Molecular insights into eukaryotic chemotaxis

MICHAEL J. CATERINA AND PETER N. DEVREOTES[1]

Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205, USA

ABSTRACT **Many cells display directed migration toward specific compounds. The best-studied eukaryotic models of chemotaxis are polymorphonuclear leukocytes, which respond to formylated peptides and *Dictyostelium* amoebas, which respond to extracellular cAMP. In both cell types, chemoattractants bind to surface receptors that contain seven transmembrane domains and interact with G proteins. Some cells, such as fibroblasts, undergo chemotaxis toward compounds whose receptors lack this motif and transmit their signals by other mechanisms. The cytosolic changes elicited by chemoattractants include increased levels of cAMP, cGMP, inositol phosphates, and calcium. These changes are correlated with actin polymerization and other cytoskeletal events that result in preferential extention of pseudopods toward the chemoattractant. *Dictyostelium* cell lines in which specific genes have been disrupted have demonstrated the necessity of a cAMP receptor (cAR1) and a G protein α-subunit ($G_\alpha 2$) for responsiveness to cAMP. Other proteins, such as myosin heavy chain and several actin binding proteins, are dispensible although their absence does affect the details of chemotaxis. The disruption of other relevant genes and the genetic reconstitution of chemotaxis in cells lacking crucial proteins should reveal many clues about this complicated and fascinating process.—Caterina, M. J.; Devreotes, P. N. Molecular insights into eukaryotic chemotaxis. *FASEB J.* 5: 3078-3085; 1991.**

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CHEMOTAXIS, THE DIRECTED MIGRATION OF cells toward regions of higher concentrations of a chemical attractant, is a behavior that is probably exhibited by all motile cells. An extensive genetic analysis has led to an elegant description of the mechanism of chemotaxis in bacteria, but little is known about the process in eukaryotes (1). Although many eukaryotic cells exhibit this phenomenon, the most well-studied examples are polymorphonuclear leukocytes and *Dictyostelium discoideum* ameobas (2). The former are mammalian phagocytic cells of myeloid origin that play a major role in defense against infection. The latter are cellular slime molds that normally live as unicellular amoebas but which, upon starvation, aggregate to form a multicellular organism (2). Leukocytes carry out chemotaxis in response to bacterial products such as N-formylated peptides and paracrine factors such as leukotrienes (2). *Dictyostelium* amoebas are attracted to nutrients such as folic acid during the growth stage, but at early stages in development they acquire sensitivity to adenosine 3'-5' cyclic monophosphate (cAMP)[2]. The nucleotide is secreted from central points and directs the cells into the aggregate (2). The dissection of some of the molecular components of chemotaxis in these two cell types has begun to shed light on the question of how eukaryotic cells are able to sense chemical gradients and respond to them appropriately.

These chemotactically sensitive cells display remarkable, quite similar properties. The accuracy of chemotaxis appears to depend on the difference in the fraction of occupied receptors at the ends of the cell, as it reaches an optimum when the midpoint of the gradient is near the dissociation constant for the binding of chemoattractant to the cell surface and is highest in steep gradients. The cells are exquisitely sensitive: a 10-μm cell can detect a concentration gradient of roughly 1%, which corresponds to a difference of only 250 occupied receptors between its ends (3). The cells have a means for background subtraction, which allows them to sense relative rather than absolute levels of chemoattractant and to respond to a wide range of gradients and to temporally increasing gradients. This capacity is believed to depend on cellular adaptation to the chemoattractant signals (2).

Chemotactic cells can sense a gradient even before they have begun to move. If a cell is placed in an existing gradient, its first pseudopod is extended up the gradient (4). The subsequent translocation of the cell can then be viewed in terms of error correction and suppression. Although the frequency of turns is the same whether a cell is moving up or down a gradient, the direction and magnitude of those turns depends on the angle between its current direction and that of the gradient. The more incorrect a cell's direction of migration, the more likely its next turn is to be in a direction that compensates for that error; the more correct a cell's direction of migration, the smaller the magnitude of its next turn is likely to be (4, 5). The result of these changes is that individual cells move up the gradient. Moreover, the attractants have a chemokinetic effect; cells translocate more rapidly in higher concentrations (5, 6).

These eukaryotic cells maintain a kind of dynamic polarization. The cells move by rhythmically extending new pseudopods about every 30 s. One region of the cortex appears to be less contracted than the rest, and therefore is more likely within a given period to extend a new pseudopod. Leukocytes are more rigidly polarized than the amoebas, which often initiate new fronts (2). In the absence of a gradient, the directions of polarization of the individual cells of a population are random. Attractants modify the direction of this dynamic polarization such that most cells become polarized up the gradient of chemoattractant (4).

The manner in which attractants alter cell morphology is dramatically illustrated by the sudden exposure of cells to a uniform high concentration of chemoattractant, such as the application of cAMP to *Dictyostelium* (**Fig. 1**). Upon administration of chemoattractant, the cells initially freeze and then undergo a rounding (or cringe) that lasts up to 30 s, followed

[1]To whom correspondence should be addressed.

[2]Abbreviations: cAMP, adenosine 3'-5' cyclic monophosphate; cAR1, cAMP receptor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor; IP_3, inositol, 1,4,5 trisphosphate; cGMP, guanylate 3'-5' cyclic monophosphate.

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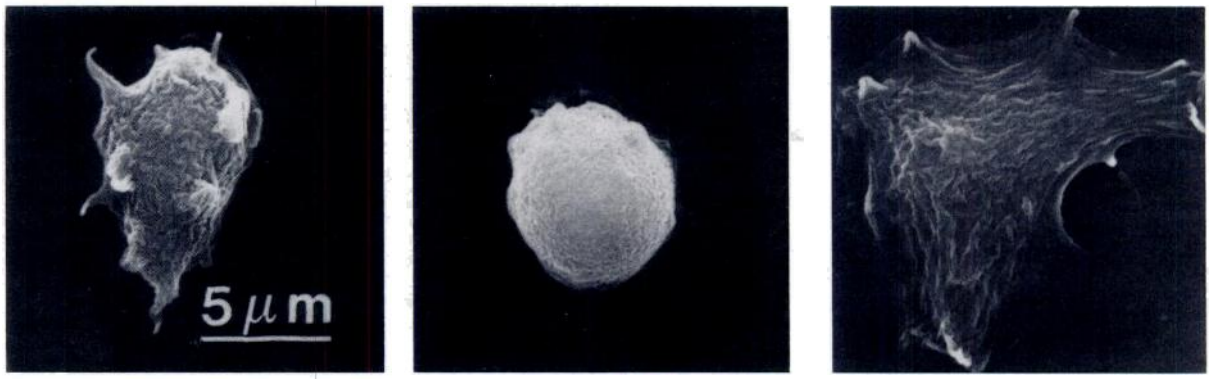

Figure 1. Scanning electron micrograph of aggregation-competent *Dictyostelium* amoebas fixed in osmium tetroxide before (left), at 25 s (center), and 60 s (right) after application of 100 μM cAMP.

by the nearly simultaneous extension of pseudopods from multiple points about the periphery of the cell. These events cause a dramatic decrease in the rate of translocation. The spreading response persists for several minutes and then the cells adapt to the new level of chemoattractant, repolarize, and resume a nearly normal pattern of pseudopod extension. If the cells are then presented with a further increment in chemoattractant concentration, the same series of events ensues. Leukocytes display a similar series of behavioral changes in response to successive increments in the concentration of chemotactic peptides (2). A parallel pattern of adaptation occurs in the biochemical responses of *Dictyostelium* amoebas and leukocytes to chemoattractants, and as discussed below, this pattern may reflect the biochemical basis of the behavioral responses. Adaptation probably accounts for the capacity of cells to carry out chemotaxis in gradients with different mean concentrations and to respond to temporally increasing gradients. It may also be required in the mechanism of moment-to-moment sensing of the gradient.

These chemotactic responses are characteristic of leukocytes and amoebas. Whereas other cell types, such as fibroblasts and endothelial cells, have been reported to carry out chemotaxis, the behavior of these cells differs in detail from that of the phagocytic cells. For instance, unlike the pseudopods extended by leukocytes and *Dictyostelium* amoebas, the processes (lamellopodia) extended by fibroblasts and endothelial cells often exhibit ruffling, a transient thickening of their limiting plasma membrane. Furthermore, fibroblasts and endothelial cells migrate about 10- to 20-fold more slowly (6, 7). Whether these differences in behavior reflect major differences in the underlying biochemical mechanism of chemotaxis remains to be determined.

How do cells manage to sense chemoattractant gradients and adjust their behavior accordingly? Three hypothetical schemes (**Fig. 2**) can be used to illustrate possible ways by which these tasks may be achieved (2). In the first model, the occupancy of chemoattractant receptors on the cell surface is directly coupled to a coordinated contractile apparatus. Because a greater contractile force will be generated at the end of the cell facing the high end of the gradient, it will win the "tug of war." In the second model, moving pseudopods detect the rate of change of chemoattractant concentration with time. Those extended up the gradient will encounter an increase and be reinforced whereas those extended down the gradient will encounter a decrease and be inhibited. In the third model, occupancy of chemoattractant receptors elicits both local excitatory and global inhibitory signals. The balance at any one point will then determine whether pseudopods will be extended from that region. The relative strengths and weaknesses of these models have been described and it is unlikely that any one completely describes the sensing mechanism (2). Nevertheless, they provide a set of functions that may be carried out by the chemotactic machinery and therefore suggest functional assays in which particular components can be tested.

CHEMOATTRACTANT RECEPTORS

The transduction of chemoattractant-elicited stimuli into the cell is mediated by specific chemoattractant receptor molecules on the cell surface. Recently, several of these proteins have been identified, purified, and cloned. Sequence analysis of the genes, in combination with biochemical analyses, has suggested that the mechanisms by which eukaryotic cells sense chemoattractants, hormones, and neurotransmitters are very similar.

The predicted amino acid sequence of the cAMP receptor (cAR1) from *Dictyostelium* suggests it is topologically homologous to a host of mammalian hormone and neurotransmitter receptors such as the β-adrenergic receptor, muscarinic acetylcholine receptor, rhodopsin, and the luteinizing hormone/thyroid-stimulating hormone/follicle-stimulating hormone class of receptors that are proposed to span the membrane seven times and to interact with and activate a variety of membrane-associated G proteins (8–12). Another common feature of these receptors, clusters of serine and threonine residues, potential targets of phosphorylation, is especially pronounced in cAR1.

It has been definitively shown that cAR1 is a cell-surface cAMP-binding protein and is required for chemotaxis. Expression of cAR1 in growth stage cells, which normally exhibit only minimal binding, resulted in the appearance of 500,000 cAMP binding sites/cell (9). The expressed protein is identical by multiple criteria to the receptor expressed in wild-type cells. The necessity of cAR1 for chemotaxis has been demonstrated by disruption of the cAR1 gene via homologous recombination (13). The resulting cAR1 null cells display negligible levels of cAMP binding sites, are insensitive to cAMP, and fail to enter the developmental program. The defect is specific for detection of cAMP, as the cells retain chemotactic sensitivity to folic acid, the growth stage attractant. Expression of an exogenous copy of the wild-type cAR1 gene in the cAR1 null cells restores a wild-type phenotype. These advances define a system for detailed structure-function studies of a chemoattractant receptor. In addition to cAR1, the genes for three homologous cAMP receptors from *Dictyostelium* have been cloned (14). The most

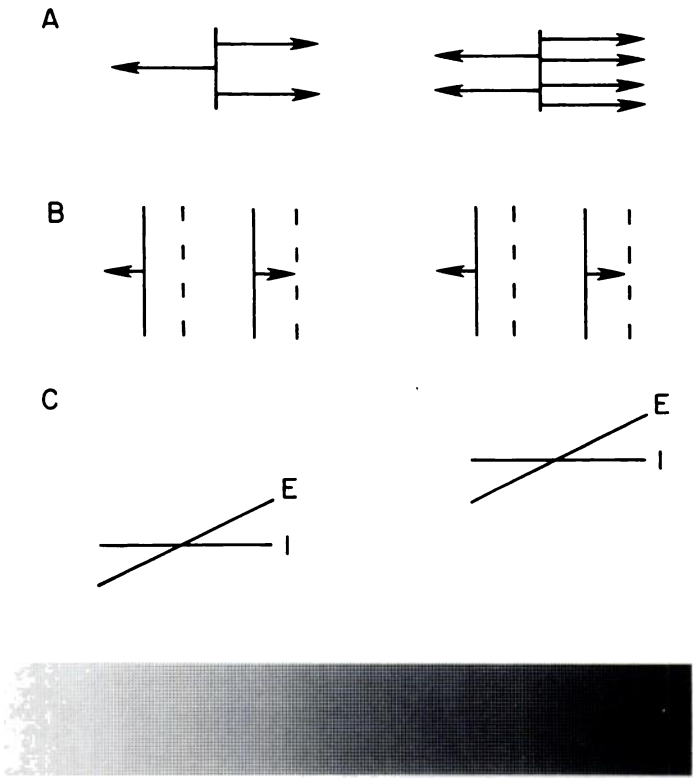

Figure 2. Three hypothetical models for gradient sensing. The shaded bar at the bottom represents the concentration of chemoattractant, which increases from left to right. For each model, a hypothetical cell has been placed at two points in the chemical gradient. The internal workings of the cell reveal how sensing is achieved at both low and high mean attractant concentrations. *A*) Each horizontal arrow represents the force elicited by the coupling of an occupied receptor to the underlying contractile elements, whereas vertical bars represent a common point within the cell at which the forces are exerted and integrated; cells move in the direction of net force. *B*) Solid vertical bars represent initial cell boundaries. Horizontal arrows represent the direction of pseudopod extension. Pseudopods extended up the gradient experience an increased receptor occupancy with time ($dC/dt > 0$) and are reinforced whereas those extended down the gradient experience a decrease ($dC/dt < 0$) and are inhibited. The resulting cell boundaries are indicated by the dashed vertical lines. *C*) Receptor occupancy elicits an excitatory signal (E) that is strongest locally and a global, uniform inhibitory signal (I). The points from which pseudopods are extended, and thus the direction of migration, depend on the difference E-I. See text for further details.

highly conserved sequences among the four subtypes are clustered within the predicted transmembrane domains and intracellular loops, whereas the COOH-terminal, putatively cytoplasmic domains are completely divergent. Gene disruption and expression studies are under way to determine whether the subtypes cAR2, cAR3, and cAR4 also serve as chemoattractant receptors during other stages of the development program.

The recent cloning of the cDNA encoding the formyl peptide receptor from rabbit neutrophils and the complement factor C5a receptor from a leukemic cell line have revealed that as with cAR1, they are also predicted to contain seven transmembrane domains and multiple clusters of serine residues in their cytoplasmic COOH-terminal domains (15, 16). Thus, these chemoattractant receptors are also likely to be coupled to G proteins, as further suggested by the biochemical evidence described below. In terms of sequence identity, however, these receptors are more closely related to mammalian peptide hormone receptors than they are to cAR1; there is no discernible region that might provide a signature for a chemoattractant receptor.

Similar forms of regulation of chemoattractant receptor function are seen in *Dictyostelium* amoebas and leukocytes. In both cell types, continuous exposure to a fixed concentration of chemoattractant results in a reversible decrease in responsiveness, termed desensitization. Three ligand-induced alterations of cAR1 play a role in this process: phosphorylation, loss of ligand binding, and decrease in cAR1 protein levels.

cAR1 undergoes a time-dependent shift in mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE), due to ligand-activated serine phosphorylation on the cytoplasmic COOH-terminal portion of the molecule (2, 17). The kinetics and dose-response of cAR1 phosphorylation correlate closely with adaptation of the receptor-mediated activation of adenylate cyclase and of the chemoattractant-elicited cell shape changes described previously. A similar phosphorylation mechanism is thought to regulate the activities of the β-adrenergic receptor and rhodopsin (18). The adaptation of chemotactic responsiveness by the reversible covalent modification (methylation) of chemoattractant receptors is an essential feature of chemotaxis in bacteria (19). The phosphorylation/dephosphorylation reactions may similarly regulate chemotactic responsiveness in eukaryotic cells. For cAR1 in *Dictyostelium*, there is evidence that although an increase in the fraction of receptors occupied leads to an increase in the fraction of receptors modified, a given receptor need not be occupied to be modified (2). Such a mechanism could thus provide the global inhibitory signal described previously. It will be interesting to learn whether the other chemoattractant receptors undergo a ligand-induced phosphorylation/dephosphorylation reaction. Chemoattractant pretreatment has been shown to attenuate ligand-mediated responses in leukocyte membranes, but the basis of this desensitization is not understood (20).

In many G protein-coupled receptor systems, exposure of cells to ligand leads to a rapid, reversible loss of surface binding sites (2). Half-maximal stimulation of this loss occurs at chemoattractant concentrations near the dissociation constants for these attractants. Thus, loss of ligand binding requires attractant concentrations about 10-fold higher than those required to stimulate increases in cAMP or inositol phosphates (21–24). Maximal stimulation results in a 70% decrease in the number of binding sites without change in the affinities of the remaining sites. The $t_{1/2}$ for the loss of ligand binding is on the order of 2 min, whereas the half-time of reappearance of sites upon removal of ligand is 1 h in amoebas and 10 min in leukocytes (2). Immunofluorescence studies have shown that cAR1 staining rapidly changes from a diffuse distribution about the periphery of the cell to a collection of condensed pockets or patches (2). In leukocytes, formyl peptide receptors shift from a plasma membrane fraction to one that cosediments with the golgi (2). Further studies are required to determine whether these receptors become internalized or clustered in a domain of the plasma membrane. Nevertheless, it is conceivable that these changes in receptor distribution play a role in chemotaxis. The ability of leukocytes to orient in a gradient is correlated with the number of receptors available for occupancy, whereas the recovery of chemotactic ability after preincubation with peptide is temporally correlated with the recovery of available binding sites

(25, 26). The loss of binding sites could serve as a coarse adjustment of sensitivity, allowing cells to detect gradients that vary widely in mean attractant concentration.

A third point of control is the ligand-induced increases or decreases in the amount of receptor protein caused by changes in receptor protein stability or mRNA levels (P. Van Haastert, personal communication). Although such regulation is likely to be important for assembly of the chemotactic apparatus, its slow kinetics (minutes to hours) suggests that it does not play a direct role in the sensing response.

Not all chemoattractant receptors are necessarily G protein-coupled receptors containing seven transmembrane domains. Platelet-derived growth factor (PGDF) stimulates chemotaxis in a variety of cell types, including Swiss 3T3 cells (27). PDGF receptors are heterodimers or homodimers of polypeptides that cross the plasma membrane once and bear intracellular tyrosine kinase domains (28). Transfection of a wild-type cDNA for PDGF receptors into endothelial cells, which normally lack this receptor, renders these cells chemotactically responsive to the growth factor. Mutant PDGF receptors lacking tyrosine kinase activity are inactive, suggesting that the tyrosine kinase domain is essential for this process (29). Among the other receptors that reportedly mediate chemotaxis are those for nerve growth factor, fibroblast growth factor, and fibronectin (30–32). Thus, it is possible that multiple types of receptors can mediate chemotaxis. The structures of these receptors suggest that they transmit their signals through a variety of mechanisms (33–35). As pointed out previously, the chemotaxis exhibited by fibroblasts and endothelial cells differs from that exhibited by the highly motile, amoeboid phagocytic cells. It is therefore tempting to speculate that different styles of chemotaxis might be mediated by different classes of receptors. An initial step in testing this hypothesis will be to determine whether these various receptor classes activate completely independent signaling pathways or all link to a common pathway.

G PROTEINS

The structure of the chemoattractant receptors in phagocytic cells suggests that they couple to G proteins. Consistent with this finding, cAMP can enhance both GTP[γS] binding to and GTPase activity of membrane preparations from *Dictyostelium* (36). In addition, GTP and GDP decrease the affinity of cAMP for its receptors in such preparations (2). Taken together, these data indicate that chemotaxis in response to cAMP is mediated by receptor/G protein interactions. *Dictyostelium* amoebas express five homologous G protein α subunits at different stages of the developmental program, and mutant cell lines have been generated by gene-directed homologous recombination (37–39; M. Pupillo, personal communication, J. Hadwiger, personal communication). The $G_\alpha 2$ null mutants display a phenotype similar to that of the cAR1 null cells in the sense that they are unable to carry out most cAMP-elicited responses, though their ability to carry out chemotaxis to folic acid is unaltered. In addition, membranes from $G_\alpha 2$ null cells show a decreased cAMP-mediated GTP hydrolysis and greatly diminished GTP inhibition of cAMP binding. The $G_\alpha 1$ and $G_\alpha 3$ proteins, which overlap in expression with $G_\alpha 2$, are present in the $G_\alpha 2$ null cells but cannot substitute for its function. Rescue of $G_\alpha 2$ null cells with a wild-type copy of the $G_\alpha 2$ gene reverses all the phenotypic alterations. Together, these results suggest that signals necessary for chemotaxis to cAMP are transduced through cAR1 to $G_\alpha 2$. There may be a distinct G protein for the folic acid pathway, as the $G_\alpha 2$ null cells can sense this attractant.

Several lines of evidence also support a role for G proteins in leukocyte chemotaxis. First, in membrane preparations, formyl peptide induces GTP binding and hydrolysis, and its affinity for its receptor is reduced by GTP (2). Second, preincubation with pertussis toxin, which is known to inactivate some classes of G protein α subunits, inhibits chemotaxis in leukocytes (2). Third, the formyl peptide receptor has been shown to copurify with two putative GTP binding proteins—a 40-kDa pertussis toxin substrate and a 26-kDa protein that is not modified by pertussis toxin (40, 41). Analogs of GTP such as GTP[γS] or AlF_4 elicit a pertussis toxin-insensitive increase in cytoplasmic polymerized actin in permeabilized leukocytes, also suggesting a role for a G protein (42). These results support the conclusion that in leukocytes as well as in *Dictyostelium*, chemoattractant receptors transmit their signals by coupling to one or more G proteins.

SECOND MESSENGERS

As illustrated in **Fig. 3**, a characteristic spectrum of changes in the levels of intracellular second messengers and in the modification or redistribution of transmembrane signaling and contractile proteins occurs in response to persistent stimulation with the chemoattractant, cAMP, in *Dictyostelium*. A similar set of changes occurs in leukocytes in response to chemoattractants (2). It is possible to roughly classify these changes kinetically as: rapid transient, slow transient, and persistent. These patterns are illustrated in Fig. 3 and seen to parallel the cAMP-induced changes in shape, shown diagramatically. However, the available data are derived from a variety of cell strains and experimental conditions.

There are several peaks in the fractional amount of actin in its polymerized form at 3 and 15 and 60–120 s after application of the stimulus (43). Intracellular inositol, 1,4,5 trisphosphate (IP_3), Ca^{2+}, and cGMP increase to peak levels within the first few seconds (2, 44). At 10–15 s, there is a rapid decrease in the extent of myosin heavy-chain phosphorylation, an influx of extracellular Ca^{2+}, and efflux of K^+ (2, 45, 46). During the slower phase of actin polymerization, myosin heavy and light chains are transiently phosphorylated, and a transient activation of adenylate cyclase produces intracellular and secreted cAMP (45, 47). There is also a concurrent transient phosphorylation of $G_\alpha 2$ (48). The point within the GTP hydrolysis cycle at which $G_\alpha 2$ is phosphorylated and the role of this modification (if any) in chemotactic responsiveness are not yet clear. As all these changes are occurring, cAR1 becomes gradually phosphorylated and the responses subside as this modification reaches a new steady-state level (2).

These biochemical changes seem to correlate kinetically with the series of chemoattractant-elicited cell shape changes illustrated in Fig. 1 and Fig. 3. The rapid freeze, then cringe, responses end as the rapid biochemical changes subside and the subsequent prolonged pseudopodial extension phase occurs concurrently with the slow, transient biochemical changes. After a few mintues of continuous stimulation, and corresponding to the time at which cAR1 phosphorylation plateaus, the cells repolarize and resume their prestimulus translocation behavior.

At present very little evidence bears on which of these biochemical changes are essential to and which are merely incidental to the cell shape changes and chemotactic response. It is clear from gene disruptions in *Dictyostelium* and pertussis toxin treatment in leukocytes that a surface chemoattractant

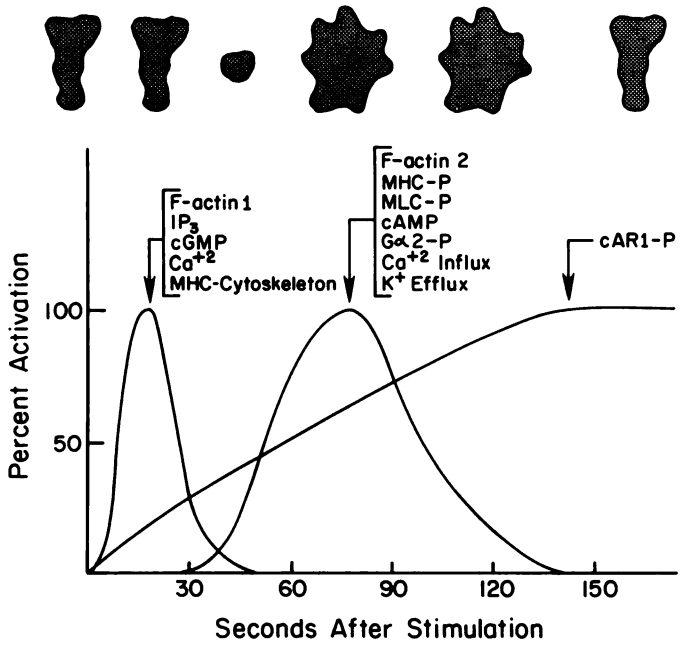

Figure 3. Responses of *Dictyostelium* amoebas to the sudden application of cAMP. The time after application of the stimulus is shown on the bottom. The graph shows the approximate times at which different biochemical responses reach their maxima. The drawings above the graph schematize the morphological changes the cells undergo at given times after application of cAMP. F-actin 1 and F-actin 2: the first and second peaks of actin polymerization. MHC-cytoskeleton: association of myosin heavy chain with the cytoskeleton. MHC-P: phosphorylation of myosin heavy chain. MLC-P: phosphorylation of myosin light chain. Gα2-P: phosphorylation of Gα2. cAR1-P: phosphorylation of cAR1. See text for further details.

receptor and its target G protein are essential components of the pathway (**Fig. 4**). The relevant target of the G protein is, at this point, unclear. A somewhat unique characteristic of these chemoattractants is the capacity to trigger activation of both phospholipase C and adenylate cyclase. Mutation or modification of a critical G protein blocks both of these activations in vivo. Thus there are a variety of alternatives to evaluate in defining the link between the G protein and the motile apparatus.

There is strong evidence that the observed activation of adenylate cyclase represents a limb that diverges from the chemotaxis pathway as the synag 7 mutation in *Dictyostelium* (which blocks activation) and adenylate cyclase inhibitors in leukocytes enhance rather than inhibit chemotactic responsiveness (2). Similarly, chemoattractant-elicited changes in ion channel activity may not be relevant because cells with collapsed membrane potential perform accurate chemotaxis (49). The potential role of the inositol pathway is more difficult to assess. Phospholipase C is a target of the chemoattractant receptor-activated G proteins, as $G_{\alpha}2$ null mutants in *Dictyostelium* and pertussis toxin-treated leukocytes fail to elevate IP_3 or cytoplasmic Ca^{2+} (50). However, in leukocytes the clamping of cytoplasmic Ca^{2+} pools (by incubating cells in Ca^{2+}-EGTA buffers in the presence of ionophore) fails to inhibit either receptor-mediated actin polymerization, motility increases or chemotaxis (2, 26). A potential role for minor or ionophore inaccessible Ca^{2+} levels cannot be excluded as it is not possible to completely deplete cells of Ca^{2+}.

Guanylate 3'-5' cyclic monophosphate (cGMP) may play an important role in the dynamic polarization of chemotaxis. Streamer mutants of *Dictyostelium* are chemotactically responsive to cAMP but exhibit a prolonged polarization phase when the gradient is withdrawn. Biochemically, these mutants are defective in cGMP phosphodiesterase and consequently exhibit prolonged elevations in cGMP when stimulated with chemoattractant (2). Furthermore, 8-bromo-cGMP and agents that increase intracellular cGMP increase the polarization of monocytes and leukocytes (2). By inference, one might expect guanylate cyclase mutants, when available, to lack polarization and exhibit impaired chemotaxis.

The other product of phospholipase C-mediated cleavage of phosphatidylinositol bisphosphate is diacylglycerol. In leukocytes, two different attractants—formyl peptide and leukotriene B4—both elicit rapid transient elevations in cellular diacylglycerol levels (51). Micromolar levels of diacylglycerol have been shown to stimulate chemotaxis of leukocytes using two different chemotaxis assays (52). This finding is suggestive of a role for diacylglycerol in chemotaxis and warrants further study in both systems. It may be important to assess whether chemoattractants stimulate the cleavage of phosphatidylcholine. In other cells, changes in diacylglycerol derived from this phospholipid appear to persist after the transient breakdown of phosphatidylinositol bisphosphate (53). Such slower changes could underlie the slower changes

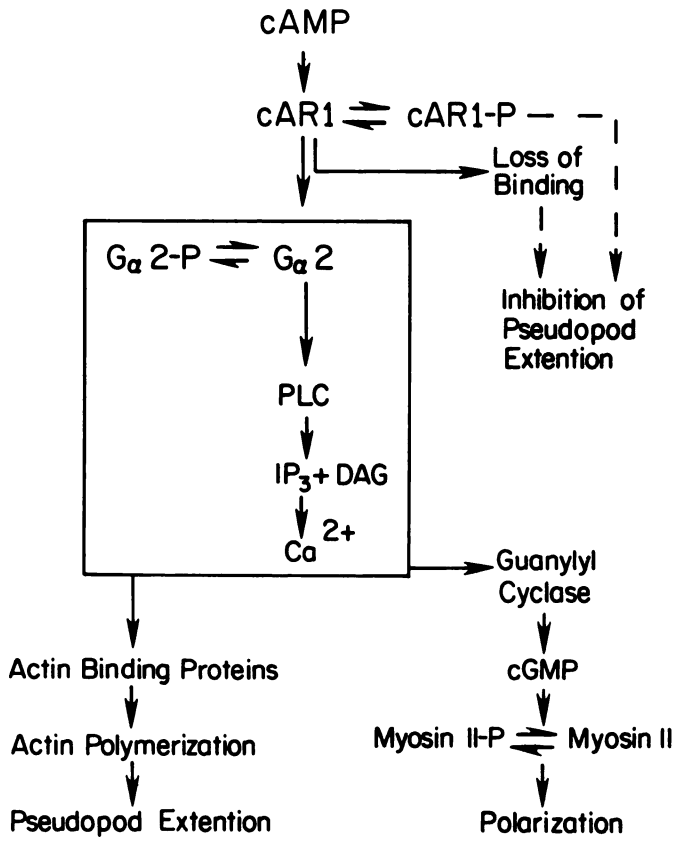

Figure 4. Schematic diagram of signal transduction events potentially leading to chemotaxis. The specific molecules indicated are those from *Dictyostelium*, but similar components are presumed to regulate PMN chemotaxis. Arrows originating on the box indicate that although Gα2 activates phospholipase C and is necessary for the indicated downstream processes to occur, the signals from Gα2 are not known. Dashed arrows represent speculative interactions.

in actin polymerization, myosin heavy and light chain, and G protein phosphorylation that correlate with the multiple pseudopod extension (spreading) phase.

Other intracellular signals have been suggested to play a role in chemotaxis. Na^+/H^+ exchange through an antiport system has been correlated with chemotaxis to formyl peptides in leukocytes (2). In *Dictyostelium* amoebas, cAMP can evoke transient H^+ efflux, possibly via activation of an H^+-ATPase (54). Although this remains a potentially important signaling mechanism, Na^+-free medium inhibits leukocyte chemotaxis only partially, and permeabilizing *Dictyostelium* amoebas by electroporation does not inhibit chemotaxis (49, 55). The significance of other signals such as phospholipid methylation, phospholipase A2 activation, increases in the levels of novel inositol phosphate species, and phosphorylation of a number of cellular proteins also remains to be determined (2).

LINK TO MOTILE APPARATUS

Regardless of the nature of the second messengers employed by chemotactic cells, they must ultimately regulate the cell motility apparatus. Our understanding of cell motility at a mechanistic level is advancing, but many questions remain unresolved (56). The components of the locomotive machinery that are currently receiving the most scrutiny are myosin heavy and light chains, actin, and various actin binding and modifying proteins. In response to cAMP, myosin heavy chain transiently becomes associated with the cytoskeleton. Phosphorylation of this molecule subsequently reverses the equilibrium between the cytoskeleton-bound and free cytoplasmic forms. In the streamer mutants mentioned previously, the phosphorylation of myosin heavy chain and its consequent dissociation from the cytoskeleton is delayed, in parallel with the delay in cGMP degradation (44). Thus, this cycling of myosin heavy chain between compartments appears to be under at least partial control of cGMP levels. However, it has been recognized that myosin heavy chain is dispensible for chemotaxis (56). Myosin heavy-chain null cells, created by homologous recombination, are still chemotactically response to cAMP, although their polarization, average velocity of translocation, and accuracy of chemotaxis are markedly compromised (57). More recently it has been demonstrated that cells lacking either myo I or abm A, members of a family of small myosins, also retain chemotactic responsiveness even though the rate of cell motility is reduced (56, 58).

It seems likely that an essential target of chemoattractant-elicited signals is the polymerization of pseudopodial actin. Lysates of *Dictyostelium* amoebas and leukocytes catalyze the polymerization of pyrene-actin in a chemoattractant-specific manner, whereas inhibition of actin polymerization by cytochalasin completely blocks chemotactic responsiveness (2, 59). In *Dictyostelium,* this activity depends on the presence of $G_\alpha 2$ (60). Conversely, removal of chemoattractant accelerates the depolymerization of pseudopodial but not cortical actin in leukocytes (61). However, all *Dictyostelium* mutants created so far that lack specific actin binding proteins retain chemotactic responsiveness (56, 62, 63). One such mutant that lacks the 120-kDa actin binding protein exhibits an abnormal morphological response to the sudden application of cAMP, but can still undergo chemotaxis in a cAMP gradient (63). It has been suggested that the ability of mutants lacking some cytoskeletal components to carry out chemotaxis is due to redundancy in function, and double mutants are being constructed to test this possibility. Recently, a soluble cAMP-regulated inhibitor of actin polymerization has been identified in *Dictyostelium* (59; J Condeelis, personal communication). The disruption of the gene for this protein may shed some light on this issue.

STRATEGIES TOWARD FURTHER UNDERSTANDING OF CHEMOTAXIS

Despite advances in our understanding of eukaryotic chemotaxis, many issues remain unresolved. For instance, how many components comprise the chemotactic machinery? What is the relationship of the sequential series of shape changes elicited by a stimulus increment to the orientation of a cell in a gradient? Where do the biochemical pathways controlling chemotactic and nonchemotactic motility converge? How is pseudopodial actin polymerization controlled by the stimulus? What is the biochemical basis of adaptation? Because of developments in the application of molecular genetics to the study of chemotaxis, the answers to many of these questions are now within reach.

Inactivation of specific genes by homologous recombination will elucidate the roles of effectors of the activated G proteins such as phospholipase C, protein kinase C, guanylate cyclase, and other proteins thought to be involved in chemotaxis. Is phospholipase C the immediate target for $G_\alpha 2$ and the analogous G protein in leukocytes? Is activation of protein kinase C part of the excitation pathway? Are cells lacking cGMP unpolarized? Is the actin polymerization inhibitor identified in *Dictyostelium* the sought-after link between cAR1 and actin polymerization? Given the unexpected results of many of the genetic disruptions created thus far, this line of experimentation promises to yield some interesting surprises.

Null cell lines could also be complemented with mutant forms of the genes of interest in order to study both structure-function relationships and the physiological roles of different protein activities. One could, for instance, complement cAR1 null *Dictyostelium* amoebas with mutant forms of cAR1. What would be the effects on chemotaxis of a cAR1 that could not be phosphorylated or did not lose ligand binding in response to cAMP? Would such a cell lose its ability to sense a cAMP gradient? Can other cARs substitute for cAR1? In $G_\alpha 2$ null cells, what would be the consequences of the expression of a $G_\alpha 2$ that could not be phosphorylated or could not couple to phospholipase C? Can other G protein-coupled receptors serve as chemoattractant receptors in the proper contexts? Can mammalian proteins substitute for *Dictyostelium* proteins at any point (or points) in the chemotactic signal transduction pathway?

Little is known regarding the spatial distribution of the key proteins and second messengers involved in chemotaxis. For example, how are cAR1 molecules distributed in the presence and absence of cAMP? Does this distribution change during chemotaxis? Do intracellular waves of Ca^{2+} emanate from a region of occupied receptors as they do in some cell types (64, 65)? The combination of video image analysis with immunological methods and with the employment of fluorescent Ca^{2+} binding compounds could be used to answer these questions.

Finally, it is likely that crucial players are missing. More chemotaxis-deficient *Dictyostelium* cell lines need to be mapped to complementation groups and characterized. The recent advent of methods to efficiently transform *Dictyostelium* amoebas with genomic DNA libraries has made the rescue of these mutants, and consequently the isolation of their

defective genes, imminent (66). The future prospects for understanding this fundamental and fascinating process at a molecular level are now very strong. FJ

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