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CHEMOTAXIS IN EUKARYOTIC CELLS: A Focus on Leukocytes and *Dictyostelium*

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OVERVIEW

Chemotaxis, cell motion directed by external chemical gradients, is a phenomenon of widespread occurrence and significance. Chemotaxis has been reported in the following eukaryotic cells: free-living microorganisms,

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leukocytes (inflammation), endothelial cells (angiogenesis), sperm (fertilization), neuronal growth cones (neurogenesis), fibroblasts (wound healing), and thymocytes (embryogenesis) (Singer & Kupfer 1986). It is perhaps most clearly displayed and extensively studied in cells of the immune system and the cellular slime molds.

We have limited our discussion to leukocytes and *Dictyostelium discoideum* since they display strong chemotactic responses to well-defined stimuli. Although these two cell types are highly specialized for different roles, there are remarkable similarities in their chemotactic responses. Polymorphonuclear leukocytes (PMN) and macrophages are specialized to migrate to sites of inflammation and carry out cytocidal functions. Chemotactic factors include *N*-formylated peptides (NFP) such as f-Met-Leu-Phe, leukotriene B_4 (LTB_4), platelet activating factor (PAF), and a cleavage product of complement component 5 (C5a) (Sha'afi & Molski 1987). *D. discoideum* amoebae (DDA) spontaneously aggregate to form multicellular structures when induced to differentiate by removal of nutrients. Chemotactic factors include pterines, which apparently serve as cues for nutrient localization, and adenosine 3′,5′-monophosphate (cAMP), which mediates the aggregation (Van Haastert & Konijn 1982). The rapid rates of locomotion, the reproducible responses to chemoattractants, and the ease of preparation of large quantities of identical cells make these systems attractive for studies of chemotaxis. In addition, the small (40,000 kb) haploid genome of DDA allows the rapid scoring and mapping of recessive mutations. In addition, mutation by homologous DNA insertion is now possible (Devreotes et al 1987; Van Haastert & Konijn 1982; Nellen et al 1987; Loomis 1987; De Lozanne 1987; Segall et al 1987).

Eukaryotic chemotaxis is an intriguing biological phenomenon that, at present defies biochemical description. This review summarizes chemotactic behavior and presents three simple schemes to illustrate features of sensing that must be explained in biochemical terms. Responses induced by chemoattractants are outlined and evaluated as a means to accomplish features of chemotaxis highlighted in the simple schemes. We then attempt to integrate the current knowledge (or ignorance) into a more realistic scheme and to identify areas where further information is needed.

FEATURES OF CHEMOTACTIC BEHAVIOR

DDA and PMN are amoeboid cells that move by pseudopod extension. Chemotaxis is achieved by orienting the direction of locomotion along a chemoattractant gradient. The orientation results from the preference of pseudopod extension toward the higher chemoattractant concentration (Zigmond 1974). Efficient translocation requires coordination of motile

activities, which is achieved by cell polarization. Pseudopod formation is favored at the anterior; the posterior contracts to form a uropod (Zigmond et al 1981; Swanson & Taylor 1982). Polarity, which is more pronounced in PMN than DDA, develops in the absence of a chemoattractant gradient. The polarity modulates the effects of chemoattractants, and chemoattractants can compromise or modify endogenous polarity. Local application of attractant from a micropipette elicits pseudopods from the near side of the cell, which results in turning and moving toward the source. While moderate concentrations of attractant preferentially stimulate pseudopods from the front of a cell, a steep gradient applied at the rear of the cell can induce pseudopod formation from the uropod (Gerisch et al 1975; Gerisch & Keller 1981; Claviez et al 1986). High uniform concentrations of attractants induce pseudopod formation over much of the surface, decreasing the rate of locomotion (Zigmond & Sullivan 1979; Painter et al 1984a; Shields & Haston 1985; Klein et al 1985b). Removal of attractants induces pseudopod retraction (Zigmond et al 1981); however, at least in DDA, simultaneous addition of even a low concentration of a second attractant and removal of a high concentration of the first evokes further pseudopod extension (Van Haastert 1983a; Fontana et al 1986).

The accuracy of orientation depends on the mean concentration and steepness of the chemoattractant gradient. Highest sensitivity is observed at mean concentrations near the apparent dissociation constant, K_D, of the receptor-attractant complex. At this concentration, a 2% change in concentration over 10 μm (cell length) can be detected (Mato et al 1975; Tranquillo et al 1988). Steeper gradients are required to observe orientation in mean concentrations several orders of magnitude above and below K_D. These observations suggest that the accuracy of the orientation correlates with the difference in number of occupied receptors across the cell (Zigmond 1981). Thus, a cell orients equally well in gradients where the number of occupied receptors at its ends varies from 700–900 and from 10,700–10,900. In this regard, sensing by eukaryotic cells is analogous to sensing in bacteria, where response is proportional to the change in receptor occupancy that occurs upon displacement along the gradient (Spudich & Koshland 1975). A cell can orient in a gradient of one chemoattractant when a homogeneous concentration of a second attractant is also present. Thus, DDA orient in a cAMP gradient in the presence of a homogeneous concentration of folic acid and vice versa (Van Haastert 1983b). PMN orient in a NFP gradient in the presence of LTB_4, but in the presence of homogeneous NFP, orientation to LTB_4 is blocked (S. H. Zigmond, unpublished observations).

Both PMN and DDA can respond to stable spatial gradients established

between a source and sink of attractant. Chemotaxis is observed at any point when the concentration can be considered constant [i.e. the concentration changes only about 0.0001%/minute (D. A. Lauffenburger, personal communication)]. Studies concluding that stable gradients can not induce chemotaxis used insufficiently steep gradients or cells that had previously been exposed to an attractant (Vicker et al 1984, 1986; Lauffenburger et al 1987). Cells can also respond to moving gradients. During aggregation, DDA respond to waves of cAMP that have a half-width of 100 μm and move at 300 μm/min (Tomchik & Devreotes 1981; Devreotes et al 1983). During a wave's approach, the average gradient rises; the back edge of the cell continually encounters the concentration that the front experienced two seconds earlier. As the wave passes, the gradient direction is reversed and the mean concentration falls. Cells orient along the rising gradient but not along the reversed, receding gradient. These natural, moving gradients are probably very steep (7% changes over 10 μm and peak concentrations saturate receptors).

Both PMN and DDA rapidly degrade their chemoattractants (Aswanikumar et al 1976; Malchow & Gerisch 1974; Yuli & Snyderman 1986). The ability to degrade the attractants is important physiologically in order to steepen gradients and possibly to limit the duration and extent of signaling. However, attractant degradation is not necessarily part of the chemotactic mechanism. In DDA, chemotaxis occurs toward non-hydrolyzable analogs of cAMP and cAMP in the presence of DTT, which inhibits cAMP phosphodiesterase (Van Haastert 1983b).

HOW DO CELLS ACCOMPLISH CHEMOTAXIS?

Critical Questions

The behavior of DDA and PMN raises issues that must eventually be understood in biochemical terms. These issues include defining the mechanisms of gradient detection and cell locomotion:

- How is the directional response achieved?
- What allows the response to be proportional to the difference in receptor occupancy?
- What accounts for the extraordinary sensitivity of the gradient detection?
- Is there amplification between stimulus and response?
- At what point do pathways for different chemoattractants merge?

Hypothetical Schemes for Chemotaxis

Figure 1 (*A*, *B*, and *C*) presents several hypothetical, simplified schemes for chemotaxis that provide a framework for discussion of the key issues.

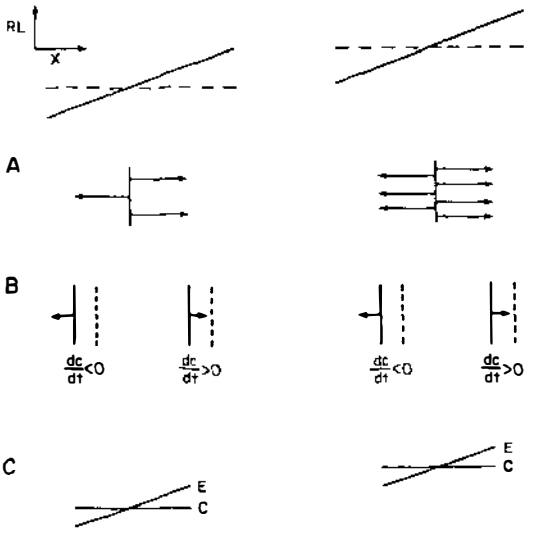

Figure 1 Hypothetical mechanisms of gradient sensing. Shown at top, in units of receptors occupied with ligand (RL), are two gradients of identical slope; the midpoint of the gradient on the right is higher. Diagrams in each column show the cell response to the corresponding gradient. Three independent schemes are illustrated (*A*, *B*, and *C*). (*A*) Horizontal arrows, extending from the vertical midline to the boundaries of the cell, represent actin filaments, which pull the cell in the direction of arrows. (*B*) Solid vertical lines delineate initial boundaries of cell; horizontal arrows represent pilot pseudopods. Dotted vertical lines represent new boundaries of the cell following response to information from pilot pseudopods, i.e. $dC/dt < \text{or} > 0$. (*C*) Slanted or horizontal lines represent concentrations within cell of excitatory (E) and counteracting (C) signals.

Although each scheme is discussed separately, they are not mutually exclusive.

A. (Figure 1*A*) Each occupied attractant receptor activates a contractile element that pulls the cell in that direction. The resulting tug-of-war produces a net mechanical force in the direction of the highest receptor occupancy. The force is proportional to the difference in occupancy and is independent of the mean occupancy. A similar scheme for polarization of net electrical charge can be envisioned by linking each occupied receptor to generation of a charge that induces pseudopod formation.

B. (Figure 1*B*) A cell extends pilot pseudopods in random directions. Those extended up the gradient experience an increase in receptor occupancy and are reinforced, while those extended down the gradient experience a decrease in receptor occupancy and are withdrawn.

Because each region of the cell surface responds only to changes in its level of receptor occupancy, the responses are independent of the mean level of receptor occupancy.

C. (Figure 1*C*) Receptor occupancy generates both excitatory and counteracting signals. The excitatory signal that stimulates pseudopod extension remains localized near the occupied receptor, while the counteracting signal distributes throughout the cell. The concentration of the counteracting signal is specified by the mean receptor occupancy. The persistent excess of an excitatory signal at the high side of the gradient and of a counteracting signal at the low side of the gradient restricts pseudopod extension to the high concentration side of the cell. This asymmetry is independent of the mean level of receptor occupancy.

THE DIRECTIONAL RESPONSE In scheme A, directional motile response is achieved by mechanical or electrical constraints; since both mechanical and electrical forces give a net directional force, a positive response at the front necessitates an inhibitory one at the rear. In scheme B, the directional response arises from the relative strengths of each locally evoked signal; there is essentially no communication between different regions of the cell. This scheme fails to explain the ability of cells to orient in rapidly rising or falling gradients since the front and back of the cell respond independently. In scheme C, communication is achieved by the distribution of the counteracting signal; the response is limited to regions where the stimulus is above the mean level.

An excitatory signal that induces a pseudopod must be localized. For instance, in scheme A, an occupied receptor may directly activate a contractile element. The excitatory signal would then be highly localized. In schemes B and C, a second messenger cascade connects occupied receptors to pseudopod extension. If these messengers rapidly equilibrate throughout the cell before acting, the directional information will be lost. To remain localized, the signal molecule must have a short space constant, determined by the product of its half-life and diffusion coefficient. [A freely diffusing molecule of $M_r = 1000$ ($D = 10^{-7}$ cm^2/sec) with a half-life of one second would have a space constant of about 3 μm (Segall et al 1985; D. Lauffenburger, personal communication).] Binding to its target or other cytoplasmic components could alter this estimated space constant. Mediators, which must communicate throughout the cell, such as the counteracting signal in scheme C, should have large space constants. Activation of counteracting signals can help localize the excitatory response.

DIFFERENCE IN RECEPTOR OCCUPANCY In each scheme the magnitude of the directional response is proportional to the absolute change in receptor

occupancy and is independent of the mean level. The net directional signals or forces in the right- and left-hand columns of Figure 1 are equal even though the mean levels of occupancy differ; this is achieved by mechanisms that allow the mean level of occupancy to be ignored. In scheme A, this is achieved by linking opposing mechanical forces. In Scheme B, only pseudopod excursions that encounter increases in occupancy generate new responses. In scheme C, the counteracting signal limits responses to regions with receptor occupancy levels above the mean.

Mechanisms that allow persistent stimuli to be ignored are often referred to as adaptation. Here, adaptation is defined as a reversible, time-dependent adjustment of sensitivity to the current level of receptor occupancy such that the evoked response ceases. Adaptation is either local (the sensitivity is adjusted to regional levels of receptor occupancy as in scheme B) or global (the sensitivity is adjusted to the mean occupancy of the entire cell). The counteracting signal of scheme C could result from global adaptation after the transient response has declined.

SENSITIVITY The ability of cells to detect small changes in attractant concentration restricts the number, affinity, and kinetic properties of chemoattractant receptors. In low attractant concentrations, random fluctuations in the number of receptors occupied within a given cell surface area limit the precision with which the local concentration of attractant is measured. The accuracy and, therefore, the sensitivity of gradient detection can be increased by: increasing the receptor density or the area involved in sampling, increasing the number of times a receptor resamples the medium in a given time period, or increasing the time period over which the cell integrates the occupancy information (Lauffenburger 1982; Tranquillo et al 1988). While schemes A and C utilize a large fraction of the receptors, scheme B is limited by the number of receptors present on the test pseudopod. Schemes A and B also place time constraints on the integration process. In scheme A, the number of activated filaments fluctuates with occupancy; in scheme B, a decision must be made during the advance time of the pseudopod.

AMPLIFICATION The extraordinary sensitivity of chemotaxis suggests that there are amplification steps in the signal transduction process. In scheme A, no amplification is necessary as long as the net force generated is sufficient to give direction to the cell movement. Schemes B and C are particularly suited for amplification. In these two schemes, the motile apparatus can be highly responsive to a specific concentration of signal generated by small changes in receptor occupancy since the magnitude of the local (scheme B) or mean whole cell (scheme C) signal is always reset to a basal level.

PATHWAYS FOR DIFFERENT CHEMOATTRACTANTS All three schemes can account for the ability to sense a gradient of one attractant in the presence of a uniform concentration of another provided that the effector systems are not saturated. However, the ability of a second attractant to evoke pseudopod formation in DDA when added at the moment of removal of the first indicates that the site of adaptation must be early in the transduction pathway. This places constraints on schemes B and C, which utilize adaptation to ignore the current level of receptor occupancy.

RESPONSES INDUCED BY ADDITION OF CHEMOATTRACTANTS

Physiological Responses

In order to identify components of cell physiology related to chemotaxis, we compare the responses induced by addition of chemoattractants to these evolutionarily diverse cell types. The spectrum of elicited responses, although complex, is surprisingly similar in both cell types.

Attractants stimulate shape changes, locomotion, adhesion, secretion, and pinocytosis (Table 1). The shape changes of DDA are illustrated in Figure 2. Within a few seconds following a large increment in attractant concentration, DDA round or "cringe" for about 20–30 seconds (Futrelle et al 1982; Klein et al 1985b; Fontana et al 1986). In PMN, a contraction

Table 1 Physiological responses induced by chemotactic factors[a]

Response	PMN	DDA	Kinetic type
Orientation in a gradient[b]	+	+	P
Shape changes[c]	+	+	
Cringe	N	+	T
Pseudopod extention	+	+	T/P
Polarization	+	+	P
Chemokinesis[d]	+	+	P
Adhesion[e]	+	+	T
Secretion[f]	+	+	T
Pinocytosis[g]	+	+	T/P

[a] Observed responses are indicated as +. Persistent or transient responses are indicated as P or T. T/P indicates a response showing both transient and persistent features. N indicates not determined. References cited are in addition to those cited in the text.

[b] See Konijn 1970; Zigmond & Hirsch 1973.

[c] See Davis et al 1982; Lewis 1934; Painter et al 1984a; Yuli & Snyderman 1984.

[d] Allan & Wilkinson 1978.

[e] See Boxer et al 1979; Smith et al 1979a; Smith et al 1979b.

[f] See Becker et al 1974; Korchak et al 1984a,b,c.

[g] See Niedel et al 1979; Davis et al 1982.

can be seen on elongated cells. Within about 30 seconds, cells spread on the substrate and extend lamellae or ruffles in random directions, a response lasting several minutes with high concentrations of chemoattractant. The ruffles then become localized and cells begin to translocate. In response to small increases in chemoattractant, cells directly form localized ruffles; the rounding and spreading steps are not apparent. Cells treated in suspension also exhibit these responses and develop a polarized morphology (Zigmond & Sullivan 1979; Stephens & Snyderman 1982). When chemoattractants are removed or diluted, pseudopods are withdrawn, surface blebbing occurs, the cells round, and (in DDA) then resume random motility (Zigmond & Sullivan 1979). In DDA, these transient spreading responses can be repeatedly elicited by switching the stimulus between folic acid and cAMP (Fontana et al 1986).

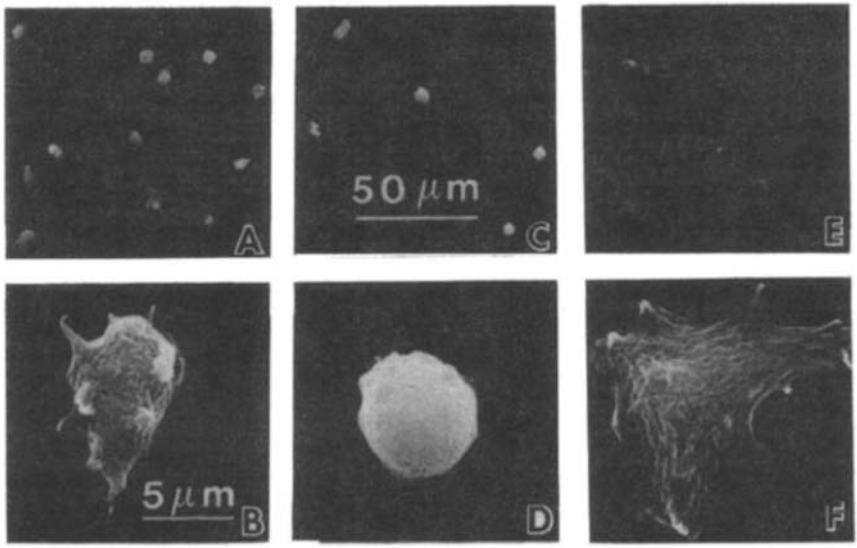

Figure 2 Cyclic AMP-elicited shape changes. NC-4 cells were starved for 5.5 hr, settled on glass, and perfused with phosphate buffer. Cells were prepared for scanning electron microscopy by rapid fixation with OsO_4 as described elsewhere (Condeelis et al 1988). Fixation was done either 30 sec before (*A*, *B*) or 25 sec (*C*, *D*) and 60 sec (*E*, *F*) after the perfusion buffer was abruptly switched to one containing 10–6 M cAMP. *A*, *C*, and *E* show fields of cells, while *B*, *D*, and *F* show representative cells at higher magnification. Before stimulation with cAMP cells have a morphology typical of normal motile amoebae (*A*, *B*). However, cells respond to cAMP in discrete morphological stages beginning with rounding (*C*, *D*), followed by dramatic protrusive activity and flattening (*E*, *F*), followed by the return to normal amoeboid morphology typical of cells before cAMP stimulation (Hall et al 1988).

In both cell types, the presence of a chemoattractant stimulates the rate of locomotion, a response known as chemokinesis. Chemokinesis is most obvious in PMN, which are immobile in the absence of a stimulant. In PMN, the rate of locomotion is optimal when the chemoattractant concentration is somewhat less than K_D (Becker 1980). DDA are motile in the absence of chemoattractant (Potel & MacKay 1979); however, acceleration in response to increasing concentrations applied in vitro and in vivo (by approaching cAMP waves) is readily measurable (Varnum et al 1985; Vicker et al 1984, 1986; MacKay 1978).

A rapid and transient increase in adhesion of PMN has been monitored by measuring cell-cell aggregation (O'Flaherty et al 1979). A chemoattractant-induced increase in the number of adhesion molecules, including CR3 and CR1, and at the cell surface may help mediate this response (Anderson & Springer 1987). Cells lacking these adhesion proteins do not aggregate upon addition of chemoattractant. A similar light-scattering response is observed in DDA (Gerisch & Hess 1974), and the cells exhibit increased adhesion to agar substrates during chemotaxis (P. N. Devreotes, unpublished observation).

Secretion and pincoytosis are stimulated by the addition of chemoattractants. PMN produce and secrete superoxide and LTB_4 (Sha'afi & Molski 1987). PMN also secrete the contents of specific and azurophilic granules; the release of these products is greatly enhanced by the presence of cytochalasin (Henson et al 1978). In DDA, the only known secretory product is cAMP (Dinauer et al 1980a) although the attractant stimulates the appearance of numerous submembranous vesicles, some continuous with the plasma membrane (Maeda & Gerisch 1977). The vesicles may be involved in secretion of additional products or in pinocytosis, which is stimulated by an attractant in PMN (Table 1; Sullivan & Zigmond 1980).

It is intriguing that chemoattractants induce all these physiological responses in both DDA and PMN. It is possible that each response arose independently during evolution to serve the needs of the cells. Alternatively, this array of responses may be closely related to the basic mechanism of chemotaxis. The formation of pseudopods and the development of cell polarity are clearly necessary for the chemotaxis. Chemokinesis could result from an increased frequency or rate of pseudopod formation. Secretion and pinocytosis are considered essential in membrane flow models of cell locomotion where at the cell front fusion of vesicles provides membrane for lamellipodia extension and at the rear pinocytosis retrieves the membrane for recycling (Pfeiffer et al 1980; Bretcher et al 1987; Singer & Kupfler 1986). Increased adhesion may be characteristic of a newly formed pseudopod (Smith & Hollers 1980).

Underlying Biochemistry

The biochemistry underlying the parallel physiological responses of DDA and PMN is also similar. Information on key molecular components and on the early events following binding of chemotactic factors is rapidly increasing and has recently been reviewed (Becker et al 1986; Sha'afi & Molski 1987; Snyderman et al 1987; Omann et al 1987a; Janssens & Van Haastert 1987; Gerisch 1987). Table 2 summarizes the intracellular changes triggered by chemoattractants in PMN and DDA. Table 3 summarizes observations testing which events are essential for chemotaxis.

CHEMOATTRACTANT RECEPTORS The first molecular event in chemotaxis is the interaction of the chemoattractant with its surface receptor. Both DDA and PMN express receptors for several different chemoattractants. These receptors all elicit a similar characteristic array of responses. Recent cloning of the cAMP receptor in DDA indicates that chemoattractant receptors share structural and functional properties with the class of hormone and neurotransmitter receptors linked to guanine nucleotide binding proteins (G proteins).

In DDA, cell surface cAMP binding proteins have been identified by specific photoaffinity labeling of intact cells with 8-N_3-cAMP. The major photolabeled protein, which appears as a doublet (M_r = 40,000-43,000) in SDS PAGE, was purified to homogeneity, and a specific antiserum was raised (Juliani & Klein 1981; Theibert et al 1984; Klein et al 1987a,b). The antiserum was used to isolate the receptor cDNA. The nucleotide sequence predicts an open reading frame of 392 amino acids containing 7 domains enriched with hydrophobic residues; the seventh domain can be wound as an amphipathic helix. The C-terminal third of the molecule of hydrophilic; 13 of the last 33 amino acids are serine and threonines, which represent potential phosphorylation sites (Klein et al 1988). These features of the primary sequence suggest that the chemoattractant receptor traverses the lipid bilayer seven times in a pattern similar to other receptors that interact with G proteins, such as rhodopsin, β-adrenergic, and muscarinic acetylcholine receptors (Dohlman et al 1987). This structure is consistent with the growing evidence that chemotactic signals are transduced via G proteins, and that phosphorylation of the receptor plays a major role in its function (see below). The amphipathic helix may form a portion of the cAMP binding site. Alternatively, it may suggest that the receptor functions as part of an ion channel.

Analogs of *N*-formylmethionylleucylphenylalanine (FMLP) label a broad band of proteins (M_r = 55,000–70,000) in membranes and detergent extracts of PMN (Niedel et al 1980; Niedel 1981; Schmitt et al 1983; Painter et al 1982; Marasco et al 1985). Only about half of the mass of this

Table 2 Biochemical responses induced by chemoattractants[a]

Response	PMN	DDA	Kinetic type
Receptor alterations[b]			
Insertion of new receptors	+	+	P
Receptor down-regulation	+	+	P
Receptor redistribution	+	+	P
Receptor modification	N	+	P
Alteration of second messengers levels[c]			
IP3 (or other IP metabolites)	+	+	T
Arachidonic acid	+	N	N
Diacylglycerol	+	N	T
Phosphatidic acid	+	N	T
Decreased pH	+	N	T
Increased pH	+	+	T
Ca^{2+}	+	+	T
cAMP	+	+	T
cGMP	–	+	T
Ion fluxes[d]			
Calcium influx	+	+	T
Calcium efflux	+	+	P
Sodium influx	+	N	P
Potassium efflux	–	+	P
Proton efflux	+	+	P
Phosphorylation[e]			
Myosin heavy chain	N	+	T
Myosin light chain	+	+	T
80 kDa, 67–69 kDa, 59–60 kDa			
47–50 kDa, 45 kDa, 40 kDa, 27 kDa	+	N	N
18 kDa	+	N	N
30 kDa	N	+	N
Enzymes activated[f]			
A-cyclase	■	+	T
G-cyclase	N	+	T
Phosphodiesterase	+	+	N
Na/K-ATPase	+	N	N
Phospholipase C	+	+	T
Phospholipase A2	+	N	N
Protein kinase C	+	N	N
Methylation	+	+	T
Oxidative metabolism	+	N	N
Glycogen phosphorylase	+	N	T
Glucose transport	+	N	P
Changes in proteins of motile apparatus[g]			
Actin polymerization	+■	+	T
Actin nucleation	N	+	T

Table 2 (*continued*)

Response	PMN	DDA	Kinetic type
Changes in proteins of motile apparatus[g] (*continued*)			
Tyrosinylation of tubulin	+	*N*	*N*
Increase in microtubules	+	*N*	*N*
Reactivation of gelsolin	+	*N*	*T*
Association with cytoskeleton[h]			
Actin	+	+	T
Myosin	−	+	T
Protein kinase C	+	N	N
65-kDa protein (acumentin?)	+	N	N
Chemoattractant receptor	+	N	N

[a] Observed responses are indicated as +. Persistent or transient responses are indicated as P or T. T/P indicates a response showing both transient and persistent features. N indicates not determined. − indicates no observable change. References cited are in addition to those cited in the text.

[b] See Weinberg et al 1981; Perez et al 1986a,b; Davis et al 1982; Sullivan et al 1984; Niedel et al 1979; Jesaitis et al 1982, 1985; Lubs-Haukeness & Klein 1982; P. Klein et al 1985; C. Klein et al 1985; Devreotes & Sherring 1985; Wang et al 1988.

[c] See Ohta et al 1985; Andersson et al 1986b; Cockcroft et al 1985; Serhan et al 1983; Bradford & Rubin 1987; Lew et al 1986, 1987; Wynkoop et al 1986; Nokoch & Gilman 1984; Simchowitz 1985a,b; Faucher & Naccache 1987; Sha'afi et al 1982; Naccache et al 1977; Lew et al 1986; Andersson et al 1986a; Becker 1980; Korchak et al 1984b; Sklar & Oades 1985; Prentki et al 1984; Simchowitz et al 1983; Jackowski & Sha'afi 1979; Bokoch & Gilman 1984; Smith & Ignarro 1975; Yuli & Oplatka 1987; Dinauer et al 1980a,b,c.

[d] See Naccache et al 1977; Aeckerle et al 1985; Simchowitz 1985a; Andersson et al 1986a; Naccache et al 1977; Gallin & Gallin 1977; Malchow et al 1978, 1982; Wick et al 1978; Korchak et al 1984a.

[e] See Painter et al 1984a; White et al 1984; Huang et al 1984; Hayakawa et al 1986; Schneider et al 1981; Rahmsdorf & Gerisch 1978; Hirata 1981; Mato & Malchow 1978.

[f] See Grady & Thomas 1986; Naccache et al 1977; Ohta et al 1985; Hirata et al 1978; Bormann et al 1984; White et al 1984; Schneider et al 1981; McPhail et al 1984; Huang et al 1983; Pike & Snyderman 1981; Becker 1980; Korchak et al 1984c; Sklar et al 1985b; Slonczewski et al 1985; McCall et al 1979; Mato & Marin-Cao 1979; Van Waarde & Van Hoof 1985.

[g] See Rao & Varani 1982; Sklar & Oades 1985; Yassin et al 1985; Howard 1985a; Howard & Wang 1987; Carson et al 1986; Painter et al 1984a; Hoffstein et al 1977; Painter et al 1984b; Boxer et al 1979; Hall et al 1988.

[h] See Yassin et al 1985; Pike et al 1986; Jesaitis et al 1985.

glycoprotein is the core polypeptide, which by electrophoresis displays a discrete band of protein (M_r = 32,000) consisting of two isoelectric forms (Malech et al 1985; Heiman et al 1986). Partially purified FMLP receptors have been incorporated into phospholipid vesicles with reconstitution of binding activity (Hoyle & Freer 1984; Allen et al 1986). Cross-linking of [^{125}I] C5a to human PMN has identified a polypeptide (M_r = 40,000) as the putative C5a receptor (Rollins & Springer 1985). The receptor appears to coisolate with a second protein (M_r = 40,000) (Rollins et al 1988). It is anticipated that these polypeptides have the prototypic structure of seven membrane spanning domains. It will be interesting to learn whether features, such as the amphipathic helix, are unique to chemoattractant receptors.

Table 3 Summary of key observations of chemotactic response

Component	Evidence	Effect on chemotaxis
G proteins	Pertussus toxin (PMN) and Frigid A mutant (DDA)	Complete inhibition
Actin polymerization	Cytochalasin (PMN)	Complete inhibition
Centrioles[a]	Cytoplasts (PMN)	Normal
Microtubules	Colchicine and nocodazole	Slight inhibition
Myosin	Transformants lacking MHC II (DDA)	Slight inhibition
Actin binding proteins	Transformants lacking α-actinin, severin, 120 kDa	Normal
Cytoplasmic calcium[b]	Calcium ionophore plus EGTA (PMN)	Normal; (TMB-8 and Quin-2 plus EGTA do inhibit chemotaxis)
Sodium influx[c]	Sodium free medium	Slight inhibition
pH elevation[d]	Inhibits Na^+-H^+ exchange	Inhibited
cAMP elevation	*Synag 7* mutants (DDA) and A-cyclase inhibitor (PMN)	Normal
cGMP elevation[e]	Streamer F mutant (DDA) and cGMP analogs (PMN)	Enhanced
Methylation[f]	Methylation inhibitors	Normal

[a] See Keller & Bessis 1975; Malawista & de Boisfleury 1982.
[b] See Marasco et al 1980; Elferink & Deierckauf 1985; Meshulam et al 1986; Europe-Finner et al 1984; Zigmond et al 1988.
[c] See Showell & Becker 1976; Naccache 1977; Zigmond 1977; Zigmond et al 1985.
[d] See Simchowitz & Cragoe 1986.
[e] See Estensen et al 1973; Stephens & Snyderman 1982.
[f] See Garcia-Castro et al 1983.

The functional properties shared by the chemoattractant receptors must be taken into account in schemes for chemotaxis. All appear to be relatively rare surface proteins, with rapid dissociation rates, and to interact directly with G proteins.

Binding sites Both cell types widely modulate the number of displayed surface binding sites. Circulating (or unactivated) PMN have 3,000 exposed NFP sites, activation by a variety of stimuli exposes up to 100,000 sites, and additional sites are revealed upon cell homogenization (Tsung et al 1980; Zimmerli et al 1986). Sensitive DDA display about 50,000 sites for cAMP under physiological conditions. Additional sites (3- to 5-fold) can be unmasked by calcium or high ionic strength treatments (Janssens & Van Driel 1984; Van Haastert 1985). Receptor expression increases during development or maturation in both cell types, as does the capacity to carry out chemotaxis.

Dissociation rates In intact cells and isolated membranes, dissociation of bound attractant is multiphasic. In PMN, binding sites for NFP have affinities of 2–25 nM (Snyderman et al 1984; Sha'afi & Molski 1987). The majority of the sites dissociate with a $t_{1/2}$ of less than 2 min (Sklar et al 1984; Omann et al 1987a). In DDA, the majority of cAMP binding sites dissociate rapidly ($t_{1/2}$ = 1–3 sec) and interconvert within 10 sec between high and low affinity forms (K_D = 50 nM and 400 nM). A smaller fraction release ligand more slowly ($t_{1/2}$ = 15 sec and 150 sec) and display a single affinity of 5–15 nM (Janssens & Van Haastert 1987). Prolonged exposure of either cell type to attractant results in the association of radioactivity with components that dissociate extremely slowly ($t_{1/2}$ = 100 min) (Klein 1979; Jesaitis et al 1984; Zigmond & Tranquillo 1986). The multiplicity of receptor forms may arise from different conformations of the same polypeptide.

Effects of guanine nucleotides Guanine nucleotides regulate the affinity of chemoattractant binding sites. In PMN and macrophage membranes, an initial mixture of high and low affinity sites (25% with K_D = 0.1–1.5 nM and 75% with K_D = 25 nM) is converted to low affinity sites by GTP and GDP (Koo et al 1982, 1983; Snyderman et al 1984). In permeabilized PMN, GTP accelerates the half-time for dissociation from 2 min to 10 sec (Sklar et al 1987). The effect is reversible upon removal of the guanine nucleotides. In DDA, guanine nucleotides reduce the affinity and accelerate the dissociation of cAMP from all receptor forms (Van Haastert 1984; Janssens et al 1985, 1986; Van Haastert et al 1986; Khachatrian et al 1987). The maximal effect of guanine nucleoside triphosphates is 85%, while that of GDP is 25–50%.

SIGNAL TRANSDUCTION EVENTS FOLLOWING ATTRACTANT BINDING

Guanine nucleotide binding proteins Several lines of evidence suggest that the initial event triggered by binding of attractant to the receptor is activation of a guanine nucleotide binding protein(s). The reduction of affinity of attractants caused by guanine nucleotides indicates a direct interaction of receptors with a G protein. Chemoattractants stimulate GTPase activity in isolated membranes (Hyslop et al 1984; Okajima et al 1985; Matsumoto et al 1986; Feltner et al 1986; Snaar-Jagalska et al 1988a). In vitro, occupancy of one NFP receptor stimulates hydrolysis of 5–25 molecules of GTP per minute. The GTPase activity stimulated by a saturating concentration of NFP is not increased by addition of C5a, which suggests that the receptors share a common pool of G protein (M. W. Wilde, S. H. Zigmond, manuscript in preparation). Chemoattractant-induced responses are blocked if the relevant G protein is inhibited (Becker et al 1986). In PMN, G proteins can be functionally inhibited with islet

activating protein of pertussus toxin, which ADP ribosylates α-subunits of G proteins (M_r = 40,000 and 41,000) designated G_i-2 and G_i-3 (Table 3). Both are expressed in the HL-60 cell line (Murphy et al 1987). Two α-subunits and a β-subunit are expressed in DDA (M. Pupillo et al, manuscript in preparation; P. Lilly & P. Devreotes, manuscript in preparation). In a DDA mutant, frigid A, the effects of GTP on cAMP binding are greatly reduced (Coukell et al 1983; Kesbeke et al 1988). Although frigid A expresses chemoattractant receptors that undergo ligand-induced modification, all other physiological responses, as well as chemotaxis, are lost (Table 3). Preliminary evidence suggests that the frigid A locus is one of the α-subunits. It will be interesting to learn whether any of these G proteins are specific for chemotaxis.

Phospholipid-induced messengers An immediate target of the receptor activated G protein is believed to be phospholipase C (PLC) (Smith et al 1985). Its activation results in hydrolysis of phosphotidylinositol bisphosphate (PIP_2) and liberation of inositol triphosphate (IP_3) and diacylglcerol (DAG). IP_3 releases calcium from internal storage sites while DAG activates protein kinase C (PKC). This scheme is supported primarily by observations of leukocytes where a mix of A23187 and phorbol myristyl acetate (PMA), which elevate cytosolic calcium and activate PKC, can bypass pertussis intoxication and stimulate O_2 production (see Sha'afi & Molski 1987). Activated DDA are reported to produce IP_3 (Europe-Finner & Newell 1987a,b), and in saponin-permeabilized cells, IP_3 can directly elicit several receptor-mediated responses (Europe-Finner & Newell 1985, 1986a,b). In addition, DDA expresses a family of PKC genes (J. Williams, personal communication).

Calcium levels elevated from internal stores are further increased by the influx of calcium from the medium. Internal calcium also opens a nonspecific cation channel that allows the flux of sodium and potassium (Von Tscharner et al 1986). The elevated cytoplasmic calcium is expected to activate calcium-calmodulin dependent protein kinases, phospholipase A2 (PLA2), and actin binding proteins (Lew et al 1986a). However, the significance of these ion fluxes for chemotaxis is difficult to evaluate since cells carry out chemotaxis in sodium- and calcium-free media (Table 3) (Marasco et al 1980).

The cascade of events triggered by DAG can be partially mimicked by phorbol esters and soluble DAGs such as 1,2-dioctanylglycerol (DiC8). Although the essential kinases and substrates are unknown, both FMLP and PMA stimulate the parallel phosphorylation of a series of bands (pp67, pp60, pp50) (Huang et al 1983; Andrews & Babior 1983) and lipomodulin,

whose phosphorylation appears to reduce its inhibitory activity against PLA2 (Hirata 1981). One of the consequences of the PKC-catalyzed phosphorylations is the activation of an ameloride sensitive Na^+-H^+ exchanger, which leads to an increase in intracellular pH (Grinstein & Furuya 1984; Simchowitz 1985b). Elevation of cytoplasmic pH is reported to correlate with chemotaxis (Simchowitz & Cragoe 1986). PMA is not effective in DDA; however, attractants stimulate numerous phosphorylations, proton efflux, and an increase in intracellular pH (Aerts et al 1987).

The calcium and PMA mediated activation of PLA2 releases arachidonic acid (AA), which is a substrate for endoperoxidases and lipoxygenases. AA is converted to a number of products including LTB_4. Since LTB_4 is a chemoattractant in PMNs, its secretion could initiate a positive feedback loop leading to amplification of the original response. There is abundant evidence that cAMP receptor–adenylate cyclase coupling in DDA does create a feedback loop that amplifies the initial stimulus (Devreotes & Steck 1979). The alterations of lipid metabolism extend beyond the release of DAG, IP_3 and AA. There are also changes in lipid kinases, other PI complexes, hydrolysis of phospholipids in addition to PIP_2, and changes in lipid methylation (Sha'afi & Molski 1987; Korchak & Lunquist 1987).

Cyclic nucleotides Chemoattractants elicit transient (2–5 min) changes in cAMP, which in intact DDA are due to transient activation of adenylate cyclase (AC) (Roos et al 1977). Since attractants do not influence enzyme activity in membranes from either cell type, the link between chemoattractant receptor and adenylate cyclase appears to be indirect and may involve calcium or other intermediates in the inositol pathway. In calcium-depleted or TMB-8 treated PMN, FMLP-stimulated increases in cAMP do not occur although responses to epinephrine and prostaglandin E_1 (PGE_1), which bind to receptors that interact directly with G_S, are unaffected (Verghese et al 1985). Stimulation of DDA appears to modify the pathway leading to adenylate cyclase; GTP-γ-S activation of adenylate cyclase is enhanced in membranes from DDA briefly pretreated with the attractant. A soluble factor is also required for GTP-γ-S stimulation of the enzyme and a mutant defective in this factor, synag 7, has been isolated (Theibert & Devreotes 1986; Van Haastert et al 1987). Increases in intracellular cAMP are not essential for chemotaxis since the chemotactic responses of cells that show little increase in cAMP, such as synag 7 and PMN treated with adenine analogs, are indistinguishable from wild-type responses (Table 3).

Chemoattractants in all species of slime molds elicit rapid transient (10 sec) increases in cGMP, which are difficult to measure (Mato et al 1977;

Wurster et al 1977). In saponin-permeabilized cells, IP_3, GTP-γ-S, or calcium are each reported to elevate cGMP (Europe-Finner & Newell 1985). DDA streamer F mutants lack cGMP-specific phosphodiesterase, and attractant-elicited cGMP levels remain persistently elevated (Ross & Newell 1981; Van Haastert et al 1982). In a cAMP gradient, these cells are able to orient themselves, but remain elongated after the gradient is removed (Table 3). When the concentration of cAMP is rapidly increased these cells maintain the "cringe" morphology for 70 sec rather than 20 sec (G. McNamara, personal communication). Stimulation of PMNs with crude attractants such as zymosan activated serum or bacterial supernatants does slowly elevate cGMP (Hatch et al 1977). However, NFP-elicited increases in cGMP have not been observed (Simchowitz et al 1980).

ACTIVATION OF THE MOTILE RESPONSE Cell locomotion involves the coordination of complex processes including protrusion of lamellipodia, formation and breakage of attachments to substrate, and maintenance of cell integrity through elasticity and/or contraction. Continued locomotion may require a cycling of cytoskeletal and membranous components between the cell front and rear (Bretcher et al 1987; Bray & White 1988). None of these processes are understood in detail. It would be useful to identify the sites where chemoattractants stimulate and direct locomotion in these processes.

Actin The chemoattractant-induced formation of lamellipodia correlates temporally and spatially with polymerization of actin. Peak levels of F-actin (filamentous actin) occur by 15–60 sec (Wallace et al 1984; Howard & Oresajo 1985a,b). Two peaks of actin polymerization are seen in DDA and sometimes in PMN (McRobbie & Newell 1983; Omann et al 1987b). The newly formed lamellipodia are brightly fluorescent when stained with nitrobenzoxadiazole (NBD)-phallocidin, which binds selectively to F-actin (Fechheimer & Zigmond 1983; Hall et al 1988). If the chemoattractant is removed, the actin rapidly depolymerizes and the lamellipodia are withdrawn (Zigmond et al 1981; Sklar et al 1985b; Omann et al 1987a). In triton-lysed cells, the polymerized actin sediments in a low speed centrifugation, which suggests that it is associated with the cytoskeleton (White et al 1982, 1983; McRobbie & Newell 1983; McRobbie 1986; Hall et al 1988).

The steady state between globular actin, G-actin, and F-actin depends on the concentration of G-actin available and the net affinity of the filaments for actin. An increase in G-actin could arise by releasing it from an actin-sequestering molecule such as profilin. An increase in free profilin has been observed after activation of platelets (Markey et al 1981; Lind et al 1987). However, the amount of profilin present in platelets and PMN

does not appear sufficient to account for the observed changes in actin concentration (Lind et al 1987; F. Southwick, personal communication).

The affinity of the filament for G-actin can be altered by proteins that stabilize filaments, such as tropomyosin, or by proteins that change the ratio of available high affinity ("barbed") and low affinity ("pointed") filament ends. Chemoattractants rapidly increase the number of barbed-ends available for polymerization (Carson et al 1986; Condeelis et al 1988). Between 60 and 200 new sites for actin growth can be produced by an occupied receptor (M. Carson & S. H. Zigmond, unpublished observations). With this modest amplification, occupancy of 2000 receptors could double the number of actin filaments. [The actin polymerized in a resting cell forms 2×10^5 filaments, 0.6 μm in length (Hartwig & Shevlin 1986).] Chemoattractant-induced polymerization in vivo in PMN is blocked by cytochalasin, which suggests that the filament growth occurs at the barbed end (Wallace et al 1984). Barbed ends could arise de novo in nucleating filaments with molecules such as ponticulin (Wuestehube & Luna 1987; Schwartz & Luna 1988) by cutting existing filaments or by removing capping proteins, such as gelsolin or severin, from the barbed ends of existing filaments. The amount of activated gelsolin (i.e. able to cap filaments) is reduced by addition of chemoattractants to activated macrophages or by various stimuli to platelets (Chaponnier et al 1987; Lind et al 1987).

The second messengers that mediate changes in actin are largely unknown. Phosphatidylinositol bisphosphate micelles release actin from profilin and gelsolin from an actin-gelsolin-calcium complex (Lassing & Lindberg 1985; Jammey & Stossel 1987). However, the presence of such micelles in vivo and the specificity of action of these highly charged surfaces has not been defined. Calcium modulates a large number of actin-associated proteins in both PMN and DDA (Stossel et al 1985; Fukui & Yumura 1986; McRobbie 1986). However, elevation of the mean cytoplasmic calcium level does not appear necessary for actin polymerization in vivo or for chemoattractant stimulation of cell locomotion (Table 3; Sklar et al 1985b; Sheterline et al 1985; Sha'afi et al 1986; Carson et al 1986; Omann et al 1987a).

Recent experiments with DDA transformants have revealed that cells lacking severin, a DDA analog of gelsolin, α-actinin, or a 120-kDa actin binding protein, or both severin and α-actinin exhibit chemotaxis (Table 3). These surprising results necessitate a reevaluation of the identities of critical actin binding proteins.

Myosin Phosphorylation of a myosin light chain is stimulated by attractants, apparently by activation of a myosin chain kinase (Fechheimer &

Zigmond 1983; Berlot et al 1985). A transient decrease and then an increase in the phosphorylation of the myosin heavy chain is observed in DDA. The increase in phosphorylation results from attractant-induced movement of myosin to the cell cortex where it interacts with a constitutively active membrane bound kinase (Yumura & Fukui 1985b; Berlot et al 1987). The myosin subsequently returns to the endoplasm. Movement of myosin to a cytoskeletal fraction has been observed in platelets but not in PMN (Fox & Phillips 1982; White et al 1983). Interestingly, the heavy chain of myosin is not essential for chemotaxis or motility. DDA transformants lacking the heavy chain of myosin, although unable to undergo cytokinesis, are motile (but slow and uncoordinated) and form the streaming patterns indicative of chemotaxis if extracellular calcium is present (Table 3; Knecht & Loomis 1987; De Lozanne & Spudich 1987). DDA have a small form of myosin, whose significance for cell motility and chemotaxis is now an important issue.

Localization of cytoskeletal proteins Immunofluorescence studies show that contractile proteins are asymmetrically distributed in a locomoting cell. Actin and actin-binding protein are concentrated in the advancing pseudopod; actin is also present in the cortex and uropod where myosin is concentrated (Oliver et al 1978; Valerius et al 1981; Fechheimer & Zigmond 1983; Stossel et al 1985; Yumura & Fukui 1985; Sawyer et al 1986). Addition of chemoattractants modulates this inherent polarity; F-actin is found in attractant-induced pseudopods. As noted above, in DDA, myosin moves transiently to the cortex. Streamer mutants of DDA, which show increased polarity, display a prolonged association of myosin with the cortex (Liu & Newell 1988).

Microtubules arise from the centriolar region, which is just anterior to the nucleus in a moving PMN (Malech et al 1977). Addition of chemoattractants increases tubulin polymerization and tubulin tyrosinylation in PMN (Hoffstein et al 1977; Nath et al 1981). Microtubule inhibitors including colchicine and nacodazole compromise the polarity of cells; the cells make more frequent and larger turns. However, cells without centrioles and without microtubules do still exhibit chemotaxis although less well than control cells (Keller & Bessis 1975; Zigmond 1977; Allan & Wilkinson 1978; Malawista & de Boisfleury 1982).

Polarity is modulated by cyclic nucleotides. Agents that increase cGMP and inhibit cAMP induce polarization of leukocytes (Stephens & Snyderman 1982). Streamer F, the cGMP phosphodiesterase mutant of DDA with persistently elevated cGMP, moves in unusually straight paths. In both cases, the increased polarity correlates with better chemotaxis (Sandler et al 1975; G. McNamara, personal communication).

CHEMOATTRACTANT-INDUCED DESENSITIZATION

The kinetics of the cellular responses can be divided into two classes as indicated in Tables 1 and 2. Some responses, such as chemokinesis, persist as long as the attractant is present and cease when it is removed. Most responses are transient even in the continued presence of the stimulus. The transient responses vary with respect to time to maximum response, time and extent of the return to baseline, responsiveness to increments in attractant concentration, and recovery of responsiveness after removal of the attractant. Mechanisms that bring about a loss of responsiveness, referred to collectively as desensitization, may include: decreases in receptor number or down-regulation, modification of receptor function, modification of G protein or effector function, or depletion of messengers or their precursors. The loss of responsiveness can be independent of the response or can result from feedback inhibition.

Receptor Alterations

DOWN-REGULATION Pretreatment of PMN with NFP or DDA with cAMP leads to rapid ($t_{1/2}$ = 1–10 min), dose-dependent attenuation of subsequent [^{3}H] NFP or [^{3}H]cAMP binding (Klein & Juliani 1977; Sullivan & Zigmond 1980; Vitkauskas et al 1980; Chenoweth & Hugli 1980; Van Haastert 1987a,b). Down-regulation can occur under conditions that preclude all other chemoattractant-elicited responses. These conditions include pertussus intoxication of PMN (M. W. Wilde & S. H. Zigmond, unpublished observations), stimulation of DDA with the antagonist cAMPS(Rp), and mutation of the frigid A locus in DDA (see Table 3). In DDA, down-regulated receptors reappear very slowly ($t_{1/2} > 60$ min) when chemoattractant is removed; in PMN the $t_{1/2}$ is between 10 and 20 min (Zigmond et al 1982; Van Haastert 1987a,b).

Chemoattractants induce rapid redistribution of receptors within or on cells. In DDA, cAMP causes cAMP receptors, visualized with fluorescent antibody, to move from the cell perimeter to the interior within 15 min (Wang et al 1988). In PMN, NFP induces translocation of receptors from a rapidly to a slowly sedimenting microsomal fraction (Painter et al 1987).

COVALENT MODIFICATION There is strong correlative evidence in DDA that covalent modification of chemoattractant receptors is required for adaptation of certain cellular responses such as adenylate cyclase activation (Theibert & Debreotes 1983), myosin phosphorylation (Berlot et al 1985), and cell shape changes (Fontana et al 1986). As noted previously, adaptation refers to a reversible extinction of responsiveness caused by

adjustment of cellular sensitivity to the current level of the stimulus. Adapted cells will respond further if the stimulus is increased and will recover when the stimulus is removed. The surface cAMP receptor exists in two molecular weight forms (M_r = 40,000 and 43,000) which are interconverted by application and removal (Theibert et al 1984) of the chemoattractant. The chemoattractant-induced increase in molecular weight is associated with an increase in serine phosphorylation from 2 moles/mole receptor to 6 moles/mole (Klein et al 1985a,b,c). The modification occurs with a half-time of about one min and plateaus as the physiological responses subside. A stimulus increment triggers an additional response and a further increase in receptor modification. When the stimulus is removed, simultaneous recovery of cellular sensitivity and reversal of receptor modification occur with a half-time of about four min. At 0°C, receptor modification does not reverse and cells do not regain sensitivity. The dose-dependence of the fraction of modified receptors at steady-state matches that for activation of the cellular responses (Devreotes & Sherring 1985). Although modification of NFP receptors has not been reported, it is anticipated based on the similarity in kinetics and adaptation properties of the responses.

In DDA, desensitization brought about by down-regulation can be readily distinguished from that caused by receptor modification since the former has a tenfold higher dose-dependence and is not readily reversible (Van Haastert 1987a,b). Cells in which receptors have been down-regulated continue to display reversible adaptation/deadaptation and receptor modification/demodification cycles. Down-regulated receptors that operationally are not exposed to the chemoattractant are modified/demodified along with exposed receptors, which suggests that the signal for modification is global (Snarr-Jagalska 1988b).

Additional Desensitization Mechanisms

MODIFICATION OF G PROTEIN FUNCTION In DDA, adenylate cyclase can be activated in vitro with GTP-γ-S in the absence of ligand. Prolonged pretreatment of cells with chemoattractant causes a complete loss in this sensitivity to guanine nucleotides, but does not alter direct activation of the enzyme by Mn^{2+} (Theibert & Devreotes 1986). GTP-γ-S and Ca^{2+} will each induce cGMP accumulation in saponin-permeabilized DDA cells. In adapted cells, GTP-γ-S is no longer effective, while Ca^{2+} remains effective, which suggests that the guanylate cyclase has not been modified (Small et al 1987). These observations suggest that G protein function is altered in desensitized cells and may indicate direct modification of the G proteins involved.

FEEDBACK INHIBITION It has been proposed that feedback inhibition could

account for response termination. Chemoattractant-elicited increases in A or C kinase could provide an attenuation signal. Treatment of leucocytes with cAMP-elevating drugs and catecholamines causes inhibition of PIP_2 hydrolysis, chlorotetracyline-monitored calcium mobilization, and dependent responses. Experiments with PMA also suggest that a PKC-mediated phosphorylation may prevent activation of phospholipase C by G protein (Matumoto et al 1986; Sha'afi & Molski 1987; Snyderman et al 1987; Van Haastert 1987c). In DDA, intracellular cAMP appears to perform at most a minor role in adaptation. In the DDA mutant, synag 7, as well as in caffeine-treated wild-type cells, chemoattractant-elicited increases in cAMP are absent yet cGMP increases, shape-change responses, and adenylate cyclase adaptation proceed with normal time courses (Table 3).

ALTERATIONS OF EFFECTOR AND DEPLETION OF INTERMEDIATES In contrast to the strong correlation between surface cAMP receptor phosphorylation and adaptation of the many physiological responses, it is difficult to envision how the phosphorylation regulates the very rapid responses. For instance cGMP accumulation becomes fully committed within a few seconds of stimulus application and is adapted before receptor modification reaches a steady-state (Van Haastert & Van der Heijden 1983). The temperature dependence of this desensitization response also indicates that it is not mediated by receptor modification (Van Haastert 1987d). Depletion of messenger stores (calcium) may contribute to early termination of responses. Rapid desensitization may also occur at the level of the effector (McRobbie & Newell 1983; Sklar & Oades 1985).

INTERDEPENDENCE OF DESENSITIZATION MECHANISMS If desensitization occurs at the level of the receptor through either down-regulation or modification, the desensitization to different chemoattractants can be independent. PMN incubated with moderate concentrations of NFP, LTB_4 or C5a exhibit desensitization that is specific to the attractant present during the incubation (Henson et al 1978; O'Flaherty et al 1979). Incubation in high concentrations of chemoattractants causes ligand nonspecific desensitization in PMN (Nelson et al 1978). As noted above, in DDA that have been adapted to folic acid, cAMP elicits normal shape change and cGMP accumulation responses. If the folic acid stimulus is withdrawn when the cAMP stimulus is introduced, the cells deadapt to folic acid while responding to cAMP. A fresh response can then be evoked by reintroduction of folic acid (Van Haastert 1983a; Fontana et al 1986).

Pretreatment of PMNs with *N*-formyl peptides leads to an attenuation of chemoattractant stimulated GTPase activity monitored in vitro. This attenuation of signal transduction occurs in the same dose range as receptor down-regulation. While down-regulation is ligand specific, the attenu-

ation of chemoattractant-stimulated GTPase activity is not ligand specific, which suggests a global nature to this form of desensitization. The receptor down-regulation in PMNs is reversible; the decrease in transduction is only partially reversible (M. W. Wilde & S. H. Zigmond, manuscript in preparation).

CURRENT VIEW OF EUKARYOTIC CHEMOTAXIS

Biochemical Events Essential for Chemotaxis

A large number of chemoattractant-induced biochemical changes have been observed (Tables 1 and 2). Table 3 summarizes key observations that a current model of chemotaxis must incorporate. Remarkably few of the observed changes appear to be essential for chemotaxis. Probably other components are essential, i.e. the ability to adapt, but this has not yet been documented. The fact that a given change is not essential does not necessarily mean that it is not involved, but merely that there are redundant mechanisms for achieving a response.

Working Model for Chemotaxis

At the outset, three schemes were presented to illustrated requirements for effective chemotaxis. Biochemical mechanisms must account for a directional response, responses proportional to the difference in receptor occupancy, sensitivity, amplification, and interdependence of responses to different attractants. Although the biochemical data relating to chemotaxis is fragmentary, we can speculate on which reactions contribute to these features of chemotaxis. Our current working model, illustrated in Figure 3, draws on elements present in each of the three original schemes. Figure 3*A* depicts a cell (moving to the right) in a uniform concentration of chemoattractant. Figure 3*B* shows the same cell shortly after focal application of an attractant. The cell extends a pseudopod and begins to turn in the direction of the source. Shown within the cell are stimulus-induced changes in states of receptors and G proteins, actin, myosin, and microtubules. Changes in second messengers are not illustrated, but are discussed below.

DIRECTIONAL RESPONSE A large part of coordination required for effective locomotion and chemotaxis is due to endogenous polarity. The cell in Figure 3*A* is polarized even though it is exposed to a uniform concentration of attractant. Disruption of either myosin heavy chain or microtubules decreases the endogenous polarity and impairs, but does not eliminate, chemotaxis (Table 3). These cytoskeletal proteins may polarize the cell by inhibiting pseudopod extension from the rear and sides. Many other

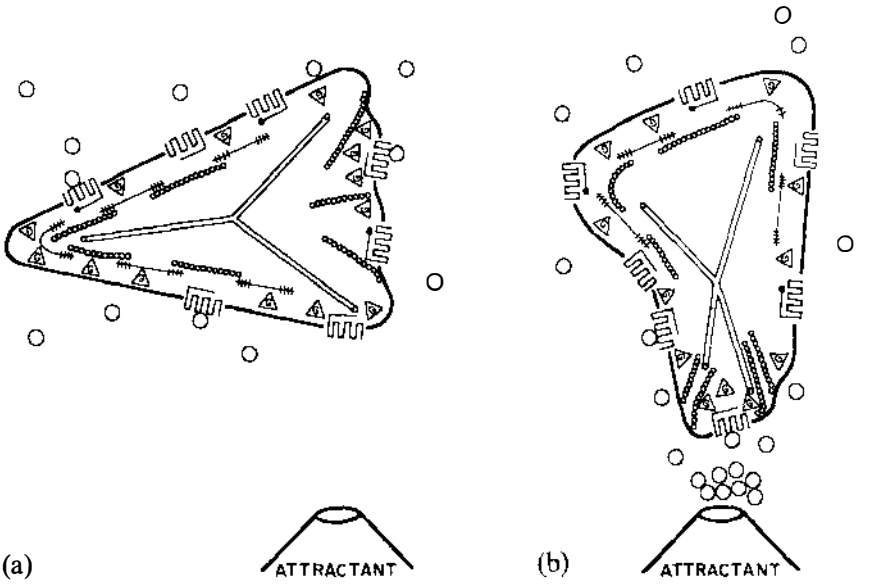

Figure 3 Diagram of chemotactically sensitive cell. (A) Cell locomoting in a uniform concentration of chemoattractant. The leading edge of the cell and the direction of locomotion are to the right. (B) Same cell shortly after release of concentrated chemoattractant from the micropipet in the lower portion of diagram. Shown are attractant molecules (○); receptors (); modified receptors (); G proteins (); actin filaments (); myosin filaments (); microtubules ().

components are certainly involved in the establishment of polarity. However, three actin-associated proteins, severin, α-actinin and a 120-kDa actin binding protein can be eliminated from DDA without any apparent effect on locomotion or chemotaxis (Table 3).

Chemoattractants modulate the endogenous polarity. The modulation may be mediated by a combination of stimulation and inhibition of pseudopod formation. In Figure 3*B*, local application of an attractant is envisioned to induce a pseudopod by locally stimulating actin polymerization and weakening the cytoskeleton. When high uniform attractant concentrations are first applied, weakening of the cytoskeleton occurs at multiple points, which induces many pseudopods. The weakening at local sites may lead to concomitant strengthening in the remaining cortex depicted in Figure 3*B* as a rearrangement of myosin and microtubules. In DDA streamer F mutants, the prolonged attractant-induced association of myosin with the cytoskeleton is correlated with the prolonged cell polarization. Microtubule polymerization, increased by addition of

chemoattractants in PMN, may also contribute to cortical stability (Table 2).

The excitatory messenger that weakens the cortex and triggers actin polymerization must be localized. The signal that stimulates the polymerization requires transduction through a G protein, which suggests that messengers such as DAG, IP_3 and calcium are involved. These molecules could potentially localize the actin polymerization since there are means for limiting their space constants, i.e. rapid sequestration or degradation. Although elevation of cytoplasmic calcium levels does not appear to be essential for chemotaxis, the importance of local calcium fluxes have not been ruled out (Cramer & Gallin 1979). The localization of pseudopod formation could, of course, also be due to the local removal of an inhibitory activity.

The effects of chemoattractants on microtubules and myosin may be mediated by cyclic nucleotides. cGMP increases cell polarity in PMN and a prolonged elevation of cGMP is the basis of the streamer F phenotype in DDA (Table 3). Since cGMP analogs added homogeneously are effective, a local concentration of cGMP does not appear necessary.

DIFFERENCE IN RECEPTOR OCCUPANCY Adaptation to chemoattractant results in responses that are proportional to changes in the fraction of occupied receptors. Adaptation has been shown in both cell types to involve early steps in the signaling pathway, i.e. the receptor and possibly the G protein. In the working model, adaptation is depicted as modified receptors. In Figure 3*A* the extent of modification is equal to the extent of receptor occupancy; in Figure 3*B* the local stimulus results in receptor occupancy that exceeds the current level of adaptation. Receptor phosphorylation could certainly mediate local adaptation. It may also serve as a global adaptation mechanism. Since dephosphorylation is relatively slow, the level of adaptation may become averaged throughout the cell as modified receptors move in the plane of the membrane. Alternatively, the receptor kinase may be regulated by a global signal. Attenuation of later steps in the transduction process may also be global in nature.

SENSITIVITY At the outset, the extraordinary sensitivity of chemotactic systems was noted. Evidence was presented that chemoattractant receptors have high affinity and rapid dissociation rates, allowing for frequent sampling and time averaging of the attractant concentration. Although a multiplicity of receptor forms exist in both systems, only forms which have relatively rapid dissociation rates ($t_{1/2} < 2$ min) and are present at the cell surface are depicted in Figure 3.

Noise can also be reduced by a mechanism that integrates over time information from several receptors. The G proteins provide a means of

time-averaging since an excited receptor can activate several G proteins and the G protein can remain active for some time before its GTP is hydrolyzed. The life time of the activated G protein and its mobility are important questions that are as yet undefined.

AMPLIFICATION Small changes in receptor occupancy can result in large changes in cell physiology. Adaptation, by keeping resting levels of second messengers near basal levels, permits the biochemical machinery to be highly sensitive to a small change in the concentration of attractant. The relatively high concentrations of G proteins in these cells suggests amplification may occur at this point. As depicted in Figure 3 a single excited receptor can activate numerous G proteins. Another obvious site for amplification of the signal due to changes in receptor occupancy is activation of phospholipase C.

PATHWAYS FOR DIFFERENT CHEMOATTRACTANTS Figure 3 depicts only a single class of chemoattractant receptors. Since adaptation to moderate concentrations of attractant appears to be ligand specific, it is depicted as occurring at the level of the receptor. Since receptors for different ligands may interact with a common pool of G proteins, integration of signals from different receptor types may occur at this point.

Techniques are now available to address many cell biological questions concerning chemotaxis. Do chemoattractant receptors move in the plane of the membrane? Do they recycle rapidly? Is new membrane inserted in response to chemotactic stimulation? At specific points? Is receptor modification necessary for adaptation? Can nonadapting cells carry out chemotaxis? Which G proteins are required for chemotaxis? Chemoattractant receptors are structurally similar to other G protein–linked receptors and stimulate similar transduction steps. Where is the specificity for chemotaxis? What molecular changes lead to actin polymerization? What localizes components of the cytoskeleton in a locomoting cell? Are cytoskeletal components recycled as a cell translocates across the substrate? The answers to these questions will give new insights to the fascinating process of directed cell migration.

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