in rat basophilic leukemia cells.[40] These data indicate that $Cdc42^{GTP}$ can induce actin polymerization at the membrane in these cells, consistent with the ability of $Cdc42^{GTP}$ to stimulate actin polymerzation in cell-free extracts from neutrophils and *Dictyostelium* cells.[37] Rac and/or Cdc42 regulate diverse downstream effectors. Most of these effectors contain a conserved Rac/Cdc42 interacting domain (CRIB domains) that binds the activated (GTP-bound) but not the GDP-bound form of Rac/Cdc42. Among these proteins are WASp, the Wiskott-Aldrich Syndrome protein, and PAKs, which are serine/threonine protein kinases related to the yeast Ste20/Cla4 kinases that control reorganization of the cytoskeleton in this organism.

The induction of Rac and Cdc42 function presumably occurs through the activation of Rac and Cdc42 guanine nucleotide exchange factors (GEFs). Although Rac/Cdc42 exchange factors such as Vav have been characterized biochemically, it is not known how the activity of exchange factors is stimulated in response to chemoattractants.[41] Understanding whether Rac/Cdc42 GEFs are activated at the leading edge may reveal how actin polymerization is localized to the leading edge.

Regulation of the actin cytoskeleton

During cell movement, including movement mediated by chemoattractants, cells are highly polarized, with the majority of the filamentous actin (F-actin) at the front or leading edge of the cell and, in the case of *Dictyostelium* cells, conventional myosin (myosin II) at the posterior cortex.[42,43] The initial step in chemotaxis is the formation of new pseudopodia or lamellipodia in the direction of the chemoattractant source, producing the leading edge. Pseudopodia and lamellipodia are actin-enriched cell extensions that result from the addition of G-actin monomers to the barbed end of filamentous actin (F-actin), which are directed "outward" toward the plasma membrane.[44,45] The G-actin is derived from an existing pool of actin-profilin complexes and the depolymerization of F-actin in other parts of the cell, including the region that was formerly the leading edge.[46]

Extension of the actin filaments at the leading edge is thought to provide the needed force for extension of the pseudopod.[44,45] Assembled myosin II filaments in the posterior of the cell, on the other hand, control the contraction of this region.[47] This contraction helps release the cell's contact with the substratum, allowing the posterior to retract and the cell to move forward. Extension of the leading edge and the retraction of the posterior portion might be two separate but normally well coordinated processes. That they can be uncoupled, however, is shown by the fact that mutations that affect the latter process have no apparent effect on pseudopod formation.[48] This independence is also supported by the observation that pseudopod extension is not accompanied by the retraction of the uropod, a specialized pseudopod-like tail projection possessing important motility and adhesive functions. Chemotaxing amoebae exhibit a 3-D behavior cycle that includes a transient pseudopod extension phase in the x,y axis which is followed by a z-axis expansion phase. Anterior pseudopod extension in the x,y axis is accompanied by a decrease in height, not by uropod retraction. The increase in height, however, is accompanied by uropod retraction.[49]

Induction of actin polymerization is mediated through the Arp2/3 protein complex.[50] Arp2/3's ability to stimulate actin nucleation is enhanced by two adaptor proteins, WASp and Scar.[40,50–52] Patients with Wiskott-Aldrich Syndrome have a reduced number of macrophage, which are defective in chemotaxis, indicating that WASp plays an *in vivo* role in cell motility.[53] WASp contains a binding domain for the activated form of Rac and Cdc42 and an N-terminal domain designated WH1 (WASp homology 1). WH1 is structurally related to pleckstrin homology (PH) domains, which are thought to bind phospholipids. Scar has a distinct N-terminal domain that lacks the two well-defined protein motifs found in WASp. Both WASp and Scar have an actin-binding domain, similar to verprolin, and a stretch of acidic amino acids at the C-terminus.

In vitro experiments indicate that the WASp and Scar C-termini interact with Arp2/3 and G-actin and are important in stimulating actin polymerization.[50] In vivo, WASp function is thought to be mediated through Cdc42 and/or Rac and possibly through the function of the WH1 domain. Chemoattractants might stimulate WASp function through the Cdc42/Rac-interacting and WH1 domains, possibly by regulating its translocation to the plasma membrane.[40] Translocation of WASp or Cdc42 to the plasma membrane results in filopodia formation, consistent with involvement of both proteins in regulating actin cytoskeletal rearrangements.

In addition to actin polymerization, pseudopod formation requires numerous actin-binding proteins, which are involved in modulating filament growth and cross-linking the actin filaments, functions that presumably stabilize and strengthen the F-actin network. This topic is discussed in other reviews.[1,32]

Role of myosins in chemotaxis

Myosin filament assembly, contraction, and disassembly are highly regulated in response to chemoattractants and are important in establishing cell polarity, the retraction of the posterior cell body, and the regulation of pseudopod extension and cortical tension. Disruption of the genes encoding conventional myosin (myosin II) heavy or light chains leads to chemotaxis defects, including loss of posterior contraction and production of pseudopodia along the lateral edges of cells due to a loss of cortical tension.[16,47,54,55] Myosin II assembly and motor activity are regulated by phosphorylation of the myosin heavy and light chains by specific heavy and light chain kinases whose activities are regulated by chemoattractants via the activation of guanylyl cyclase in *Dictyostelium*. Myosin heavy chain kinase (MHCK) phosphorylates myosin II in the C-terminal tail of the protein, resulting in myosin II filament disassembly.[56,57] *Dictyostelium* cells that express a myosin II mutation causing the three mapped phosphorylation sites at the tail to be converted to Ala do not maintain their shape and are unable to suppress the formation of lateral pseudopodia, indicating that the regulation of myosin II assembly is important during chemotaxis. This idea is also supported by the analysis of the Rac1-regulated Ser/Thr kinase PAKa (see below).[48,58] Two distinct myosin II kinases, PKC-MHCK and MHCKA, have been characterized.[59,60] One is a member of the PKC (protein kinase C) superfamily (PKC-MHCK); its N-terminal region is homologous to the phospholipid binding domain of PKC. The role of PKC-MHCK in chemotaxis has been elucidated through the analysis of mutant cells. Overexpression of myosin heavy chain kinase leads to myosin II disassembly, whereas the knockout of the gene results in increased myosin II assembly.[59] Chemoattractant stimulation results in PKC-MHCK translocation to the plasma membrane and an increase in its kinase activity.[61] Translocation of PKC-MHCK to the leading edge should thus disassemble myosin II in this region of the cell. As the presence of assembled myosin II is expected to impede pseudopod extension, the localized disassembly of myosin at the front of the cell should play an important part in regulating cell movement.

Genetic analysis suggests that the Ser/Thr kinase PAKa might be a negative regulator of PKC-MHCK and/or MHCKA.[58] Myosin contraction and thus myosin II assembly are required for retraction of the posterior part of the cell. PKC-MHCK would inhibit this process by promoting myosin II filament disassembly. PAKa (p21-activated protein kinase) is a structural homologue of mammalian PAK1 that co-localizes with assembled myosin II in the posterior of the cell and is localized to a region where myosin II disassembly must be inhibited. Disruption of the gene encoding PAKa results in a disassembly of myosin II, whereas expression of a constitutively active PAKa results in a hyperassembly of myosin. These phenotypes are the opposite of those of the mutants lacking one of two MHCKs.[59,62] PAKa co-localizes with myosin II in the posterior of the cell, suggesting that PAKa may inhibit MHCK activity in this region, thus maintaining myosin II assembly and permitting myosin contraction.

These results all support the idea that myosin II assembly is closely regulated in connection with chemotaxis. Like PKC-MHCK, PAKa activity is stimulated in response to chemoattractant signaling.[58] In response to chemoattractants, MHCK phosphorylates myosin II, leading to its disassembly. PAKa activity is also activated, however. PAKa's localization at the posterior of the cell spatially restricts its activity and leads to an inhibition of PKC-MHCK in this region and to a localized increase in myosin II assembly.

PAKa contains an N-terminal regulatory/targeting domain with an acidic region, a putative SH3 binding domain, and a Rac interacting domain.[58] The N-terminal domain (containing the acidic and SH3 interacting domains) is sufficient for PAKa's subcellular localization, suggesting that this localization is mediated through the interaction of this region of PAKa with another protein. Direct biochemical analysis of PAK1 in mammalian cells suggests that its Rac-interacting domain is a negative regulatory domain that controls kinase activity and that the inhibitory effect of this domain is released by the interaction of GTP-associated Rac (activated Rac).[63] This observation is consistent with analyses suggesting that the kinase domain of PAKa by itself behaves as a constitutively active kinase, whereas a construct carrying both the kinase and Rac interacting domains does not exhibit activity when expressed in cells. In addition to contraction of the posterior of the cell, myosin II maintains cortical tension around the sides and posterior of the cell, helping to repress pseudopod projection in these areas.[48,64] *paka* null and *myosin II* null cells exhibit similar cytokinesis defects. Null or dominant negative PAKa mutants lead to a loss of this lateral inhibition of pseudopod formation, suggesting that a major function of PAKa is to regulate myosin II.

The motor activities of myosin I molecules are also required for proper regulation of chemotaxis.[1,32,65,66] Cells with myosin I gene disruptions exhibit abnormal pseudopod formation and decreases in the rate of cell motility. Unlike conventional myosin (myosin II), which is primarily found in the posterior of the cell, myosin I localizes to the leading edge.[67] The motor activity of myosin I heavy chain is controlled by phosphorylation events mediated by myosin I heavy chain kinase (MIHCK). Several myosin I heavy chain kinases (MIHCKs) have been purified from *Dictyostelium* and *Acanthamoeba*[68,69] MIHCKs share a common C-terminal catalytic domain, which has a highly similar sequence to that of the kinase domain of mammalian PAKs. The N-terminal regions of MIHCKs share similarities with those of PAKs; they contain a Rac-interacting domain and their kinase

activities are stimulated 10-fold by $Rac1^{GTP}$, indicating that this pathway is also regulated through Rac exchange factors. MIHCK kinase activity is stimulated by binding to acidic phospholipids such as phosphatidylserine, phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate, suggesting that an increase in MIHCK activity might be associated with a translocation to the plasma membrane.[34,69]

Mechanisms regulating directional responses

How are cells able to sense and respond directionally to a chemoattractant gradient? In lymphocytes and leukocytes, redistribution of chemoattractant and chemokine receptors in the direction of a new chemoattractant gradient may be, in part, responsible for the formation of a new leading edge and a change in the direction of movement. In mammalian neutrophils and *Dictyostelium* cells, however, a growing body of experimental evidence suggests that cells locally activate distinct sets of signaling pathways specifically in the front of the cell, which leads to localized actin polymerization, the formation of a new leading edge, directional cell movement, and an establishment of cell polarity.[5,24,31,70,71] If this notion is correct, then all parts of a cell's perimeter should be essentially equipotent in responding to a chemoattractant. The analysis in Figure 2 supports this model and demonstrates that cells produce pseudopodia at any position on the perimeter of the cell, as the direction of the cAMP source changes. In cells already showing strongly biased polarization, however, it takes longer for a new pseudopod to form toward the posterior of the cell than on the side. This is presumably due to the organized myosin II cytoskeleton found in the posterior of polarized, chemotaxing cells that would need to be disassembled and reorganized prior to the formation of a pseudopod in this region of the cell.

The first molecular evidence supporting a localized response in the direction of the chemoattractant source derives from experiments using GFP-tagged chemoattractant receptors. The cAMP receptor cAR1 is the major chemoattractant receptor that controls aggregation in *Dictyostelium*. These receptors are uniformly distributed around the periphery of the cell and, more importantly, the receptors remain uniformly distributed when the cells chemotax or change direction,[72] providing strong evidence that it is not receptor localization that controls polarity. Similar observations in neutrophils have been made recently, using the C5a chemotaxis receptor fused to GFP.[73] As pseudopodia extend from any position along the cell's surface, it is expected that the heterotrimeric G protein that couples to cAR1 would exhibit a uniform distribution. Thus, localized responses are not due to prior localization of the receptor and coupled components. The uniform distribution of chemoattractant receptors in *Dictyostelium* suggests the need for inhibition of localized activation or pseudopod formation in lateral or posterior surfaces of the cell, as will be discussed in more detail later.

How does a cell extend pseudopodia in the direction of the highest concentration of a chemoattractant but not along the side or posterior of the cell? As illustrated in Figure 2, when the direction of the chemoattractant gradient is changed, cells respond rapidly with a change in their polarity in the direction of movement. *Dictyostelium* cells moving in one direction rapidly reorient when the direction of the chemoattractant source is altered. In a step chemoattractant gradient, as used in the experiment shown in Figure 2, this does not occur, however, by the rotation of the cell, with the maintenance of the same leading edge and posterior, as in a ship changing course. Instead, the cell puts out a new pseudopod in the direction of the new cAMP gradient, resulting in a change in the cell's polarity. In shallower, possibly more physiologically relevant gradients, the formation of a new pseudopod does not exclusively occur in the direction of the chemoattractant source, but the pseudopod formed in the direction of the chemoattractant source shows a greater persistence. Strikingly, *Dictyostelium* cells and leukocytes respond to a chemoattractant gradient in which the difference in the level of chemoattractant between the front and back of the cell is $<2\%$.[74,75] The formation of a lamellipod in the direction of the chemoattractant source, however, is not due to a difference in responses elicited by the level of chemoattractant sensed by the front and posterior of the cell, as stimulation of cells with concentrations of chemoattractants that differ by 10% does not lead to distinguishable differences in second messenger responses. Thus, other differences between the front and the sides and posterior of the cell lead to a repression of lateral pseudopod extension. In shallow chemoattractant gradients, the formation of a new pseudopod does not exclusively occur in the direction of the chemoattractant source but pseudopod extension is clearly biased in that direction. We suggest that this biased pseudopod formation in a shallow chemoattractant gradient, like that which occurs in a step gradient as illustrated in Figure 2, results from a biased, preferential activation of signaling pathways in the direction of the chemoattractant source. We propose that the pathways required for these responses are similar and that the main difference is the strength of the response, which is dependent on the concentration of the chemoattractant.

Cytosolic signaling components localize to the position of the future leading edge in response to chemoattractants

Evidence for localized activation of responses leading to directional cell movement was obtained from in vivo experiments. These demonstrated that two cytoplasmic, PH domain-containing proteins Akt/PKB[24] and CRAC (which is required for receptor activation of adenylyl

cyclase,[70]) transiently localize to the plasma membrane in response to cAMP. These experiments employed GFP fusions, and showed that CRAC and the Akt/PKB PH domain transiently translocate to, and are uniformly distributed along, the plasma membrane when *Dictyostelium* cells are bathed in cAMP, which activates receptors uniformly on the cell surface. Impressively, the membrane localization of the *Dictyostelium* CRAC-GFP and Akt/PKB-PH-GFP fusion is very rapid and brief, with the membrane localization peaking at 5-8 sec. These kinetics are consistent with membrane translocation preceding Akt/PKB activation. Since the proteins rapidly come off the membrane, the binding sites are presumably rapidly lost. Because membrane translocation of CRAC occurs in the presence of latrunculin A, which disassembles F-actin,[70] it seems that rearrangements of the actin cytoskeleton are not required for the initial chemoattractant-mediated responses.

Further insights were obtained when the response was examined in chemotaxing cells. Under these conditions, the two GFP PH domain-containing fusion proteins were found to localize to the leading edge of cells, strongly indicating that receptor-mediated responses are locally activated at the leading edge (Fig. 2 insert).[24,70] A similar observation has been made in neutrophils with a GFP-PH domain fusion derived from mammalian Akt/PKB,[31] suggesting that the mechanism from these processes has been conserved between *Dictyostelium* and mammalian cells. Interestingly, as cells move towards the chemoattractant source, the membrane localization of the *Dictyostelium* CRAC and Akt PH domains is transient and appears to relocalize briefly to the leading edge with each chemotaxis step. This observation suggests that with each step, a chemotaxing cell "resets" itself. It also suggests that each chemotaxis step requires a separate activation of all of the chemotaxis responses, i.e. the responses are not continuously activated.

Formation of "activation domains"

The PH domains of CRAC and Akt/PKB contain a consensus binding site for $PI(3,4)P_2/PI(3,4,5)P_3$. Given the requirement of PI3 kinase for Akt/PKB activation, it is possible that the translocation is mediated by the binding of the PH domain to newly generated domains or patches that are enriched in $PI(3,4,5)P_3$ as a result of activation of PI3 kinase. These domains would be preferential binding sites for PH domain-containing proteins, as suggested by Parent and Devreotes,[5] and would result in the formation of "activation domains" at the leading edge of cells (Fig. 4). This idea is concordant with present models for the membrane localization of Akt/PKB in mammalian cells in response to growth factors that activate PI3 kinase through receptor tyrosine kinases. We expect that transient membrane localization of the PH domain-containing proteins in *Dictyostelium* requires the localized activation of PI3 kinase and upstream pathways. The kinetics of *Dictyostelium* Akt/PKB activation are consistent with it occurring when the protein translocates to the plasma membrane and the idea that there is a localized activation of Akt/PKB at the leading edge.

The inability of *akt/pkb* null cells to polarize and chemotax properly suggests that a localized activation of Akt/PKB may be important in establishing and/or maintaining cell polarity. Such a model is supported by experiments using an Akt/PKB construct containing an N-terminal myristoylation signal from c-Src. Myr-tagged Akt/PKB from *Dictyostelium* and mammalian cells is constitutively targeted to the plasma membrane along the entire cell, which results in a high, constitutive Akt/PKB kinase activity.[24,26] Such cells are unable to properly polarize or chemotax efficiently, which presumably reflects loss of the spatially localized activation of Akt/PKB at the leading edge.[24]

It is expected that other pathways required for chemotaxis are similarly activated at the leading edge (Fig. 5). Key components would be the exchange factors for small G proteins such as Rac, Cdc42, and Ras and molecules from other second messenger pathways such as guanylyl cyclase in *Dictyostelium* (Fig. 3). The mammalian Rac exchange factor Vav has a PH domain that may target it to the plasma membrane in a PI3K-dependent fashion.[41] Localized Vav activation to the leading edge could result in a localized activation of Rac and Cdc42, which might cause activation of WASp and Arp2/3-mediated actin polymerization at the leading edge resulting in pseudopod formation. PAKs, including mammalian PAK1 and *Dictyostelium* myosin I kinase (a PAK family member) might be activated by Rac and/or Cdc42 at the leading edge. Other PAK family members such as *Dictyostelium* PAKa[58] could be activated at the posterior edge of cells through prelocalization of the protein, at least in cells in which polarity is already established. If this is the case, then in a chemotaxing cell in which polarity has been established, localization of components such as PAKa would be an important mechanism for maintaining polarity and efficient chemotaxis. Understanding how polarity and subcellular localization of components such as PAKa are established will require identifying which cellular proteins bind to and help localize PAKa.

How do cells reorient their polarity and establish directionality in a gradient?

Cells become polarized to the direction of migration. Locomoting cells maintain their polarity even without external cues, although some cells (e.g. *Dictyostelium* cells) become significantly more elongated and have more extreme differences in the localization of actin and myosin when they are in a chemoattractant gradient. Crawling lymphocytes move with relatively strong persistence in a homogeneous solution of chemoattractant[76] and *Dictyostelium* cells can

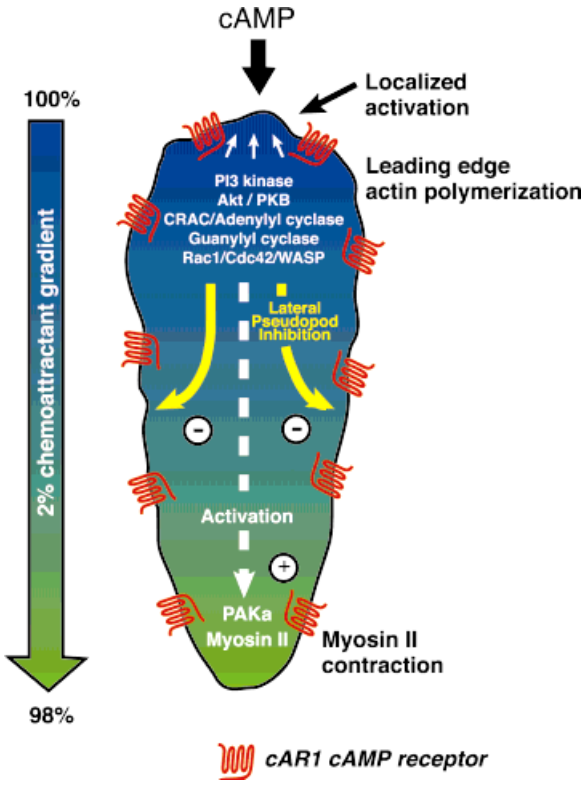

Figure 5. Model for localization of responses required for chemotaxis in *Dictyostelium*. The cartoon shows chemotaxing cells with a leading edge and a retracting posterior cell body. The serpentine, G protein-coupled chemoattractant receptors (cAR1) are uniformly distributed around the cells. F-actin is localized at the leading edge, and to a lesser degree, at the posterior. Assembled myosin II and the PAK PAKa are preferentially found and function in the posterior of the cells. The leading edge exhibits a proposed localization and activation of signaling pathway components required for chemotaxis and aggregation (activation of adenylyl cyclase), as discussed in the text. Akt/PKB and CRAC localize to the leading edge and are thought to control downstream pathways. Guanylyl cyclase is required for regulating chemotaxis and is proposed to be activated at the leading edge. The reason for suggesting that PI3K may localize to the leading edge is discussed in the text. WASp functions similarly at the leading edge and thus it is thought that activated Rac1 and Cdc42 must also be localized there. The cartoon shows that cells are capable of distinguishing a $<2\%$ gradient difference between the front and back of the cell. The cartoon depicts "lateral pseudopod inhibition," namely the proposed block in the ability of the lateral sides of cells to extend pseudopodia in wild-type cells, as discussed in the text.

migrate in the absence of a chemoattractant, suggesting that these cells maintain an inherent polarity. This inherent polarity might also be maintained in neutrophils in the absence of chemoattractant stimulation. The origin of such inherent polarity is not well understood. It might confer on cells the ability to orient themselves effectively in the direction of the chemoattractant, by helping to establish differences between the leading edge and the sides of the cells. Cells have an ability to reorient their polarity, however, either in response to a change in direction of movement that might occur stochastically in the absence of external cues, or in response to a chemoattractant gradient. We will focus on the mechanism by which cells change their polarity in response to chemoattractant gradients.

Chemotaxing *Dictyostelium* cells have a strongly biased polarity, with F-actin assembled primarily in the front and myosin II in the rear cell body. The above discussion suggests that localized activation of signaling pathways stimulating F-actin assembly at the leading edge and myosin II assembly in the rear cell body in response to a new chemoattractant gradient is a first step in resetting cell polarity and initiating chemotaxis in the direction of the new chemoattractant source. The assembly of myosin II is very important in defining axial polarity and in biasing the direction of movement by repressing extensions of lateral pseudopodia through cortical tension.[16,47] PAKa appears to play an important part in regulating this process. When cells are depolarized by bathing in cAMP, they round up and the actin and myosin cytoskeletons become more uniform around the cell's cortex. Similarly, PAKa remains membrane/cortically associated but redistributes from a posterior localization to be distributed fairly uniformly along the entire cell cortex.[58] Such experiments, combined with analyses demonstrating that cells respond to a change in the direction of the chemoattractant by putting out pseudopodia in the new direction of the chemoattractant source, suggest that the cell's cytoskeleton is rapidly remodeled by the activation of responses at the leading edge. Differential activation of PKC-MHCK/MHCKA and PAKa would lead to a differential disruption of myosin II in the old posterior of the cell and reassembly of myosin II away from the chemoattractant source, respectively. The levels of activity of PKC-MHCK/MHCKA and PAKa in different parts of the cell will determine the kinetics of the response and where myosin II is disassembled and reassembled. Similarly, F-actin depolymerization in the old leading edge, whether specifically induced in response to chemoattractant signaling or constitutive, is an essential part of this remodeling.

Once polarization is accomplished, cells are set to continue moving up the chemoattractant concentration gradient. Because of the time it takes to put out a pseudopod in a new direction in response to a change in the direction of the chemoattractant, it is probable that the cytoskeletal rearrangement is the time-limiting step in the process. We expect that once cell polarity is established in the direction of a chemoattractant source, it stabilizes the biased movement up the chemoattractant gradient. The inertia to reset a cell's polarity is presumably high and may require a strong

directional chemoattractant signal. In more shallow gradients, cells can change direction by turning rather than resetting their polarity by forming a new leading edge.

Temporal responses and lateral inhibition

How do cells perceive a change in the chemoattractant gradient? The simplest explanation is that the part of the cell closest to the source receives the signal first and that signaling pathways are locally activated in this region of the cell, with the remainder of the cell becoming less responsive or unresponsive to the direction of the new gradient. Lymphocytes and leukocytes appear to achieve this, in part, by redistributing chemoattractant receptors such as fMLP receptor and chemokine (CCR2 and CCR5) receptors to the leading edge.[77,78] Redistribution of receptors to the leading edge would presumably leave the rest of cell body unresponsive to the chemoattractant. As discussed above, however, this method of spatially localizing responses is not applicable to neutrophils and *Dictyostelium* cells, in which the receptors remain uniform around the sides of cells. As all regions of the *Dictyostelium* plasma membrane are able to respond, any mechanism of localized activation at the leading edge must take this into consideration and explain why the pathways are not also activated on other parts of the cell, thus restricting pseudopod formation and further extension to the leading edge. To accomplish this, there must be an inhibition of some signaling responses on the sides and posterior of the cell. Such an inhibition must be very rapid to account for the rapid diffusion of the chemoattractant. We refer to this reduced ability to form lateral pseudopodia as ''lateral pseudopod inhibition'' (LPI) (Fig. 5). We expect that there must be very active coupling between localized activation at the leading edge and pseudopod inhibition at the lateral edges and posterior cell body to effectively coordinate the onset of a new polarity upon the change of chemoattractant gradient.

One component of LPI is cortical tension mediated through myosin II assembly at the cortex of the posterior cell body.[16,47,48,55,64] Mutant strains that lack myosin II or strains that are unable to properly regulate myosin II assembly (e.g. mutants in myosin light chain kinase or PAKa) often form dominant lateral pseudopodia, which can result in a change in the direction of movement.[16,48,58] There appears to be another component of LPI, however, that is an ''active'' process and which is mediated through or resulting from the activation of responses at the leading edge. The fact that it is an active process is demonstrated by the phenotypes of *akt/pkb* null cells, which exhibit an almost complete lack of cell polarity and put out pseudopodia almost equally in all directions.[24] It is not known whether Akt/PKB is actively part of this mechanism (i.e. Akt/PKB substrates mediate this process) or if Akt/PKB function is required for this process to occur.

There are three possible models for an active process to mediate LPI. In the first model, LPI could function through rapidly diffusing small molecules such as cGMP, a membrane-transmitted response such as an ion channel, or the sequential activation of a pathway (e.g. kinase) along the cortex. In the second model, LPI could be controlled by the sequestration of an essential but limiting component of the pathway. One possible component is PI3K, which is expressed at very low levels in *Dictyostelium*. If PI3K is translocated to the leading edge in response to chemoattractants and if the amount of PI3K in the cell is limiting, then it may be impossible for pathways to be activated along the lateral sides of cells. In the third model, LPI inhibition could be due to a global activation of adaptation pathways that regulate the normal turning off of signaling pathways in response to chemoattractants. All of the characterized pathways activated in response to chemoattractants in *Dictyostelium* are rapidly stimulated and rapidly adapt.[6,8] The adaptation could result from intrinsic mechanisms (e.g. RGS or effector stimulation of Gα subunit GTPase activity) or through feedback loops. At present, we cannot decide between models in which reorientation of cells in a new chemoattractant gradient is due to cells directly sensing spatial differences in the level of chemoattractant between two sides of a cell or in which reorientation results from cells responding to both temporal and spatial recognition.

The future

Although significant progress has been made in understanding the signaling pathways required for chemotaxis, much remains to be learned. The downstream substrates of kinases such as Akt/PKB or other pathways activated by second messengers such as cGMP must be determined. How these pathways couple to changes in the actin cytoskeleton and regulate other cytoskeletal proteins such as actin binding proteins or proteins that control myosin I and II must also be elucidated. We currently do not understand the mechanism by which exchange factors for Rac and Cdc42 are activated and how this leads to a localized polymerization of actin at the leading edge. Moreover, many matters that have been discussed in this article, concerning activation of leading edge pathways need to be firmly established; at present, for instance, ''lateral pseudopod inhibition'' is only a hypothesis to explain known phenomena. Expansion of molecular genetic approaches to other motile cells will be important in determining how much of the understanding we have derived from *Dictyostelium* is generally applicable to mammalian cells. From our present knowledge of chemotaxis in both *Dictyostelium* and metazoan cells, I expect that conservation of pathways will be the rule, not the exception.

Acknowledgments

We thank Raffi Aroian, Scott Emr, Eric Davidson, and Sally Zigmond for helpful scientific discussions and comments on the manuscript and Jennifer Roth for critical reading of the manuscript. Because of space limitations, we apologize if certain relevant references were omitted.

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