Signal Transduction in Chemoreception

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I. INTRODUCTION

Every organism has sensory systems designed to extract information from its environment and to transduce this information into a useful form that triggers a response. When the external information is a chemical or a mixture of chemical cues, the sensory process is referred to as “chemoreception” and can range from taste and smell, familiar to all of us, to the attraction of motile bacteria. The incentive for reviewing chemoreception in this chapter comes from the relatively recent, rapid developments in the understanding of signal transduction in a multitude of systems, including photoreception, mitogenesis, and chemoreception. Therefore this chapter is devoted to chemosensory signal transduction, i.e., the conversion of chemical cues into intracellular messengers that provide useful information to the receptor cell and hence to the organism about its chemical environment.

A. Chemicals as Primary Messengers

There is no overall unifying theme among the chemical stimuli that are utilized in chemosensory transduction, but within this confusion of compounds there is order because each stimulus fits into the context of the life of the organism: Sugars attract bacteria that ferment them; folic acid attracts paramecia and slime mold amoebae that feed on the stimulus source, bacteria; amino acids attract lobsters and catfish that prey on muscle; pheromones (or pheromone blends) unique to insect or protozoan species attract their own for mating.

The participation of chemical compounds as primary messengers subjects chemosensory transduction to constraints that do not exist for photosensory or mechanosensory transduction. For a stimulus to be effective it must be transient [Atema, 1987]. If a stimulus remains in place too long, the sensory system will adapt, become insensitive to the presence of the stimulus, and stop responding. Unlike light or touch, when the source of the stimulus is removed, chemical stimuli remain. Therefore, there must be a means of destroying the signal, including enzymes to degrade the chemical stimulus, proteins to bind it, and the endocytosis of receptor–ligand complexes (see section II.F.)

B. Diverse Chemosensory Systems and Responses

The chemosensory systems commonly studied are diverse and their responses varied. In unicellular organisms, detection and transduction of chemical signals brings about the changes in the cells’ motility apparatuses that allow bacteria and protozoa to accumulate at sources of nutrients or to escape nonoptimal
environments; allow slime mold amoebae to crawl to seek food or to aggregate for development into a multicellular slug; facilitate the meeting of sperm and egg or cells of different mating type; or, considering circulating blood cells as honorary unicellular systems, mediate the migration of leukocytes to sites of infection. In metazoans, chemoreception underlies the sensory processes of taste, smell, and common chemical sense that in turn facilitate the processes of identifying (safe) food sources, mates, and sites for settling on substrates. The behavioral mechanisms by which the organisms achieve the same ends of the chemosensory pathway—location of food, for example—can range from indirect movement by biased random walk (klinokinesis) to direct crawling or swimming up a gradient of chemical stimulus (chemotaxis).

One solution to covering sensory transduction with all this diversity would be to focus on general, common aspects. Therefore this chapter attempts to survey many of these systems in order to make emerging, common themes apparent.

C. Common Pathways of Reception and Transduction

While wide-ranging, chemoreception systems do have many aspects in common, particularly at the cellular and molecular levels. They all appear to be initiated at the membrane surface of a receptor cell by the interaction of a stimulus with a receptor molecule (or perhaps in some cases the membrane directly) and subsequent transduction of this interaction into intracellular messengers. The second and third messengers are limited in number and for the most part are recruited from cyclic nucleotides, permeant ions, phosphoinositides, diacylglycerol, arachidonic acid, and internal pH levels. It is becoming increasingly apparent that GTP-binding proteins (G proteins) mediate at least some of this transduction. Therefore receptors, internal messengers, and transduction mechanisms are discussed in turn in order to examine common themes with variations among diverse chemosensory systems.

II. RECEPTORS

A. Bacteria

Bacterial chemoreceptors mediate a change in flagellar motion that biases the individual cell’s random walk and indirectly causes a population of bacteria to accumulate in attractant stimuli or disperse away from repellents. In the most extensively studied bacteria, *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis*, the cells swim smoothly in response to attractants (with flagella rotating counterclockwise) and in a “tumbly,” frequently turning response to repellents (with flagella rotating clockwise with each tumble). The general scheme of things is shown in Figure 1, taken from an overview by Parkinson (1988). Bacterial chemoresponse is treated in depth in this volume.
Fig. 1. Schematic of information transfer in bacterial chemoresponse. Ligand (+) binds to receptor, which signals the presence of ligand by way of cheW to cheA by enhancing the autophosphorylation of A (nonphosphorylated states are O and phosphorylated states are O'). The phosphates on A proteins are then physically transferred from A to Y and B. Activated (phosphorylated) Y interacts with the motor to promote clockwise (CW) rotation and tumbling. Z functions to inactivate the signal by receiving phosphates from Y (or A, not shown). cheB and cheR affect the adaptation state of the receptor by adding or removing methyl groups. [Reproduced from Parkinson, 1988, with permission of the publisher.]

by M. Eisenbach. However, because such a great deal is known about protein components of the bacterial chemoresponse pathway, bacterial chemoreception is also included in this chapter, especially for the purposes of comparison to other, often lesser known systems.

The 20 or so receptors for Esch. coli and the highly homologous receptors in Sal. typhimurium fall into two classes: those carrier proteins loosely associated with the membrane and found in the periplasmic space and those intrinsic membrane proteins that also serve as transducers. Within this latter group are four proteins that have been referred to as "MCPs" (methyl-accepting chemotaxis proteins) and are known as the products of the tsr, tar, trg, and tap genes (Fig. 2). The aspartate receptor (MCP II) serves as a good example from which to generalize. This one MCP mediates both aspartate and maltose chemoresponse by slightly different mechanisms.

The aspartate receptor resides in the membrane as a dimer of Tar proteins [Milligan and Koshland, 1988], each with two membrane-spanning groups, a periplasmic domain with ligand-binding sites and a cytoplasmic domain with its sites for signal transduction and covalent modification [Russo and Koshland, 1983]. The bacterial aspartate and other MCP receptors fall into a family of "simple" receptors, including the epidermal growth factor (EGF), nerve growth factor (NGF), low-density lipoprotein (LDL), and insulin receptors based on the small number of transmembrane-spanning regions through which information about binding of ligand to the extracellular domain must be conveyed to the interior of the cell [Mowbray and Koshland, 1987]. Using site-directed muta-
Fig. 2. The receptors and membrane signal transducing proteins of E. coli. The gene names for the methyl-accepting chemotaxis proteins (MCPs) are given in italics. (The sensory transduction pathway for phosphotransferase sugars joins with these transduction pathways downstream of the MCPs). [Reproduced from Adler, 1987, with permission of the publisher.]

Genesis to create sites of disulfide crosslinking, Falke and Koshland [1987] have manipulated the aspartate receptor. From their studies, the protein appears to be flexible, and the binding of aspartate directly to the Tar protein induces "global" conformation changes from the periplasmic domain through the membrane-spanning region to the cytoplasmic domain to facilitate intracellular signalling and adaptation. (The nature of the signalling is discussed in section IV.)

Adaptation is thought to occur through the methylation of the glutamate moieties of the receptor's cytoplasmic domain. A truncated aspartate receptor that is missing these residues can signal but cannot adapt [Russo and Koshland, 1983]. These glutamate residues become accessible to a methyltransferase following the binding-induced conformation change [see Adler, 1987, for a review]. The number of methyl groups on the MCP is a function of the nature of the stimulus (attractant stimuli increase the number of methyl groups over basal levels; repellent stimuli decrease this number) and the concentration of the stimulus. With the addition of more (but not saturating) stimulus such as the attractant aspartate, the number of methyl groups increases in an additive fashion [Mowbray and Koshland, 1987], with the consequences that the MCP stops transducing and the response adapts. Removal of aspartate leads to a rapid removal of methyl groups until basal levels are again attained (Fig. 3). In the
Fig. 3. Schematic of signaling and adaptation with maltose and aspartate: a vertical slide model. 
A: The unstimulated receptor is shown with an external domain that is responsible for ligand binding, two transmembrane segments, and a cytoplasmic domain that can send a signal and be methylated. The glutamic acid groups are symbolized here by the unmethylated OH groups to emphasize the net change of methylation in the following steps. B: The conformational change induced by the binding of aspartate causes the transmembrane region to be pulled up slightly (shown as three notches) into the external space. This in turn causes an alteration of the cytoplasmic portions (shown here as a compression against the membrane), which brings about changes in the signaling region (indicated by the jagged line) and in the methylation region (indicated by the exposure of the OH groups of the relevant glutamic acids). As a result, the aspartate-bound receptor becomes more highly methylated, which somehow releases the stress at the signaling site caused by binding of aspartate. C: At the new steady state, the methylation sites are sufficiently modified to bring the structure back to the nonsignaling mode, even though aspartate remains bound to the protein. D: The receptor adapted to maltose is shown here. The binding of maltose-binding protein, charged with its own ligand, at a different site has effects similar to those of aspartate, but smaller in degree (e.g., the transmembrane segment is moved only one notch). E: When both maltose and aspartate are bound, the effects are greatest (four notches), with adaptation occurring at the highest levels of methylation. An additional state, shown in F, exists when the attractant is removed from the methylated protein. This receptor sends a signal that is opposite in polarity to the one previously described. [Reproduced from Mowbray and Koshland, 1987, with permission of the publisher.]

interim, the cell responds behaviorally as though it has been exposed to a negative or repellent stimulus. This titration of methyl groups is thought to reset the system in an unchanging environment so that it is poised to respond to a change in stimulus concentration, even a change riding on top of a background level of stimulus.

The number of methyl groups on MCPs is kept in balance by the actions of a methyltransferase (CheR product) and an esterase (CheB product). A mutant in either cheR or cheB genes renders a bacterium defective in chemoresponse.
Curiously, the double mutant does retain some chemoresponse, bringing into question the role of methylation and adaptation in chemoresponse [Stock and Stock, 1987]. In particular, double mutants accumulate in capillaries of aspartate where they arrive by diffusion and move by smooth swimming and methylation-independent partial adaptation up the steep concentration gradient at the mouth of the capillary [Weiss et al., 1990]. Serine, which does not elicit any adaptation, also does not elicit any accumulation of the double mutant in capillaries, despite the presence of functional receptors and other sensory transduction components in the double mutant. It appears that the methylation-dependent adaptation that resets the response system is required for the chemoresponse to shallow gradients of stimuli and that without methylation only weak responses to very steep gradients of a few stimuli, such as aspartate, are operative.

The *Esch. coli* aspartate receptor also mediates the response to the attractant maltose. Maltose binds to its receptor, maltose-binding protein (MBP), one of the class of receptors that is found in the periplasmic space [see Brass, 1986, for a review of such proteins]. The ligand–receptor complex in turn binds to the aspartate receptor at a site probably distinct from that for aspartate binding [Mowbray and Koshland, 1987]. From a study of combined and separate responses to aspartate and maltose, Mowbray and Koshland [1987] conclude that there most likely is only one population of receptors that mediates both responses. Maltose–MBP, like aspartate, induces global MCP conformation changes that signal to the cell interior and make glutamates available for methylation. However, the number of methyl groups added under saturating stimulus conditions is lower and the order of priority of the methylation of sites is different from the aspartate response. A model that takes the conclusions of Mowbray and Koshland into account is shown in Figure 3.

As mentioned above, the MCPs fall into the class of chemoreceptors that includes intrinsic membrane proteins. Joining this class are the phosphotransferase sugar transport proteins that mediate both the transport of and chemoresponse to mannose and glucose [Lengeler et al., 1981]. The as yet unknown adaptation mechanism of these receptors does not involve methylation, and one prospect is the phosphorylation that regulates their transport function [Postma and Lengeler, 1985; Taylor et al., 1988]. The MCP-dependent and -independent systems do seem to share common requirements for the *che* Y, *che* W, and *che* A proteins and presumably a significant portion of the chemosensory transduction pathway [Taylor et al., 1988].

Like *Esch. coli*, *Bac. subtilis* is attracted to aspartate and phosphotransferase (PTS) sugars. However, there are many significant differences between the two bacterial systems [Thoekele et al., 1990]. The PTS sugars act through MCPs in *B. subtilis* and elicit a turnover of methyl groups on all the MCPs concurrently and fairly uniformly. Aspartate, which in *Esch. coli* affects the methyl-
ation state of its MCP receptor only, causes changes in methyl distribution on the three MCP species, but not uniformly as do the PTS sugars. The methylation/adaptation of Bac. subtilis differs from that of Esch. coli in that attractants stimulate demethylation and repellents stimulate no methylation change in MCPs. Additionally, there can be methyltransfer among one class of MCPs and between intermediates and other classes of MCPs [Thoeke et al., 1987; Beadle et al., 1988]. These observations hint that the methylation modifications may subserve different or additional functions from originally envisioned.

There are still other interesting chemoresponses of bacteria to oxygen, for example [Shioi et al., 1987; Taylor et al., 1988], that cannot be dealt with here. Likewise there are other bacteria with equally interesting responses, such as Caulobacter in which MCP synthesis is controlled in time and space within the cell [Nathan et al., 1986] and photosynthetic bacteria such as Rhodobacter sphaeroides and Rhodospirillum rubrum in which there are methylation-independent and -dependent chemoresponses and a role for transport in chemoreception [Ingham and Armitage, 1987; Sackett et al., 1987; Armitage, 1988, 1990]. Rhodob. sphaeroides offers an interesting contrast to the enteric bacteria, because its chemoattraction is limited to chemoeffectors and cations that are transported and do not interact with MCPs [Armitage, 1990]. The attractant stimuli (no repellents have been identified) induce increased swimming speed and decreased stopping not directly by alteration of proton motive force or pHt, but the responses do require transport and metabolism of the stimuli, implying interaction of the metabolic intermediates with the flagellar motor or acting as second messengers.

B. Neutrophils

Polymorphonuclear neutrophil leukocytes (PMNs) crawl their way to sites of inflammation from infection or wounds. They use as chemotactic stimuli fragments of complement such as C5a and fragments of bacterial proteins such as N-formyl-methionyl-leucyl-phenylalanine (FMLP); leukotriene B4; and a multitude of other endogenous and exogenous factors that diffuse from the inflammation site and set up a concentration gradient for the cells to follow [see Allen et al., 1988, for a review of stimuli]. At the site, in response to a higher concentration of stimuli than elicits chemotaxis, the PMNs release hydrolytic enzymes and superoxide that serve both to kill the infectious agent and to release more attractant stimuli. Most research on leukocyte chemotaxis has centered around FMLP and C5a as ligands, and therefore this discussion focuses primarily on the FMLP and the analogous C5a receptor-mediated transduction that causes cells to orient, polarize, migrate up concentration gradients, and secrete cytotoxic agents.

Receptors for FMLP and C5a have been identified by affinity crosslinking as integral membrane proteins of 50,000-60,000 and 40,000 M., respectively
[Niedel and Cuatrecasas, 1980a,b; Rollins and Springer, 1985; see Allen et al., 1988, and Painter et al., 1984, for reviews]. There are \( \sim 60,000 \) FMLP receptors elaborated on the plasma membrane and at least as many inside the cell. These glycoproteins are found minimally in two isoforms that can be separated by \( M, \) (50,000 and 60,000) and by \( pI \) (6.0 and 6.5, respectively), even when their substantial component of carbohydrate is removed. There may be even more isoforms within the two \( pI \) classes, because affinity labeling of receptor with radiiodinated FMLP characteristically results in a broad band of labeled protein from 50,000 to 60,000 \( M, \) on gels [see Allen et al., 1988, and Sha'afi and Molski, 1988, for reviews].

Apparently one heterogeneous set of FMLP receptors (\( K_d \) 0.6 nM [Sklar et al., 1984a,b]) mediates both chemotaxis and superoxide release, even though these responses differ in \( ED_{50} \) by one order of magnitude or more [Allen et al., 1988; Lohr and Snyderman, 1982; Yuli et al., 1982]. This difference in concentration dependence may reflect the different percentages of occupied receptors necessary to elicit each response [Painter et al., 1984; Sklar et al., 1987]. There is an alternative view that the different \( ED_{50} \)s reflect the two receptor affinity states and that the receptors in the high-affinity state mediate chemotaxis and those in the low-affinity state mediate other responses [Lohr and Snyderman, 1982; see Sklar et al., 1987, for discussion]. Both views allow for receptor affinity modulation with ligand binding, but the percent occupancy view would have the high-affinity state uncoupled from response, preparatory to down-regulation.

The different views arise in part because the FMLP-binding sites are highly dynamic, changing affinity, number, mobility, and distribution with ligand occupancy, guanine nucleotides, and methylation. Figure 4 represents a model that accounts for the effects of stimulation on receptor affinity and distribution. Upon stimulation with FMLP, bound receptors release the associated G protein in an active form that continues the signal transduction process (see section III). The receptor–ligand complex now changes affinity to become a very slowly dissociating, almost irreversibly bound form that is no longer coupled to the G protein, but is associated with the cytoskeleton [Painter et al., 1987; Jesaitis et al., 1984]. Its lateral movement to a different lipid domain and its association with the cytoskeleton are sensitive to cytochalasin D but not to pertussis toxin (a G protein inhibitor) or to guanyl nucleotides [Painter et al., 1987; Jesaitis et al., 1984]. The high-affinity receptors of the cytoskeleton-enriched fraction are still capable of interaction with G proteins, and the lateral segregation of these receptors into membrane regions poor in G proteins suggests a mechanism for desensitization and response termination [Jesaitis et al., 1989]. These desensitized receptors should correspond to the form Sklar et al. [1989] measure in real time as "LRX." These receptors are high affinity, not G associated, insensitive to pertussis toxin, and derived from "LR,"
a lower affinity form that is generated when the G protein subunits rapidly dissociate from the receptor once it is bound with ligand ("LRG").

Dissociation of the G proteins from the LRG complex is essential for the rapid (<1 min) segregation of the receptors to the cytoskeleton-associated, G protein–depleted membrane domain, because toxins have no effect while the GDP analog GDPβS drastically reduces association of receptor with cytoskeleton [Sardahl et al., 1989]. Presumably, dissociation of the G proteins reveals an actin-binding site that allows association of the receptor with the actin filaments of the cytoskeleton. The half-life of the transient association of the receptor with the cytoskeleton is ~50 sec, similar to that for the internalization of receptor [Jesaitis et al., 1984; Painter et al., 1984]. There is a loss of receptor number and sensitivity during chemotaxis for which this internalization may be responsible in part. Likewise, internalization may be a necessary process in the orientation of receptors during chemotaxis by replacing external receptors with new or cryptic ones primarily at the leading edge of the cell [Zigmond and Sullivan, 1979; Sullivan and Zigmond, 1982].

The model is based on acute stimulation with 1 nM FMLP for 2 min. Chronic stimulation, i.e., stimulation at lower temperatures that prevent endocytosis and secretion with 100 nM FMLP for 20–60 min, renders cells desensitized, unresponsive to FMLP yet still able to produce superoxide in response to phorbol ester [Jesaitis et al., 1986, 1988a,b]. As with acute stimulation, the occupied
receptors of desensitized cells convert to a high-affinity, very slowly dissociating form, still at the cell surface but now associated with cytoskeleton and in a different membrane domain from the G proteins. The relationship of these high-affinity binding sites in desensitized cells to the receptor isoforms discussed above is not yet clear, although in acutely stimulated cells the high-affinity sites associated with the cytoskeleton seem to be the 50,000–60,000 Mₚ proteins [Painter et al., 1987].

Homologous desensitization in chronically stimulated neutrophils appears to result from the physical separation of the surface receptor from the G proteins that are essential for transduction. Certainly internalization of receptors also is occurring, but the conversion of surface receptors to an almost irreversibly bound and sequestered form probably accounts for most of the desensitization process, because the ability of preincubated cells to respond to FMLP is relative to the number of surface receptors not complexed with the cytoskeleton [Jesaitis et al., 1986]. Densensitization could also involve covalent modification in addition to sequestration, as for the β-adrenergic receptor in its control of adenylate cyclase [Sibley et al., 1987]. However, there is no evidence for covalent modification with desensitization of the neutrophil receptor at this time.

This model, shown in Figure 4, is only a starting point. Filling in more details will require answers to the following questions. Is receptor occupancy all important, or are there functioning, G protein–associated receptors with different affinities that mediate different responses (chemotaxis vs. superoxide production and secretion)? As sequela: Is there only one population of receptors that interconvert by guanine nucleotides between high- and low-affinity and thereby between different functions [Painter et al., 1987]? What is the function of the unoccupied receptor found associated with the cytoskeleton in unstimulated cells? (Its Kᵣ of 1 nM curiously correlates with the ED₅₀ of chemotaxis, while the Kᵣ of cytoskeleton-free receptors correlates with the ED₅₀ of superoxide production [10 nM] [Allen et al., 1988].) Regardless of the answers, it will be important to know whether there is only one type of G protein associated with FMLP receptors and, if so, how one second effector produces several different responses to the same stimulus [see Sha’afi and Molski, 1988, for discussion].

Eventually a comprehensive model of neutrophil chemoreponse must account for the mechanism by which cells respond over several orders of magnitude of FMLP concentration and shift half-maximal responses to higher concentrations with prestimulation (somewhat similar to the bacterial adaptation system) [Sklar et al., 1984a,b; Seligmann et al., 1982]. The neutrophils detect a 1%–2% drop in FMLP concentration across the cell, and the model must take this restriction on receptor occupancy into account [Zigmund and Sullivan, 1979; Sullivan and Zigmund, 1982]. (As Zigmund [1989] points out, desensitization of receptors sets transduction and levels of second messengers back to basal levels and allows
the neutrophil to detect changes in second messenger elicited by changes in receptor occupancy from 200 to 400 receptors as well as from 4,000 to 4,200 receptors.) Additionally, a model must account for potentially different roles for the two or more isoforms of the receptors and the phenomenon of priming, a receptor-mediated process by which the FMLP receptor complement in the membrane increases upon prestimulation with a variety of extrinsic or cellular factors [see Allen et al., 1988, for review]. Many questions surrounding neutrophil receptors will be better resolved now that the cloning of the FMLP and C5a receptor genes has been accomplished [Thomas et al., 1990; Coats and Navarro, 1990; Murphy et al., 1990; Gerard and Gerard, 1991; see Goldman and Goetzl, 1982; Grob et al., 1990, for still other leukocyte receptors and coexpression of C5a, CR1, CR3, Fc, and FMLP receptors; Van Epps et al., 1990].

C. Unicellular Eukaryotes

In the category of unicellular eukaryotes are protists such as *Euplotes*, *Blepharisma*, *Paramecium*, *Chlamydomonas*; slime molds such as *Dictyostelium discoideum*; and yeast. Among these, receptors for the slime mold and yeast mating factor have been identified, and, for the rest, putative receptors have been described [see Van Houten and Preston, 1987, and Van Houten, 1990, for reviews and Devreotes and Zigmond, 1988, for a comparison of *Dictyostelium* and neutrophils].

1. Slime mold. When amoebae of *D. discoideum* run out of bacteria to feed on, they begin the process of developing into a multicellular slug. In this process, they respond to pulses of cAMP that emanate from focal cells. The cells migrate up the pulsatile gradient of cAMP until they can touch and aggregate as a prelude to forming the slug. When each cell is stimulated with cAMP, it in turn releases a pulse of cAMP (the relay) in addition to orienting and transiently moving toward the origin of the wave of stimulus. The chemoresponse can therefore be divided into the orientation of the cytoskeletal motile apparatus for chemotaxis and the activation of adenylate cyclase to produce cAMP for the relay, and the two processes can be studied independently.

Upon starvation, the amoebae acquire cAMP receptors. These receptors were first studied for ligand-binding properties, and, through pharmacological studies, a fairly detailed picture of the ligand-binding site has emerged, as chronicled by Jansens and Van Haastert [1987]. The binding sites were found to be heterogeneous and positively cooperative (Table I). Those binding sites associated with chemotaxis response are likely to be the high-affinity "B" sites that are coupled to the guanylate cyclase that is responsible for the production of an internal messenger for chemotaxis (Fig. 5). The "A" sites are thought to be coupled with the adenylate cyclase of the cAMP relay system. Within both classes of sites there are high- and low-affinity or fast- and slow-dissociating sets that can interconvert in the presence of ligand, and binding to members of both classes can be modulated by the presence of guanine nucleotides (Table
TABLE I. Kinetic cAMP Receptor Forms Observed in *D. discoideum* Cells and Isolated Membranes

<table>
<thead>
<tr>
<th>Receptor form</th>
<th>Apparent <em>K</em>ₐ (nM)</th>
<th>Dissociation rate constant (k⁻¹, s⁻¹, 20°C)</th>
<th>No. of sites per cell</th>
<th>Effect of guanine nucleotides on abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A⁺</td>
<td>60</td>
<td>4 × 10⁻¹</td>
<td>77,000</td>
<td>Decrease</td>
</tr>
<tr>
<td>A⁻</td>
<td>450</td>
<td>10 × 10⁻¹</td>
<td></td>
<td>Increase</td>
</tr>
<tr>
<td>Bˢ</td>
<td>6–13</td>
<td>4.3 × 10⁻²</td>
<td>2,300</td>
<td>Decrease</td>
</tr>
<tr>
<td>Bˢˢ</td>
<td>6–13</td>
<td>4.7 × 10⁻³</td>
<td>1,100</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

Reproduced from Janssens and Van Haastert (1987), with permission of the publisher.

I). As with the neutrophil chemoattractant receptor [Klar et al., 1984a,b], the rate of ligand binding rather than occupancy alone may be significant for transduction [Van Haastert et al., 1981].

Two groups have identified the protein counterpart of a cAMP receptor by photoaffinity labeling cells with ³²P-N₃-cAMP [Klein et al., 1985b; Juliani and Klein, 1981; Thiebert et al., 1984]. The protein is ~40,000 in *M*ᵣ and, despite the lack of consensus about the size, it is agreed that the protein is a phosphoprotein existing in two phosphorylation states [Klein et al., 1986,

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**Fig. 5.** Signal transduction pathways in *Dictyostelium discoideum* leading from cell surface cAMP receptors to the regeneration of the cAMP signal (signal relay) and to events of chemotaxis. More recent findings could add action of synag 7 gene product at the G protein (G) and adenylate cyclase (AC) interaction and the *frgA* gene product as the G protein for chemotaxis [Snaar-Jaglasta and Van Haastert, 1988; Keskbeke et al., 1988]. [Reproduced from Newell et al., 1988. with permission of the publisher.]
1987b]. Once bound with cAMP, the receptors become phosphorylated (approximately seven phosphates per peptide) [Klein et al., 1987b] and consequently shift to lower mobility on sodium dodecyl sulfate (SDS) gels.

The 40 kD protein has been purified to homogeneity [Klein et al., 1987a], and this phosphoprotein fits several criteria for the receptor: 1) specificity for labeling by the photoaffinity cAMP analog that parallels potency or affinity of analogs for chemoreceptor binding; 2) appearance at the expected time in development; 3) presence in membranes and not cytosol; and 4) nonidentity with the phosphodiesterase that is responsible for degradation of the extracellular stimulus [Klein et al., 1987b]. An additional 70 kD protein has been identified by photoaffinity labeling [Meyers-Hutchins and Frazier, 1984]. It is not clear how this protein relates to the 40 kD protein, but it is possible that this represents an aggregation product of the 40 kD protein [Janssens and Van Haastert, 1987].

As mentioned above, the adenylate cyclase of the relay and the guanylate cyclase that produces an internal messenger for chemotaxis appear to be activated by separate sets of receptors (Fig. 5). Judging from the kinetics of the high- to low-mobility transitions of the 40 kD protein on gels, this phosphoprotein could qualify for the receptor involved with adaptation of adenylate cyclase, but not with activation of the adenylate cyclase or either activation or adaptation of guanylate cyclase [Janssens and Van Haastert, 1987]. Therefore, as in the bacteria, a covalent modification of the receptor is coincident with adaptation, a common theme of vertebrate receptors as well [Sibley and Lefkowitz, 1985; Sibley et al., 1987].

The relationship of the phosphoprotein to the identified binding sites (Table I) is not known at this time [Janssens and Van Haastert, 1987]. cAMP is thought to activate the relay adenylate cyclase through the "A" sites, and the abundance of the phosphoproteins would suggest that they represent the majority of cAMP-binding sites, i.e., the "A" sites. One could extrapolate that the phosphorylation of the 40 kD protein corresponds to the transition of the "A" sites from high- to low-affinity forms and that the low-affinity form no longer activates the adenylate cyclase and thereby is responsible for adaptation of the relay. However, the time course of transitions of the high- to low-affinity "A"-binding sites and the high- to low-mobility phosphoprotein do not match [Van Haastert and De Wit, 1984; Devreotes and Sherrington, 1985]. A correspondence of the phosphoprotein with the "B" sites that govern chemotaxis seems unlikely, but has not yet been ruled out. Indeed, it is not clear whether the forms of the surface receptor (two A and two B) represent states of the same protein [Van Ments-Cohen and Van Haastert, 1989].

Relatively recently, screening of a cDNA library yielded cloned genes corresponding to cAMP receptors [Klein et al., 1988; Saxe et al., 1988; Saxe, personal communication]. A primary amino acid sequence deduced from one DNA clone predicts seven transmembrane-spanning regions, a G protein inter-
action site, and phosphorylation sites on the C-terminal cytoplasmic tail, all characteristics of a new member of the superfamily of receptors typified by rhodopsin and β-adrenergic receptors. Dictyostelium transformed with antisense constructs of the receptor gene do not express the mRNA for the receptor protein and fail to aggregate and proceed through development [Klein et al., 1988]. By following the appearance of mRNAs from the cloned genes during Dictyostelium development, the receptors for chemotaxis, relay, and developmental gene expression will be identified, and the discussions of the number and coincidence of receptors with binding states will soon be settled by molecular genetics. (See Section V for new additions to this gene family.)

The Dictyostelium amoebae when feeding on bacteria and not starved are responsive to folic acid and pteridines. Like the cAMP receptor, the folate receptor was first characterized among the membrane folate-binding proteins for its binding kinetics [see Janssens and Van Haastert, 1987, for review]. There are a total of five kinetic binding site forms, and among these three "B" sites have a selective binding that corresponds to the specificity of chemoresponse and therefore are likely to be the ones coupled with the guanylate cyclase for signal transduction in chemotaxis. (The two "A" sites probably couple with the adenylate cyclase and account for the ability of folic acid to induce cAMP in early aggregative cells.) The three "B" sites are interconvertible with ligand binding. As with the cAMP receptors, G proteins are implicated in receptor function because guanine nucleotides modulate the binding to all five binding subtypes and folic acid modulates the binding of GTP to cell membranes. The availability of chemoresponse mutants should facilitate the search for the still-elusive folate receptor [Segall et al., 1987]; however, it appears that no folate mutants to date are null mutants [Segall et al., 1988], and it remains for techniques of gene disruption and homologous recombination to produce the necessary cell lines [Segall and Gerisch, 1989]. Alternatively, the folate receptor gene may be identified among the growing number of cAMP receptor genes [Saxe et al., 1991].

2. Chlamydomonas. Chlamydomonas gametes begin by sticking to cells of the complementary mating type as a prelude to mating. This sticking at first is random, later is confined to the flagella, and then in Chlamydomonas reinhardtii is further limited to the tips of these organelles. The species-specific agglutinin molecules that mediate this process in Chlamydomonas and C. reinhardtii are large (>10^3 kDa, 228–320 nm) glycoproteins, rich in hydroxyproline, with characteristic shaft, hook, and globular head regions [Goodenough et al., 1985; Adair et al., 1983; Crabbendam et al., 1986]. The hook anchors the molecule in the membrane. It is the head that shows morphological differences between + and − mating type gametes and is essential for the agglutination mating response [Goodenough et al., 1985; Crabbendam et al., 1986]. The + and − C. reinhardtii mating type cells each express the corresponding + or − agglutinin along the long axis of the flagella. After contact with the complementary gamete,
the agglutinins accumulate at the tip, hence the "tipping" process in which the cells contact each other only at the flagellar ends. The + cell of the pair puts out a fertilization tube filled with polymerized actin. This tube contacts the − cell at a swelling specifically prepared for this contact, and fusion of the cells occurs here. The cells secrete autolysin to loosen or shed their cell walls in preparation to cell fusion [see Pasquale and Goodenough, 1987, and Musgrave and van den Ende, 1987, for reviews and Van Ende et al., 1990, for differences between species].

The agglutination process has set in motion a series of events. It is not clear how the agglutinin membrane proteins signal to the cell that this series should commence, but the adenylate cyclase is activated early in the process, and an increase in internal cAMP is sufficient to trigger the cascade of mating responses [Pasquale and Goodenough, 1987]. Crosslinking of the agglutinins in place on the membrane seems essential; solubilized agglutinins or isolated flagella interact weakly if at all [Musgrave and van den Ende, 1987]. Studies with antibodies to the agglutinins imply that crosslinking of complementary agglutinins is sufficient and that invoking the binding of agglutinins to yet other unknown surface receptors is not necessary [Homan et al., 1987]. The N-glucosamine sugars of the + mating type agglutinin and the O-linked oligosaccharide with terminal galactose residues of the − mating type agglutinin of C. eugametos are obligatory for mating-specific binding domains [Musgrave and van den Ende, 1987]. There is still much to discover about these agglutinins that mediate this contact chemoresponse that is reminiscent of immune cell and sperm−egg receptor-mediated interactions.

3. Ciliates. Paramecium tetraurelia responds to chemicals in solution around it. In particular, fermentation and other bacterial products are attractants, probably signifying the presence of food [Van Houten, 1978; Van Houten and Preston, 1988]. Stimuli such as folic acid, acetate, and cAMP hyperpolarize the cells [Van Houten, 1979], and thereby causing changes in ciliary beating: The cells move more smoothly and turn less frequently as a consequence. This in turn causes populations of cells to accumulate indirectly by a biased random walk, not unlike that of the bacterial chemoresponse [Van Houten, 1978; Van Houten and Van Houten, 1982]. The stimuli are thought to interact with the cell at specific receptor sites, because radiolabeled stimuli bind specifically and saturably to the cells [Schulz et al., 1984; Smith et al., 1987] and single-site mutations eliminate both binding and chemoresponse [DiNallo et al., 1982; Schulz et al., 1984; Smith et al., 1987; Isaksen and Van Houten, unpublished results]. Cilia are not essential for chemoreception: Deciliated cells show the characteristic hyperpolarization in attractants [Preston and Van Houten, 1987a,b], and only a small minority of binding sites are on the cilia [Schulz et al., 1984; Smith et al., 1987]. An exception to this may be the response to L-glutamate [Preston and Usherwood, 1988].

One candidate receptor has been partially purified. A doublet of cAMP-binding proteins from cell body membranes shows an elution profile from cAMP
affinity columns that would be expected for the receptor [Van Houten et al., 1990]. A protein of the same Mr (48,000) can be labeled by 32P-N3-cAMP photolysis of whole cells. Covalent linking of N3-cAMP to whole cells specifically eliminates chemoresponse to cAMP and not to other stimuli, implying that the receptor should be among the proteins crosslinked with this photoaffinity analog [Van Houten et al., 1991]. Both bands of the doublet are glycosylated, and other covalent modifications remain to be determined, as does the relationship between the two proteins. Total amino acid analysis is consistent with the two proteins having one origin at the gene level. Most importantly, polyclonal antibodies produced against this doublet specifically block chemoresponse to folate, and the preimmune serum does not (Baez and Van Houten, unpublished results). Other receptors for folate are being identified by similar approaches [Sasner and Van Houten, 1989]. There is a gradient of responsiveness to folate from anterior to posterior, and it will be interesting to determine whether receptors follow this gradient [Preston and Van Houten, 1987b].

As Chlamydomonas, ciliates have different mating types, but, unlike Chlamydomonas, some ciliates signal their presence to complementary mating types by soluble pheromones. These pheromones cause physiological changes in cells in preparation for mating. The two mating types of Blepharisma japonicum each have a soluble "gamone." Type I cells secrete blepharione (gamone I, a glycoprotein of 20,000 Mr), and type II cells put out blepharisonone (gamone II, a tryptophan derivative) [see Van Houten and Preston, 1987, and Nobili, 1987, for reviews]. Blepharisonone is a chemoattractant to cells of mating type I and is a common gamone to all Blepharisma species. In contrast, gamone I is species specific. Binding studies using 125I-gamone II imply that there is a specific binding site, perhaps a receptor on the cell surface. However, receptor proteins for these interesting stimuli have not been identified.

Species of another ciliate, Euplates, have multiple mating types. The genetic analysis implicates three to four codominant alleles of the mating type locus, depending on the species. The expression of a homozygous or heterozygous state determines the cell's mating type and, presumably, both the pheromones released and receptors displayed at the cell surface. At present there is no agreement about the nature of this complex system: whether cells synthesize receptors for the pheromones they produce or only for the pheromones they do not produce [see Beale, 1990, for overview; Nobili et al., 1987; Heckmann and Kuhlmann, 1986].

In the self-recognition model for Euplates raikovi, a homozygous cell expresses a single receptor to which homologous (self)-pheromone or nonself-pheromones from cells of other mating types can bind. If there is sufficient nonself-pheromone to displace the self-pheromone from the receptor, the mating process will commence. This model is supported by competition binding studies of purified pheromones [Luporini and Miceli, 1986]. An interesting prediction of this model is that the receptor and pheromone (∼ 14kD) will be
closely related, indeed identical, except for the portion of the receptor necessary to anchor it in the membrane. The cDNA for one pheromone has been characterized [Miceli et al., 1989], and cloning of the receptor gene will determine whether the receptor and pheromone are related. The $M_r$ of the dimeric pheromone–receptor complex is $\sim 28$ kD in molecular mass, which is compatible with this model [Beale, 1990].

A different model for Euploïdes octocarinatus predicts not one but many receptors per cell recognizing all pheromones but the ones produced by the cell itself [Heckmann and Kuhlmann, 1986]. Genetics of Eup. octocarinatus lend support to this model, and the recent cloning of the pheromone genes will help to clarify the details [Meyer et al., 1991, and personal communication; see Van Houten et al., 1981, for overview of protozoan chemoreception].

4. Yeast. There are two mating types, $a$ and $\alpha$, of haploid cells of the yeast Saccharomyces cerevisiae. Each mating type produces a pheromone that arrests cells of the complementary mating type in G1, induces changes in the cell wall and the characteristic shmoo shape, and alters gene expression as a prelude to mating. The pheromones $a$ and $\alpha$ are small peptides of 12 and 13 amino acids each [Thorner, 1981]. The $\alpha$-factor binds to approximately 8,000 sites on an $MATa$ cell with a $K_d$ of $6 \times 10^{-9}$ [Jenness et al., 1986, 1987]. A haploid cell will express the $a$ or $\alpha$-receptor gene, but not both, and likewise secretes only the pheromone to which it will not respond. The mechanism by which the $\alpha$-factor is secreted is unconventional and may involve a specific ATPase pump [see Featherstone, 1990, for review]. Diploid cells that result from mating are not responsive to either pheromone. The genetics of mating types in yeast is fascinating [Nasmyth, 1982], but here only the genes for the receptors are the focus of attention [see Fields, 1990, for a short review of the entire pheromone response].

The receptor for $\alpha$-factor is coded for by the STE2 gene and for $a$-factor by the STE3 gene [Burkholder and Hartwell, 1985; Nakayama et al., 1985; Hagen et al., 1986]. The amino acid sequence that is inferred from the DNA sequence gives a picture of two receptors that are similar in structure. It is curious that while their hydropathy plots are virtually superimposable with seven potential membrane-spanning regions, the proteins are utterly different in primary amino acid sequence [Hagen et al., 1986]. However, their deduced structures resemble those of members of the class of receptors that interact with G proteins: the rhodopsin/$\beta$-adrenergic, muscarinic acetylcholine family [Herskowitz and Marsh, 1987; Marx, 1987; Marsh and Herskowitz, 1988]. This classification based on structure is supported by recent reports of G protein involvement in the mating process (see below).

D. Invertebrates

This section focuses on chemoreception in sea urchin spermatozoa and arthropods, to the exclusion of other interesting, but less well-characterized invertebrate receptor systems [see Ache, 1987, for a review].
Secretions from the eggs of the sea urchins *Strongylocentrotus purpuratus* and *Arbacia punctulata* stimulate spermatozoan motility and metabolism to facilitate fertilization [Trimmer and Vacquier, 1986]. Upon stimulation, internal levels of cAMP and cGMP increase, there is a net $H^+$ and $K^+$ efflux and calcium influx, and guanylate cyclase is dephosphorylated (Fig. 6). The stimulus activity comes from two peptides, speract and resact, consisting of 10 and 14 amino acids each [Dangott and Garbers, 1984].

Speract and resact stimulate spermatozoa in a species-specific manner through receptor proteins in spermatozoan membranes. Speract- and resact-binding proteins have been identified through crosslinking studies as proteins of $M_r$ 77,000 and 160,000, respectively [Dangott and Garbers, 1984; Shimomura et al., 1986; Bentley et al., 1987]. The 160,000 $M_r$ resact receptor and the guanylate cyclase are the same protein [Shimomura et al., 1986] and represent one example of a new paradigm for second-messenger signal transduction [Bentley et al., 1986b; Paul et al., 1987; Thorpe and Garbers, 1989; Garbers, 1989a,b; Schulz et al., 1989]. The speract peptide of *Strong. purpuratus* likewise activates a guanylate cyclase, but it is not yet evident whether the 77 kD protein to which speract binds is the receptor or a subunit thereof or how it relates to the guanylate cyclase [Schulz et al., 1989]. When spermatozoa are incubated with egg jelly or resact, the guanylate cyclase is first transiently activated and then inactivated by dephosphorylation [Bentley et al., 1986a,b]. This loss of phosphates coincides with a change in mobility of the enzyme on gels from 160,000 to 150,000 $M_r$. Therefore, like receptors in bacteria and *Dictyostelium*, spermatozoan receptors may undergo covalent modification, and like *Bac. subtilis* in particular, attractant receptor occupancy would
be associated with removal of covalently attached groups. Whether this modification in spermatozoa is part of the adaptation process is not yet known. The spermatozoa receptacle serves as a model for the mammalian atriopeptide factor receptors, which similarly are guanylate cyclases and therefore represent yet other members of this relatively newly described signal transduction receptor family that crosses phylog [Lowe et al., 1989; Schulz et al., 1989; see Paul et al., 1987, and Bentley et al., 1986b, for discussions].

Among the arthropods, lobsters (both spiny and American) and insects share very similar structures and receptor cell mechanisms for taste and smell, even though the medium by which the stimuli arrive is in water for crustacea and air for insects [Atema, 1987]. The long, aesthetasc sensilla on the lobsters antennules and the sensilla trichodea of moth antennae subserve olfaction. Both have permeable chitinous coverings over the dendrites of the bipolar receptor neurons that send information about odors in trains of impulses to the central nervous system (CNS) [Ache, 1987; Kaislling, 1987]. The thick hedgehog sensilla on the lobster walking legs and the tarsal sensilla of insect feet are chitin-covered dendrites of receptor and mechanosensory cells with access to taste stimuli only through one tip pore. The animals taste as stimuli enter the sensilla, usually upon direct contact with food. As in olfaction, information about stimuli is sent to the CNS in trains of action potentials from the receptor neurons.

Sensilla on insect legs respond to contact with sugar solutions [Dethier, 1978] and those on insect antennae are tuned to components of pheromones [Vogt, 1987; Kaislling, 1987], while the lobster and other crustacea find amino acids, nucleotides, and peptides to be stimulatory [Ache, 1987; Carr et al., 1987]. The spectrum of stimulatory amino acids is large and overlaps between antennules and legs, characteristically with glutamate receptors prominent on legs and hydroxyproline and taurine receptors prominent on antennules [Atema, 1987].

As Atema [1985] notes, signals must not persist. They must be removed from the area of the receptor cell if fresh information is to be processed. The female pheromone is rapidly degraded by the abundant esterase in the lymph that surrounds the receptor dendrites in the male silk moth sensillum [Vogt et al., 1985; Vogt, 1987]. It is possible that the stimulus survives on its traverse from pores in the chitin covering to the receptor cell, because it is promptly bound to a lymph protein that protects it until it can stimulate the receptor [Vogt, 1987; Vogt et al., 1989]. The lobster, likewise, has a system for degradation of signal in the aesthetasc sensilla (Fig. 7). Here a primary stimulus, ATP, is degraded to ADP, AMP, and, finally, adenosine, all of which are active as weaker stimuli or inhibitors of the degradation enzymes or are actively transported into the receptor cell [Trapido-Rosenthal et al., 1987]. One end result of this cascade is the removal of stimulus from the receptor interaction, but certainly there are other modulatory effects as well.
Multiple receptors and signal degrading enzymes of the lobster olfactory sensilla. The cross section through the sensillum shows lymph surrounding dendrites, on which are separate receptors for adenine nucleotides and a ubiquitous adenosine uptake system. ATP, ADP, and AMP are stimulatory while adenosine is not, and ADP and AMP are antagonists of the ATP response. In the sensillar lymph surrounding the dendrites are enzymes that rapidly degrade ATP to adenosine and, by doing so, create complex mixtures of stimuli and ultimately adenosine that is removed by uptake. (There is no evidence that the classes of receptors always reside on separate dendrites.) (Reproduced from Trapido-Rosenthal et al., 1989, with permission of the publisher.)

Insects and crustacea have very low thresholds for response of a receptor cell [Kaiissling, 1987; Ache, 1987]. It is estimated that one molecule of pheromone can elicit an action potential [Kaiissling, 1987]. The action potentials originate in either dendrite or soma [Ache, 1987; Kaiissling, 1987], and their ionic mechanism(s) are not yet fully characterized [Schmiedel-Jakob et al., 1989; McClintock and Ache, 1988, 1989a,b]. A cell can be specialized to respond best to one stimulus, or it can be a more generalist in its response. In either case, the rate of discharge is a function of stimulus concentration, but a unique pattern of discharge does not code each individual stimulus in the cell's response spectrum [Ache, 1987]. Therefore, when odorant stimuli from natural sources arrive at the receptor cells in mixtures, it is thought that the collective pattern of depolarized cells across the olfactory sensillum provide the CNS with information about the quality and quantity of the odor. However, it is now clear (for vertebrates as well as invertebrates) that com-
ponents of odorant mixtures can be excitatory to one cell and inhibitory to another, and, while both individual cells are depolarized by the mixture, the inhibitory components can reduce the magnitude and delay the onset of the evoked depolarization [McClintock and Ache, 1989; Michel and Ache, 1990; Dionne, 1990]. Therefore, the first level of integration of information is at the level of the receptor cell [Michel and Ache, 1990] and not in the CNS, as previously believed.

The lobster responds over many orders of magnitude of stimulus concentration [Ache, 1987; Atema, 1987]. A means of expanding the dynamic range of a cell is the shift up of the entire stimulus–response curve of leg ammonium receptor cells after adaptation to background amounts of ammonium [Borroni and Atema, 1987]. (This is very reminiscent of the neutrophil’s ability to respond to an increase in 200 receptors occupied regardless of whether the change is 200 to 400 or 4,000 to 4,200 [Zigmond, 1989]; see section II.B.) Adaptation may allow these cells to distinguish signal from background ammonium, which ranges from $10^{-6}$ M in seawater to $10^{-2}$ M in prey tissues [Atema, 1987]. The molecular mechanism of adaptation is not yet understood, but must somehow be accounted for in the receptor function or the transduction mechanism [see Atema, 1985; Derby and Atema, 1987; Schmitt and Ache, 1979; and Ache, 1987, for consideration of adaptation and section II.G. for other means of expanding a cell’s dynamic range].

The receptor proteins of arthropod sensilla have been elusive, even though moths provide reasonable amounts of membrane for receptor biochemistry. Recently, however, Vogt et al. [1988] used a photoaffinity analog of the Antheraea polyphemus pheromone (E,Z)6,11-3H-hexadecanethyldiazoacetate to label not only the soluble receptor lymph-binding protein but also a 69 kD protein from dendrite membranes. This protein is labeled specifically by the photoaffinity probe and is found in dendritic membrane only and in male moths only [Vogt et al., 1988].

E. Vertebrates

Vertebrates monitor their chemical environment through taste, smell, and common chemical sense. In all these sensory modalities, neurons or neuroepithelial cells serve as receptor cells.

1. Taste. Taste buds generally are comprised of 50–150 neuroepithelial cells in pear- or spindle-shaped clusters [Kinnamon, 1987]. They make contact with the environment at their apical end with the microvilli that protrude through the taste pore. The receptor cells are innervated by fibers that penetrate or abut the bud. Generally, sweet, saline, sour, and bitter tastants are detected by receptor cells that are not particularly finely tuned, but do respond best to one tastant class [Teeter and Brand, 1987b]. Amino acids are taste stimuli, particularly in fish. As in crustacea, the spectrum of amino acids overlaps for taste and smell [Caprio, 1988; Atema, 1987].
Upon stimulation, a taste receptor cell will release neurotransmitter and alter the rate of spontaneous firing of primary nerve fibers that traverse to the CNS. In this case, interaction of stimulus with the apical membrane of the receptor cell is transduced into signals for neurotransmitter release and generally not into active electrogenic response as in taste receptors in invertebrates and all olfactory receptor cells (although some taste cells have been shown to be capable of generating action potentials [Roper, 1989]).

There is no unifying mechanism for taste stimulus transduction. The ion movements and second messengers in response to saline, sweet, bitter, sour, and amino acid tastants are still being deciphered [Kinnamon, 1988; Teeter and Brand 1987b]. However, some generalizations can be made, and all of the following mechanisms are likely to work (Fig. 8): The release of neurotransmitter is a calcium-dependent process, and a tastant could increase internal calcium by one of several ways. 1) Depolarization would open calcium channels and allow an influx of internal calcium. The depolarization could be a direct consequence of the tastant, e.g., salt entering the cell through voltage-insensitive amiloride blockable channels [DeSimone et al., 1981; DeSimone and Ferrell, 1985; Schiffmann, 1990]; H+ transiently blocking resting K+ conductance, thus decreasing a hyperpolarizing conductance [Kinnamon and Roper, 1988]; or a tastant binding to receptor that in turn opens a channel or activates a transport system [Mierson et al., 1988; Teeter et al., 1990]. 2) Alternatively, no depolarization is needed if a tastant alters surface potential and consequently opens channels in the membrane, or if the tastant, through a receptor such as the sugar receptor or the catfish alanine receptor, generates internal messengers that liberate calcium from internal stores [see Teeter and Brand, 1987b; Teeter et al., 1987, 1989; and Roper, 1989, for reviews].

There is evidence for receptors in the mediation only of sweet and amino acid taste [Sato, 1987; Teeter and Brand, 1987b; Cagan and Boyle, 1984; Dionne, 1988]. Perhaps the best characterized are the catfish receptors for alanine and arginine for which binding kinetics have been measured and blocking antibodies have been produced [Cagan, 1981; Brand et al., 1987; Bryant et al., 1987; Kalinoski et al., 1987a,b]. A heterogeneous group of proteins of 110,000 daltons exclusively from taste tissues are recognized on immunoblots by an antibody that blocks alanine binding [Bryant et al., 1987, 1989]. The receptor among these proteins has yet to be purified or cloned, but it is expected that the arginine receptor will be a ligand-gated cation channel and the alanine receptor will be a G protein-associated, transmembrane receptor [Teeter et al., 1990].

The only other partially characterized taste receptors are for sapid stimuli [Persaud et al., 1988] and the sweet-tasting protein thaumatin [see Sato, 1987 for a review]. A 3H-photoaffinity analog of thaumatin was used to label specifically a 50,000 dalton protein in taste but not other papillae. Likewise, proteins eluting from a thaumatin affinity column included one of ≈50,000 daltons.
2. Olfaction. Olfactory receptor cells are bipolar primary neurons. The cilia (or, less commonly, microvilli) at the apical ends make contact with the immediate environment, the mucous layer that sweeps across the olfactory and respiratory epithelia. The mucus is secreted in part by sustentacular cells that along with the receptor and basal cells make up the epithelium. Odorants partition into the mucus, where at least some kinds are bound by an olfactory-binding protein (OBP) [Pevsner et al., 1985; Bignetti et al., 1985] that is secreted into the mucus [Pevsner et al., 1986]. It is likely that this OBP from cow and mouse as well as a newly described protein from frog [Lee et al., 1987] are analogous to the insect lymph pheromone-binding protein and protect odorant...
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until it contacts the cilia, the site of olfactory transduction [Adamek et al., 1984; Rhein and Cagan, 1981]. Alternatively, the OBP may function to clear odor from the mucus [see Getchell and Getchell, 1987, and Getchell et al., 1984, for review]. As the intake of air washes odorants over the epithelium, the odorants partition into the mucus as a function of their solubility, imposing a differential distribution of odorants on the epithelium. Superimposed on this is the inherent pattern of receptor cells. Individual receptor neurons are broadly tuned with a spectrum of response that overlaps that of other cells [Gesteland, 1986; Sicard and Holley, 1984]. However, receptor cells are not evenly distributed across the epithelium. Maps of activity across the epithelium show differential clusters of activity to different odorants [Edwards et al., 1988]. The axons of the receptor cells project to the olfactory bulb, and the pattern of activity across the axons must encode the quantity and quality of the odor [see Kauer, 1987, for a review].

A receptor cell will respond to an excitatory odorant stimulus with a decrease in input resistance, current flow of monovalent cations, and subsequent depolarization receptor potential that is responsible for opening voltage-dependent channels of the axon hillock [Gesteland, 1986; Persaud et al., 1987]. The opening of these channels converts the transducing receptor current into action potentials that are transmitted to the CNS [see Getchell and Getchell, 1987, for review]. The important questions in olfactory transduction regard the generation of a receptor current and depolarization by the interaction of stimulus with dendritic ciliary membrane.

Because olfactory receptor cells are broadly tuned, it is possible that each has many receptors for different stimuli. The number of stimuli that a human can detect is thought to be $10^6$ [Dionne, 1988], and therefore the number of receptors necessary for this detection could be very high. There could be many gene products of similar size but with different odorant-binding capacities, perhaps generated through differential gene rearrangement analogous to the immune system or through differential splicing of one transcript or transcription of a family of genes. Minimally there appear to be two transduction mechanisms (see below) and therefore at least two sets of receptor types. However, to date no vertebrate olfactory receptor protein has been isolated, although there are some candidates (see next paragraph). This failure to identify an external olfactory chemoreceptor may be due in part to the difficulties of dealing with low abundance membrane proteins of relatively low binding affinity [Price, 1981]. Alternatively, there may be mechanisms of olfaction that do not require receptors, in which volatile lipophilic molecules interact directly with the membrane [Dionne, 1988; Anholt, 1987; Lerner et al., 1988]. The recent cloning of putative receptor genes will help to resolve these issues [Buck and Axel, 1991; Nef and Dionne, personal communication; see section V].

Candidates for receptors are the anisole- and benzaldehyde-binding proteins identified by affinity chromatography of dog epithelium [Price and Willey,
These proteins are found in the olfactory but not respiratory epithelium. Antibodies against the anisole proteins block electrical responses to anisole stimulation best, but block all odorant stimulation to some degree, perhaps reflecting epitopes common to all receptors. This idea is supported by studies of electro-olfactograms (EOGs) in the presence of monoclonal antibodies against the same proteins [Price and Willey, 1988]. Some of the monoclonal antibodies were specific in their inhibition of the electrical stimulation by anisole or benzaldehyde, but others were nonspecific. It is curious that the anisole- and benzaldehyde-binding proteins are of the same M, (62,000) and perhaps share an epitope in common to a family of olfactory receptors.

Lancet and coworkers have found a membrane glycoprotein of 95 kDa (gp95) from frog olfactory epithelium that is of particular interest because antibodies against it coprecipitate the ciliary adenylate cyclase, which they argue is an important component in sensory transduction [Lancet et al., 1987; Lancet and Pace, 1987; Chen et al., 1986]. While this protein has the tissue specificity and location in ciliary membrane to qualify for a receptor, it has not been demonstrated to bind odorant and appears to be secreted into the mucus [Menco, 1991].

Fesenko et al. [1987] reported odorant binding to a membrane glycoprotein specific to the olfactory epithelium. However, as Vogt et al. [1988] point out, there is no demonstration that this protein is enriched in the ciliary fraction. In other examples, binding sites for 3H-alanine [Rhein and Cagan, 1981; Cagan, 1981], amino acids on skate olfactory epithelium [Novoselov et al., 1988], and pyrazine [Pelosi et al., 1982] are described, but the protein moieties are at best only partially purified and characterized.

3. Common chemical sense. In common chemical sense, chemosensation is due to the stimulation of epithelial or mucosal free nerve endings from branches of the trigeminal nerve [Silver, 1987]. Receptor studies lag behind those in taste and olfaction and will not be treated further here.

F. Perireceptor Events

Ligand binding to receptor is thought to begin the transduction process that leads to the second messengers and ion conductance changes that are essential for chemosensation. However, there are essential events that occur prior to and after binding, the so-called perireceptor events that play a significant role in the chemoreception process [Getchell et al., 1984]. As mentioned for insect pheromone and vertebrate olfaction, there are molecules that are likely to be carriers, i.e., protectors, of the odorant molecule until it binds to receptor. Stengel et al. [1990] have shown that pheromone complexed with pheromone-binding protein is less effective than pheromone alone at opening ion channels in cultured insect cells, and therefore pheromone-binding proteins may also function to sequester stimulus in preparation for the next wave. Recently, an mRNA for a protein analogous to the OBP has been demonstrated in von Ebner's glands, salivary glands located directly beneath and ducting into a
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...through at the base of rat taste bud papillae [Schmale et al., 1990]. The protein is a member of the same carrier protein superfamily to which OBPs belong, suggesting that this salivary protein might function in the concentration or delivery of sapor molecules to the taste receptor. Whether these binding proteins can act to facilitate diffusion or cause concentration of stimulus [Pevsner et al., 1986] is a matter of discussion [Snyder et al., 1988; Pelosi and Dal Monte, 1990]. However, there clearly are other mechanisms to remove the stimulus from the region of the receptor: phosphodiesterase of Dictyostelium degrades extracellular cAMP; neutrophil enzymes degrade the attractant stimulus FMLP; phosphatase, and nucleotidases in lobster, destroy nucleotide stimuli; the movement of mucus and active and facilitated transport remove stimuli from the area of receptors in vertebrates and crustaceans; and intracellular degradation by cytochrome P-450 and UDP-glucuronosyltransferase prevent the diffusion of hydrophobic odorants back out of cells where they can be confused with newly arriving stimuli [Lancet et al., 1989; Lazard et al., 1990, 1991; see Burchell, 1991, for review]. The relative rates of these reactions greatly influence the sensitivity, duration, and adaptation of receptor-mediated events and therefore indirectly affect sensory transduction [see Getchell et al., 1984; Getchell and Getchell, 1987; Carr, 1989; and Trapido-Rosenthal et al., 1989, for reviews].

G. Reception in Mixtures

Stimuli rarely come singly, but more usually in a bouquet with other components. Mixtures are the most effective stimuli in eliciting behavioral responses in crustacea [Carr et al., 1984] and insects [Linn and Roelofs, 1989; Linn et al., 1985; O'Connell, 1986; Vogt, 1987]. The components of natural stimulatory mixtures for these organisms are not generally equimolar or equipotent; some components act additively or synergistically to stimulate, and others suppress the behavioral response [see Derby and Atema, 1987; Caprio 1987a,b; Ache, 1987, 1989; and Derby et al., 1989, for discussion].

The individual receptor cells inside both taste and olfactory sensilla can be finely tuned to only one component of the mixture, and indeed narrow tuning predominates in the lobster taste and smell [Atema, 1985] and insect olfaction [Kaisling, 1987]. However, despite the narrow tuning, these receptor cells do not extract information about their "best" stimulus to the exclusion of other components of a mixture. Indeed, mixture components, to which the finely tuned cell will not respond individually, often suppress the receptor cell impulse output from its "best" stimulus [Derby and Atema, 1987]. This mixture suppression is thought to allow the cell a large concentration range for response [Atema, 1987; Johnson et al., 1989]. The lobster receptor cell response saturates over two to three orders of magnitude, but the receptor populations must be able to respond over a range of $10^{-8}$ to $10^{-2}$ M amino acids, for example [Atema, 1985; Ache, 1987]. Not all stimuli in mixtures are excitatory or neutral. As discussed under invertebrate olfaction (section II.D.2.),
stimuli can be excitatory to some cells and inhibitory to others by hyperpolarizing the cell. Mixtures of the hyperpolarizing stimuli can influence the time of onset and the magnitude of the evoked depolarization, thus allowing for integration of mixture information at the periphery as well as at the CNS.

Lobsters and insects are not the only organisms to show mixture suppression and synergy, but they serve to point out that, when examining receptor function and receptor-mediated transduction, one must be aware of mixture effects. Additionally, mixture suppression occurs in the CNS as well as the periphery and therefore should not be interpreted solely as a modification of receptor or receptor cell function [Derby et al., 1984; Derby and Ache, 1984].

III. SECOND MESSENGERS IN CHEMORECEPTION

A. Overview of Second Messengers

Now that several chemoreceptor systems have been introduced, it is time to discuss their common aspects of signal transduction. None of these transduction pathways are known from ligand to response end, but there is clear evidence for each system that one or more second messengers are involved. There are a limited number of second messengers, and, as Margolis [1987] notes for olfaction, there is no reason to suspect that any of these organisms have had to utilize a completely novel pathway to deal with the fairly common process of signally the presence of an external ligand to the interior of a cell. It is important to recognize that there can be two or more "second" messengers in series. To avoid confusion, these will be referred to generally as "internal" messengers and more specifically as "second" and "third" messengers if this hierarchy has been established.

B. Cyclic Nucleotides

Changes in levels of intracellular cAMP or cGMP have been identified as responses to ligand binding in Chlamydomonas agglutination [Pasquale and Goodenough, 1987; Musgrave and van den Ende, 1987], D. discoideum aggregation [Janssens and Van Haastert, 1987], sperm chemotaxis [Garbers et al., 1986], neutrophil chemotaxis [Sha'afi and Molski, 1988], and vertebrate olfaction and gustation [see Teeter and Gold, 1988; Lancet and Pace, 1987; and Anholt, 1987, for overviews]. In Chlamydomonas and D. discoideum it is most clearly established that cyclic nucleotides are internal messengers that function as links in the sensory transduction pathways.

In Chlamydomonas, intracellular cAMP is increased 10-fold upon flagellar agglutination [Pasquale and Goodenough, 1987]. Permeable dibutyl-cAMP and phosphodiesterase inhibitors will elicit flagellar tip activation, cell wall loss, and mating structure activation with actin polymerization in gametes of
a single mating type, and an inhibitor of cAMP protein kinase, H8, antagonizes these effects. The flagella have adenylate cyclase (albeit different from vertebrate enzymes) and phosphodiesterase activity [Pasquale and Goodenough, 1987]. Therefore the site of signal transduction that controls second-messenger levels could be either of these enzymes.

In *D. discoideum*, cGMP is not the only internal messenger generated as a result of receptor binding (Fig. 5), but it does indeed appear to be a causative agent in the half of the bifurcated response pathway that mediates chemotaxis. The transient increases in internal cGMP in response to external cAMP or folic acid stimuli occur over the time course expected for the second-messenger response for initiation of chemotaxis, and for some time cGMP was considered the second messenger in chemotaxis [Mato et al., 1977; Wurster et al., 1977; see Van Houten and Preston, 1987, for an overview]. Now it is clear that increases in inositol phosphates, in particular inositol-1,4,5-trisphosphate (IP$_3$), and Ca$^{2+}$, precede the stimulation of guanylate cyclase and the rise in cGMP (Fig. 5) [Small et al., 1987; Europe-Finier and Newell, 1985, 1986]. Therefore cGMP may be considered a later, perhaps fourth, messenger and functions in the accumulation of myosin in the cytoskeleton of the *Dictyostelium* cell that is preparing for a change in cell shape and oriented movement [Liu and Newell, 1988; Newell et al., 1988]. Actin polymerization likewise is implicated in change of cell shape and as a driving force in pseudopod extension [Newell, 1986; Condeelis et al., 1988]. The "B" receptor, mediated chemotaxis pathway that couples through G$_{2a}$ to phospholipase C (PLC) can be examined separately from the "A"-mediated relay pathway that is coupled through the G protein to the adenylate cyclase, and it appears that G$_{2a}$ is directly or indirectly responsible for transduction of stimulus by cAMP to actin nucleation centers [Hall et al., 1989]. Intracellular Ca$^{2+}$, not cGMP, appears to be the second messenger for actin polymerization, although both second messengers are the consequences of "B"-receptor activation of PLC [Newell, 1986]. Mutants with defective phosphodiesterase and hence abnormally elevated and prolonged rises in cGMP are defective in chemotaxis [Ross and Newell, 1981; Van Haastert et al., 1982]. In these mutants, myosin association with the cytoskeleton but not actin polymerization is affected [Newell, 1986; Liu and Newell, 1988].

In *sea urchin sperm* chemotaxis, the stimulus resact increases cAMP levels 300-fold [Garbers and Kopf, 1980] and alters cGMP levels by transiently stimulating and then inhibiting the receptor guanylate cyclase [Bentley et al., 1986a]. The adenylate cyclase requires an influx of calcium for its activation (Fig. 6), and cAMP in turn activates the cAMP-dependent protein kinase that figures into the stimulatory effects of resact on respiration and motility [Garbers et al., 1980; Garbers, 1986; see Satir, 1985, Bonini and Nelson, 1988, and Tash et al., 1987, for effects of cyclic nucleotides on flagellar motility]. Because the guanylate cyclase is both resact receptor and generator of internal messen-
ger, it seems safe to assume that either the absolute increase or decrease (as in vision) or the changes in cGMP figure into the chemotaxis signal transduction pathway.

*Olfactory cilia* have a very high adenylate cyclase activity [Lancet, 1986], and this activity is stimulated 1.5–2.5-fold with some odorants in a GTP-dependent manner [Pace and Lancet, 1986; Sklar et al., 1986; Shirley et al., 1986]. At present, odorants are categorized by their ability or inability to stimulate adenylate cyclase. The latter class is thought possibly to work by stimulation of phosphoinositide lipid (PIP$_2$) hydrolysis to generate internal messengers [Lancet and Pace, 1987]. (cGMP is not a candidate as an internal messenger here because its levels do not change with odorant stimulation [Shirley et al., 1986].)

What has not been apparent is the role of the cAMP that clearly increases in response to some odorants: Is cAMP a second messenger that opens ion channels in receptor cells either directly or by way of a protein kinase A activity, or is it outside the sensory transduction pathway and functions to desensitize the receptor or close ion channels by processes such as phosphorylation (Fig. 9)? The time course of the adenylate cyclase stimulation in some systems would suggest that it functions in a slower process such as adaptation/desensitization [Bruch and Teeter, 1988; Bruch et al., 1987a,b, 1989; Anholt, 1987]. Also, there are ion conductances that are directly odorant stimulated, obviating the need for a second messenger to open channels [Labarca et al., 1988]. However, cyclic nucleotide—sensitive conductances have also been identified by voltage- and patch-clamping of receptor cells. These conductances respond equally well to cAMP or cGMP [Nakamura and Gold, 1987; Kolesnikov et al., 1990; Bruch and Teeter, 1990].

The magnitude of the EOG, a summed response of olfactory epithelial cells elicited by an odorant, correlates with the magnitude of adenylate cyclase activity, possibly implying a transduction and not adaptation role for cAMP [Lowe et al., 1988]. Which one or more of these nucleotide-dependent and -independent conductances is involved in sensory transduction and generation of the receptor potential in the olfactory receptor cells remains to be established. However, it seems clear that cAMP gating of channels will be among the mechanisms [Firestein and Shepherd, 1989, 1990].

Recently, stop-flow kinetics analyses have provided evidence that cAMP can indeed increase and drop off sufficiently rapidly in a GTP-dependent manner to qualify as a second messenger for a set of odorants [Breer and Boekoff, 1991; Breer et al., 1990a,b]. It appears that odorants stimulate either cAMP or IP$_3$ production in rat olfactory cilia on a subsecond time scale. In comparison, a moth pheromone stimulates second-messenger production on a time scale sufficiently fast to be involved in conductance changes, but only IP$_3$ is generated in the antennal preparations. In all cases, the effects on second messengers are GTP dependent, reinforcing the idea that odorant receptors will eventually be isolated. Excitable cells other than olfactory receptor cells did not respond to odorants
with increases in second messengers, helping to discount some of the concern that the lipophilic nature of some odorants would allow them to induce conductance changes by direct membrane interactions without benefit of receptors or specific binding sites on channels [Dionne, 1988; Lerner et al., 1988; Kashiwayanagi et al., 1990].

There may be a second mechanism by which odorants stimulate the adenylate cyclase [Anholt and Rivers, 1990]. In frog epithelium, adenylate cyclase is stimulated not only by odorants, but also by Ca\(^{2+}\)-calmodulin in a GTP-dependent manner [Anholt and Rivers, 1990]. The two modes of stimulation are additive, providing for alternate mechanisms by which odorants can produce second messengers and for "cross-talk" between pathways. In particu-
lar, Anholt and Rivers [1990] propose that low concentrations of odorants directly open Ca\(^{2+}\) channels to provide the Ca\(^{2+}\) for calmodulin stimulation of adeny- 
late cyclase and that, at high concentrations of odorants, the olfactory specific G protein (G\(_{olf}\)) is activated, resulting in further stimulation of adeny- 
late cyclase. This model implies that receptor cells could have the potential for cross- 
talk between these pathways to add a dimension of integration of information about quantities of stimuli in mixtures, for example. (See next section for parallel pathways for cAMP and IP\(_3\) in catfish.)

Similarly in taste cells, cyclic nucleotide internal messengers are implicated in the depolarization of the receptor cell. Reports of GTP-dependent cAMP synthesis in response to sugars in rat taste tissue [Lancet et al., 1987; Striem et al., 1989] and to amino acids by catfish [Bruch and Teeter, 1988, 1989; Kalinoski et al., 1988; Bruch et al., 1989] are further supported by direct measurements of depolarizing conductance changes mediated by cyclic nucleotides. Rat taste 
cells injected with cGMP (and to a lesser extent with cAMP) depolarize the cells by decreasing K\(^{+}\) conductance, the same conductance that decreases during depolarization with a sugar stimulus [Tonosaki and Funakoshi, 1988]. Frog taste 
cells under patch-clamp likewise decrease K\(^{+}\) conductance with cAMP (cGMP was not tested), and in this case the conductance decrease has been traced to a cAMP-dependent protein phosphorylation [Avenet et al., 1988].

The stimulation of taste adeny late cyclase may be sufficiently fast to allow cAMP levels to participate in transduction [Kalinowski et al., 1988, 1989], but not all tastants in catfish activate adeny late cyclase, thereby requiring at least a second transduction mechanism. (The recent reports of tastants L-arginine and L-proline activating ion channels directly neatly provides one answer [Teeter et al., 1990; Kumazawa et al., 1990; Kohbara et al., 1990]; see section IV.D.). The modulation of ion channel gating by cAMP-dependent protein kinase is a departure of the gustatory from the olfactory system with its direct gating by cyclic nucleotides [Nakamura and Gold, 1987], but there are bound to be multiple mechanisms of transduction in taste cells, perhaps some of which will include direct gating by cyclic nucleotides, calcium, inositol phosphates, and other internal messengers (see Teeter and Gold, 1988, for an overview; also see sections III.C. and IV.B.).

**Neutrophils** experience transient increases in cAMP with chemotactic peptide stimulation not as a result of activation of the adeny late cyclase, but probably through inhibition of the phosphodiesterase [Jackowski and Sha’a fi, 1979; Sha’a fi and Molski, 1988; Smollen et al., 1980; Keller et al., 1970; Verghese et al., 1985]. However, exogenously applied cAMP, epinephrine, PGE\(_1\), cholera toxin, and isoproterenol inhibit chemotaxis, phagocytosis, and degranulation [see Sha’a fi and Molski, 1988, for an overview].

Interestingly, dibutyryl-cAMP inhibits the FMLP-stimulated phosphoinositol lipid hydrolysis [Della Bianca et al., 1986; Takenawa et al., 1986; Kato et al., 1986] and may do so indirectly through the action of the cAMP-dependent
protein kinase A on either the FMLP receptor or the sensory transduction G protein characteristic of neutrophils and associated with the receptor [Sha'afi and Molski, 1988]. Taken together, these observations suggest an indirect, modulatory role for the FMLP-induced transient rise in cAMP as opposed to a role of an internal messenger in the sensory transduction pathway.

C. Phosphoinositil Lipid Hydrolysis and Sequelae

There is a renewed appreciation of the role of lipids and lipid metabolites in receptor functioning. In particular, arachidonic acid (AA) and the inositol phospholipid hydrolysis products 1,4,5-IP₃ and diacylglycerol (DAG) have been found to figure into a diverse range of sensory transduction pathway steps [Berridge, 1987; Kikkawa and Nishizuka, 1986; Piomelli et al., 1987; Axelrod et al., 1988], many of which have been documented to be mediated by G proteins [Gilman, 1987; Berridge, 1987; Stryer and Bourne, 1986; Axelrod et al., 1988]. 1,4,5-IP₃ and DAG are generated by the action of phospholipase C (PLC) on PIP₂, although DAG can be derived from other sources as well. AA is generated by the action of DAG lipase on DAG or phospholipase A₂ on membrane lipids. Therefore, AA synthesis can be linked to or separate from the PIP₂ metabolism. The functions of IP₃ and DAG appear to be the liberation of calcium from internal, nonmitochondrial stores [Berridge, 1987] and the activation of protein kinase C, respectively [Kikkawa and Nishizuka, 1986]. The direct site of interaction of AA or its metabolites is not yet clear. It is clear, however, that IP₃, DAG, and/or AA are produced as a result of ligand–receptor interactions in Dictyostelium, neutrophils, and olfactory and taste cells.

A neutrophil’s response to FMLP is many faceted and mediated by several different internal messengers. The facets of the response differ in concentration of stimulus and time: Chemotaxis requires a lower concentration of FMLP, and changes in cytoskeleton preparatory to oriented movement occur immediately; activation of NADPH oxidase that is seminal in the oxidative burst requires a 10–50-fold high stimulus concentration [Snyderman, 1984] and occurs later in the process of activating the cell [Truett et al., 1988]. Therefore, to simplify the discussion and yet touch upon the major issues of neutrophil activation, two aspects will be examined, i.e., actin polymerization in preparation for chemotaxis and NADPH oxidase activation for the oxidative burst that produces bactericidal O₂⁻ and H₂O₂.

Receptor–FMLP binding through the intervention of a G protein stimulates PLC activity and thereby increases the cellular content of 1,4,5-IP₃ and DAG [Dillon et al., 1987a]. 1,4,5-IP₃ affects internal calcium stores and also serves as a precursor to 1,3,4,5-IP₄ (IP₄), a potential stimulus for opening calcium channels [Irvine and Moor, 1986; Houslay, 1987]. (External Ca²⁺ entry appears to be essential for some sustained responses to stimuli, but the mechanism by which surface membrane calcium channels open after the initial stimulation
of IP₃ production is a matter of debate [Putney, 1987; Petersen, 1989; Schulz et al., 1989]. Additionally, the source of this secondary Ca²⁺ for sustained response need not be external [Krause et al., 1989].

The metabolism of 1,4,5-IP₃ follows two separate routes: 1) at ambient intracellular Ca, it is dephosphorylated to 1,4-IP₂, 4-IP, and inositol; and 2) at increased calcium levels, it is phosphorylated to 1,3,4,5-IP₄, which is then dephosphorylated through a different sequence of intermediates. The differential dependence on calcium implies different functions for the inositol polyphosphates in neutrophil activation. There undoubtedly are other levels of control, as the finding of S-adenosylhomocysteine as a competitive inhibitor of phosphatidylinositol kinase implies [Pike and DeMeester, 1988]. The activation of protein kinase C by DAG appears to feed back and inhibit phosphatidylinositol hydrolysis by a yet undefined interaction with the G protein that intervenes between receptor and PLC [Dillon et al., 1987a]. DAG also serves as an important source of AA that in turn serves many functions by providing a precursor to leukotrienes (also chemoattractants), prostaglandins, and thromboxanes [Balsinde et al., 1988] and by liberating internal calcium pools [Nasmith and Grinstein, 1987a,b; Beaumier et al., 1987]. Additionally, phospholipase A₂ can produce AA from sources other than DAG, and activation of phospholipase A₂ is an early event in activation of neutrophils by ionophore [Lackie, 1988; Balsinde et al., 1988].

Upon stimulation, a neutrophil immediately changes shape by extending pseudopods and lamellipodia along the edge in contact with the highest stimulus concentration. Subsequently, it reorients its cytoskeleton to produce the characteristic polar shape and moves up the gradient of attractant [Cochrane, 1984]. Underlying the shape change and motility are rearrangements of the cytoskeleton, including a rapid polymerization, slower depolymerization, and redistribution of actin within the cell [Howard and Wang, 1987; Sha’afi and Molski, 1988; Cassimeris et al., 1990]. There appear to be two populations of actin in the cell: one stable, cortical population that is unaffected by attractants and a second labile population rapidly turning over in the lamellipodia, where chemoattractants stimulate polymerization for oriented movement and removal of attractants triggers rapid depolymerization [Cassimeris et al., 1990]. In contrast to Dictyostelium, PIP₃ and not PIP₂ seems to be involved in the modulation of actin after receptor binding [Eberle et al., 1990].

There are roles for both calcium and protein kinase in chemotaxis as evidenced by studies of oriented locomotion using ionophores, internal calcium buffers and chelators, calmodulin inhibitors, and inhibitors and activators of protein kinase [Wight et al., 1988; Harvath et al., 1989; Roos et al., 1987; Sha’afi and Molski, 1988; Laskin et al., 1987]. The roles must be complex, because manipulations of protein kinase C and intracellular Ca alone or in combination are not sufficient to account for the in vivo changes in F-actin that precede chemotaxis [Howard and Wang, 1987]. However, there are complications in the interpretation of the results with phorbol myristate acetate (PMA) treatment [Webster et al., 1986; Roos et al., 1987] that do not satisfac-
Signal Transduction in Chemoreception

The oxidative burst, as chemotaxis, appears to involve multiple pathways [see Bagdian and Wymann, 1990, for review] and is initiated through the receptor-mediated stimulation of PIP2 metabolism with consequent increases in internal calcium levels. One emerging scenario for regulation of the oxidative burst is that NADPH oxidase is activated by three mechanisms, all of which may be active under normal physiological conditions. The first is a calcium-dependent mechanism that is part of the "classic" signal transduction liberating DAG and calcium via IP3 and possibly IP4. This mechanism is supported by the observation of calcium ionophore A23187 and ionomycin stimulation of the oxidative burst and the inhibition of this stimulation by trifluoperazine [see Sha'afi and Molski, 1988; and Grinstein and Furuya, 1988, for details].

The second is a protein kinase C-dependent mechanism that requires a minimum but not a change in the internal levels of calcium [Wymann et al., 1987; Grinstein and Furuya, 1988]. Indeed, some of the strongest evidence comes from PMA induction of the oxidative burst and inhibition of this induction by protein kinase C inhibitors [Grinstein and Furuya, 1988; Sha'afi and Molski, 1988]. Additionally, PMA pretreatment can circumvent this inhibition of the oxidative burst, PLC activation, and production of IP3 and DAG brought about by buffering internal calcium with Quin-2 [Lew et al., 1984]. The presence of these first two mechanisms, one calcium dependent and the other protein kinase dependent, would fit with the observed synergism of calcium and DAG or permeable analogs [White et al., 1984; Volpi et al., 1985].

The third pathway is a very rapid NADPH oxidase activation that is not dependent on calcium and may be an effect of the lipid environment on the membrane-bound enzyme [Grinstein and Furuyma, 1988].

Truett et al. [1988] and Reibmann et al. [1988] have compared leukotriene B4 and FMLP for their internal messenger production, because, while they are equipotent as chemotaxis stimuli, they are very different in their ability to stimulate the oxidative burst. In these comparisons, leukotriene B4 and FMLP elicited similar initial increases in calcium, IP3, IP4, and DAG (<30 sec), but FMLP sustained the increased calcium and IP3 levels and elicited a second peak of DAG (at ~120 sec). This second peak of DAG was not derived from phosphoinositid lipid hydrolysis and probably was dependent on the sustained higher calcium levels. These results predict that a stimulus that supports a
sustained phosphoinositol lipid hydrolysis will also sustain increased calcium levels not only by liberating internal stores but also by stimulating influx from external sources (possibly mediated by calcium-stimulated calcium conductance or possibly IP$_3$-gated channel [Baggioni and Wymann, 1990]. This prolonged new high level of internal calcium will elicit a second wave of DAG production perhaps from phosphatidylcholine. The DAG may participate in protein kinase C membrane translocation for the oxidative burst, because leukotriene B4 that fails to elicit much of a burst also fails to stimulate much translocation [Nishihara et al., 1986].

The substrates of protein kinase C are being cataloged and include a 60 kD protein that is associated with the activation of the Na$^+$/H$^+$ antiporter by FMLP, possibly the α-subunit of the G protein that activates PLC; a 47 kD protein associated with degranulation; and several membrane- and cytoskeleton-associated proteins [White et al., 1984; Suzuki et al., 1990]. These substrates will be the next components of the multiple pathways to be fit into the puzzle of the neutrophil sensory transduction.

Activation of the calcium–phospholipid-dependent protein kinase C includes its translocation from cytosolic to membrane compartments of the cell. The binding to the DAG that is transiently present because of hydrolysis of phosphoinositol lipids brings the enzyme in contact with the membrane and lowers the calcium requirement [Sha'afi and Molski, 1988]. It is tempting to speculate that priming of neutrophils (i.e., synergistic enhancement of respiratory burst by pretreatment with low concentrations of agonists) also could be due to the translocation of the protein kinase C to the membrane, where it stands ready for immediate response to stimulus. This would fit with Alkon and Rasmussen's idea [1988] of biochemical memory. However, priming as with all aspects of neutrophil physiology, is not simply accounted for by protein kinase C translocation, but alternatively may be due to protein kinase C activation in the cytosol by increased calcium or possibly to the actions of AA [Bass et al., 1987; Costa-Casnellie et al., 1986; Korchak et al., 1984].

As depicted in Figure 5, the D. discoideum transduction systems are two-fold, with one set of receptors (A sites) associated with the adenylate cyclase of the relay and another set of receptors (B sites) poised to activate the cytoskeletal alterations necessary for chemotaxis by way of PIP$_2$ hydrolysis, calcium liberation, and production of cGMP. The implication of IP$_3$ comes from its mobilization of calcium from internal stores and polymerization of actin when applied to permeable cells [Newell et al., 1988]. Additionally, $^3$H-inositol was used to trace the increased cycling of phosphoinositol lipids through IP$_3$ with cAMP stimulation [Europe-Finner and Newell, 1987a]. The liberation of calcium was found to be downstream from the phosphoinositol lipid step in the transduction pathway; calcium mimicked cAMP-induced actin polymerization and cGMP formation in permeabilized cells without stimula-
tion of IP₃ formation [Newell et al., 1988]. The actin polymerization in slime mold amoebae is not sustained [Newell et al., 1988], but exhibits a cyclical change in polymerization–depolymerization–repolymerization that corresponds to the initial pseudopod extension, rounding up (cringing), and cell elongation necessary for oriented movement. Calcium seems to be directly responsible for these cycles of F-actin formation [Newell, 1986]. Calcium also activates the guanylate cyclase, and the resultant cGMP in turn regulates the association of myosin with the cytoskeleton, independent of calcium [Liu and Newell, 1988]. Presumably the actin and myosin associate with the cytoskeleton and contribute to orientation and motility. Chemotaxis, although not completely normal, occurs in the absence of myosin II heavy chain [Knecht and Loomis, 1987; DeLozanne and Spudich, 1987; Wessels et al., 1988], while actin polymerization correlates with pseudopod extension in Dictyostelium and neutrophils [Devreotes and Zigmond, 1988]. In light of redundant functions within cells, interpretation of these results are suggestive but not conclusive that actin and not myosin II is necessary for chemotaxis.

The phosphatidylinositol metabolism of slime molds is being determined by Van Haastert's group in as much detail as in neutrophils, and it is indeed an integral part of the sensory transduction in Dictyostelium. It is interesting that both the neutrophil and D. discoideum amoebae polymerize actin in preparation for chemotaxis, but that in D. discoideum the internal levels of calcium appear to be sufficient to account for the state of F-actin [Newell, 1986], whereas in neutrophils acidification may also be necessary [Yuli and Oplatka, 1987].

To account for the odorants that do not activate adenylate cyclase and other considerations of the adenylate cyclase as transducer [Anholt, 1987], the PIP₂ hydrolysis in olfactory epithelium has been scrutinized. GTP and odorants stimulate IP₃ production in ciliary preparations of catfish olfactory tissue [Huque and Bruch, 1986; Bruch and Huque, 1987] and do so more than additively when odorant is combined with GTP [Bruch et al., 1987]. L-alanine and L-arginine bind to distinctly separate receptor sites whose affinities are regulated by guanine nucleotides [Kalinoski and Bruch, 1987], and both amino acids elicit rapid (<15 sec) two-to threefold increases in IP₃ in a GTP-dependent manner [Bruch et al., 1989]. (The catfish olfactory PLC that is responsible for generating IP₃ is being characterized [Boyle et al., 1987].)

The L-amino acid-stimulated EOG of catfish olfactory receptor cells is abolished with the removal of external Ca²⁺, and Ca²⁺ channel blockers and patch-clamping of the receptor cell membranes reveal an IP₃-gated calcium channel [Restrepo et al., 1990]. Therefore in catfish two parallel receptor–G protein pathways exist, one coupled to PLC that increases IP₃ and thereby directly activates Ca channels and another coupled to adenylate cyclase that increases cAMP and thereby activates cation channels [Restrepo et al., 1990; Bruch and Teeter, 1989]. The relationship of the receptors, G proteins, and
channels within a single cell is of great interest because of the extra latitude multiple pathways provide for integration of information at the periphery and for cross-talk between pathways.

The IP₃ pathway in olfaction promises to be a common theme across phyla. In rat olfactory cilia and in moth antennae, odorants or pheromones can elicit an extremely rapid, GTP-dependent peak of IP₃ [Breer et al., 1990a,b], qualifying IP₃ as a second messenger. However, unlike in catfish, the set of odorants that stimulate IP₃ in rat olfactory cilia is not overlapping with the set that activate adenylate cyclase, and the rate of cAMP accumulation is clearly fast enough to qualify it as a second messenger [Breer et al., 1990a,b; Breer and Buckoff, 1991; see Restrepo et al., 1990, and Bruch and Teeter, 1989].

Similarly, in catfish taste tissues, the taster L-alanine rapidly stimulates IP₃ formation, as does Na-fluoride (NaF) [Huque et al., 1987; Huque and Brand, 1988], implicating a GTP-dependent system for taste. Bitter stimuli elicit increases in internal calcium from internal stores [Akabas et al., 1987, 1988], also indirectly pointing to IP₃ as an internal messenger [see Teeter and Gold, 1988, for a review]. The details of the involvement of PLC in taste and olfaction remain to be elaborated.

D. Calcium, pH, and Membrane Potential

Calcium, pH, and membrane potential are grouped together because often changes in one are inseparable from changes in the other two. Calcium has been described in some detail earlier (see sections II.B. and III.C.). Briefly, calcium figures into cytoskeletal changes in F-actin for neutrophil chemotaxis and into at least one pathway in oxidative burst. Lamellepodia are the sites of F-actin formation for pseudopod extension and also the site of at least some of the elevated Ca²⁺ during chemotaxis [Sawyer et al., 1985; Jacobi et al., 1988]. However, calcium-sensitive dyes over very short sampling times (0.5 sec) show no consistent localization of Ca²⁺ during chemotaxis [Marks and Maxfield, 1990a,b].

H⁺ content and membrane potential (Vm) have been shown to change rapidly with FMLP stimulation [see Sha'afi and Molski, 1988, for review]. There is a rapid (maximal in <10 sec) K⁺-dependent hyperpolarization that usually is masked by a subsequent depolarization when FMLP exceeds 10⁻³ M [Lazzari et al., 1990]; a rapid acidification follows the depolarization time course and is in turn followed by a more slowly developing H⁺ extrusion by an Na⁺/H⁺ antiporter that is activated probably by protein kinase C [see Sha'afi and Molski, 1988, and Fletcher and Seligmann, 1986, for discussion]. Yuli and Oplatka [1987] suggest that the transient rapid acidification is responsible for triggering the rearrangement of the cytoskeleton in preparation for actin polymerization and chemotaxis. Fletcher and Seligmann [1986] [although not others; see Lazzari et al., 1990] have found a correlation between the depolarization,
which varies greatly in individual cells from a neutrophil population, and the oxidative burst. The slower alkalization caused by the activity of the Na\(^+\)/H\(^+\), amiloride-sensitive antiporter can be inhibited with no significant effect on the stimulated cell responses [Sha'afi and Molski, 1988].

Calcium figures prominently in the *D. discoideum* sensory transduction pathway that results in chemotaxis (Fig. 5). The source of this calcium is internal, nonmitochondrial stores, and apparently calcium is mobilized from these stores by IP\(_3\) [Newell, 1986]. Calcium levels correlate with the polymerization of actin [Newell, 1986], and, as in neutrophils [Cassimeris et al., 1990], it is not quite clear how the polymerization is controlled and localized for pseudopod formation and motility. Calcium also acts to stimulate guanylate cyclase activity. The enzyme in vitro is not sensitive to calcium levels; therefore the details of how calcium regulates this enzyme are not yet clear [see Newell et al., 1988, for discussion]. Depolarizing changes can be made membrane potential with no consequences for chemotaxis or cGMP accumulation with stimulation by cAMP [Van Duijn et al., 1990], and therefore membrane potential seems to have no role in the chemotaxis sensory transduction pathways of these amoebae.

In spermatozoan activation by resact, there must be a calcium influx from external sources both to activate the adenylate cyclase and to elicit chemotaxis (Fig. 6). Calcium is necessary or a receptor– or G protein–adenylate cyclase interaction modulates the enzyme activity. Although the sources of calcium may differ, there are interesting parallels of the calcium regulation of guanylate cyclase activity in *D. discoideum* amoebae with the direct calcium activation of adenylate cyclase in spermatozoa and *Chlamydomonas*. Calcium influx is an absolute requirement for chemotaxis in spermatozoa. Although there also is a resact stimulation of H\(^+\) efflux, this alkalization is slow and cannot account for some of the initial response of sperm to ligand binding. However, there may be yet undescribed roles for the ligand-activated H\(^+\) efflux, because internal pH correlates with activation of motility and exposure of sea urchin sperm to pH 9 buffers causes reversible dephosphorylation of the guanylate cyclase–resact receptor similar to that caused by resact [Trimmer and Vacquier, 1986, for review; Garbers et al., 1986; Garbers, 1989a,b].

On a similar note, the cAMP that serves as a second messenger in *Chlamydomonas* agglutination responses is generated by a calcium–calmodulin (CM)-dependent adenylate cyclase [Pasquale and Goodenough, 1987]. CM inhibitors block postadhesion responses; cAMP relieves this block. The inhibitors also reduce adenylate cyclase activity in vitro. Calcium ionophores do not elicit the agglutination responses, implying that perhaps a minimum level of calcium is necessary for enzyme activation and that sensory transduction does not include an increase in internal calcium.

Taste cells function by releasing neurotransmitters that in turn stimulate the synapsing neurons. Calcium presumably is a requirement for neurotransmit-
ter release, and there are several potential mechanisms by which internal calcium levels are raised preparatory to release: Voltage-sensitive calcium channels are opened by cell depolarization through an increased influx of cation through passive or ligand-gated channels, increased membrane resistance or surface potential, or ligand–receptor interactions that result in second messengers that inhibit ion pumps. Alternatively, in the instances when cells do not change input resistance, second messengers may be liberating calcium from internal stores [see Teeter and Brand, 1987a, b for discussion]. Only in the case of sour taste is calcium thought both to carry the depolarizing current and to stimulate release of neurotransmitter [Sato et al., 1987]. Taste cell transduction varies greatly with cell type, and it is likely that all these mechanisms of increasing internal calcium are at work.

*Paramecium* respond to chemical stimuli with a change in membrane potential that is predictable from their change in swimming, hence ciliary beating patterns [Van Houten, 1979]. Ciliary beating frequency and angle (hence efficiency of swimming) are controlled by Vm [Machemer, 1976, 1989]. The electrophysiological bases of the change in Vm have been elusive; the hyperpolarization in response to folate or acetate is not dependent on either external K⁺ or Na⁺, has no reversal potential, correlates with a small increase (for folate acid) or decrease (for acetate) in membrane resistance, and is perhaps due to the activation of a calcium pump activity [Preston and Van Houten, 1987a]. Calcium efflux has been implicated in yet other ways: Lithium, which causes an inhibition of chemoresponse, reduces the normal calcium efflux and apparently the normal functioning of the surface Ca²⁺-ATPase pump [Wright and Van Houten, 1988, 1990; Van Houten et al., 1991b]; a mutant, K-shy [Evans et al., 1987], with defects in calcium homeostasis is not responsive to most stimuli [Van Houten, 1990]. Not all responses to attractant stimuli are affected by lithium, and the stimuli have been divided into groups based on this lithium effect [Van Houten et al., 1991b]. Interestingly, the stimuli that are thought to stimulate the Ca²⁺-ATPase to generate the hyperpolarization elicit a lithium-sensitive response and are not attractants to mutant K-shy, while NH₄Cl, for example, that is thought to affect internal pH and not affect Ca²⁺-ATPase through a receptor-mediated mechanism, does not elicit a lithium-sensitive response, and serves as a good stimulus for K-shy [Van Houten, 1990]. Therefore the working hypothesis is that the 0.2 namp current that is elicited by folate stimulation of cells, for example, could be accounted for by a voltage-insensitive calcium pump current (Van Houten and Preston, unpublished data).

The Ca²⁺-ATPase activities of the complex surface membranes (pellicle) of *Paramecium* have been partially characterized [Wright and Van Houten, 1990], and a corresponding protein has been identified both as a phosphoenzyme intermediate and as a calmodulin-binding protein [Wright and Van Houten, 1990, and unpublished data], but a definitive demonstration of its role in che-
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mokinesis has yet to be made. Calcium pump fluxes may also contribute to the calcium fluxes of FMLP-stimulated neutrophils and Dictyostelium [Foder et al., 1989; Böhme et al., 1987].

Both pH and Vm contribute to the proton motive force (PMF) that is the energy source for the gram-negative bacterial flagella [Boyd and Simon, 1982]. Levels of PMF also appear to be sensed by the flagellar switch that is responsible for the bacterial chemoreponse. In particular, oxygen concentrations affect Sal. typhimurium swimming; the "aerotaxis" pathway by-passes the MCPs and other membrane receptors but does converge with the other chemoreponse pathways at the flagellar switch for clockwise and counterclockwise rotation. Shioi and Taylor [1984] find that "aerotaxis" is mediated not by pH or Vm alone but by the PMF. Likewise in the photosynthetic bacteria Rhodob. sphaeroides, the response of the cells to light or O₂ depend on the PMF generated by electron transport, but not on the electron transport directly [Armitage et al., 1985].

E. Integrating Multiple Messengers

It should be evident from the descriptions of neutrophils, D. discoideum, olfaction, and taste systems that multiple internal messengers are set loose upon chemostimulation. Some are necessarily generated together, as in PLC action on PIP₂ to produce IP₃ and DAG. However, DAG can come from other sources and apparently does in neutrophils. Others are generated separately, like cAMP and calcium, but work either synergistically or in opposition to control physiological responses [Rasmussen, 1981; Alkon and Rasmussen, 1988]. While control by and presence of multiple messengers is not new [Belardetti and Siegelbaum, 1988; Imagawa et al., 1987; Rasmussen, 1981], it complicates the neat dissection of the sensory transduction pathways, particularly if the pathways are not parallel but interactive.

Multiple messengers can be generated by separate sets of receptors or the same set causing a cascade of sequential messenger production. The sensory transduction pathways in D. discoideum appear to have two sets of cAMP receptors coupled with different G proteins (frgA product vs. Gₛ) that stimulate PLC and adenylylate cyclase, respectively, along two separate sensory transduction pathways. Within the PLC pathway, IP₃, calcium, and cGMP all are generated and calcium, in particular, has further multiple effects. In neutrophils, it still is debatable whether multiple receptors or one population of receptors associated with different G proteins or other effectors allows the cells to respond differentially to a range of concentrations of stimuli or over different time courses and to have such a large repertoire of intracellular effects. Likewise, in olfaction and taste it is becoming clear that the cell population is heterogeneous and that several transduction pathways can exist within one cell with responsiveness to more than one stimulus, i.e., more than one receptor (see section IV.C. for a discussion of the interactions of cyclic nucleotides and Vm).
IV. TRANSDUCTION MECHANISMS

A. An Overview

Signal transduction begins with the stimulus–receptor interaction and ends in a response such as directed motility or synaptic transmission. The signal is first an external chemical cue and is transformed into an internal, diffusible messenger. The transfer of information from receptor to effector enzyme that produces the internal messenger very often is mediated by a G protein. G proteins are implicated in activation of adenylate cyclase, PLC, and phospholipase A₂ and in opening ion channels directly [Gilman, 1987; Axelrod et al., 1988; Dunlap et al., 1987; Miller, 1988]. However, G proteins are not the exclusive agents that transfer information to enzymes or ion channels. Calcium can do this directly [Hockberger and Swandulla, 1987], as can Vm (see section III.D.). Cyclic nucleotides interact with some ion channels directly [Hockberger and Swandulla, 1987] or indirectly influence the activity of enzymes and channels through protein kinases [Edelman et al., 1987; Hanks et al., 1988]. Likewise, there are calcium- and CM-dependent protein kinases. An interesting exception to this pattern of diffusible internal messengers is the bacterial chemoresponse system. Here information is passed from protein to protein as a phosphorylation without intervention of a diffusible messenger. The yeast mating system may involve G proteins without diffusible messengers and be yet another interesting exception in sensory transduction.

B. G Proteins

In receptor-mediated sensory transduction, often a G protein is interfaced between the receptor and effector enzyme that generates the internal messenger. The control adenylate cyclase by stimulatory (Gₘ) and inhibitory (Gᵢ) G proteins is now a classic example, as is transducin inhibition of the cGMP-phosphodiesterase in retinal cells [Stryer and Bourne, 1986] (the reader is referred to one of several reviews of G protein function for details [Stryer and Bourne, 1986; Gilman, 1987; Neer and Clapham, 1988]). A brief sketch of G protein function is as follows: G proteins are multimeric, consisting of α-, β-, and γ-subunits. GDP is bound to the α-subunit, and upon ligand binding to receptor there is a conformational change transduced to the α-subunit facilitating its binding of GTP. In this bound form, the α-subunit dissociates from the β- and γ-subunits and is active in stimulating or inhibiting its target enzyme or channel. The α-subunit also has GTPase activity, and, upon hydrolyzing GTP to GDP, the α-subunit becomes inactive and reassociates with the β- and γ-subunits and the receptor. The complexity of control of G proteins is becoming evident as accessory proteins that influence G protein function (e.g., by activating GTPase activity) are being characterized [Parsons, 1990]. Additionally, there are increasing numbers of reports of functions for the β- and
γ-subunits in transduction, but these are controversial at present [Neer and Clapham, 1988; Dunlap et al., 1987]. Cholera and pertussis toxins ADP-ribosylate and thereby perturb the functions of the α-subunit [Neer and Clapham, 1988].

The criteria for the involvement of G proteins in a sensory transduction process are [Gilman, 1987] 1) both ligand and GTP are required to initiate the response; 2) nonhydrolyzable analogs or NaF should provoke the response; 3) there should be a decreased affinity in ligand binding in the presence of GTP, and, conversely, ligand should enhance the binding of GTP to membranes; 4) cholera or pertussis toxin or antibodies against G proteins could perturb the response; and 5) reconstitution of the pathway in vitro and dependence on GTP and a G protein for the response are the ultimate criteria.

Both calcium and protein kinase C are heavily implicated in neutrophil chemotaxis and oxidative burst (see above). Both calcium and protein kinase C are modulated by PIP₂ metabolism, calcium by IP₃, IP₄, and AA, and protein kinase C by DAG and calcium. Therefore the major second messengers are IP₃ and DAG and third messengers are calcium, AA, and possibly IP₄. The generation of these messengers is dependent on G proteins at several levels.

Neutrophil receptors are coupled to PLC through G proteins. The evidence for this includes the requirement for GTP and FMLP to stimulate PIP₂ hydrolysis in membrane preparations; the same hydrolysis is inhibited by pertussis toxin; guanine nucleotides regulate receptor affinity; FMLP stimulates GTP binding and GTPase activity in membranes [Dillon et al., 1987a,b; Cockroft, 1987; Sklar et al., 1987]. The α-subunit involved is not the same as that in Gₛ or Gₓ, but instead is a smaller Gₛ α-subunit that is substrate for both pertussis and cholera toxins [Cockroft, 1987; Polakis et al., 1988; Sha’afi and Molski, 1988]. A pertussis toxin substrate that can be copurified with the receptor decreases affinity of receptor for FMLP upon GTP binding [Polakis et al., 1988]. This 40 kD protein is the likely candidate for Gₛ α-subunit.

Neutrophil phospholipase A₂ apparently is regulated by interactions with a G protein [Burgoyne, 1987]. Additionally, the sustained high calcium levels from calcium influx may be mediated by a G protein interaction separate from the one regulating PLC [Nasmith and Grinstein, 1987a,b; Lu and Grinstein, 1990], as may some of the other aspects of neutrophil activation that Sha’afi and Molski [1988] have cataloged as pertussis toxin sensitive: chemotaxis; degranulation; oxidative burst; aggregation; rise in intracellular Ca²⁺; actin polymerization; PI, PIP, and PIP₂ hydrolysis; Na⁺ influx; increase in intracellular H⁺; increase in internal pH; protein phosphorylation; membrane potential change; GTPase activity; AA release; and generation of phosphatidic acid, phosphatidylinositol, IP₂, and IP₃.

There are several lines of evidence that G proteins are integral parts of the D. discoideum sensory transduction. At the receptor level, GTP affects the
binding of cAMP to the cell surface receptors of both the relay and chemotaxis pathways [Janssens and Van Haastert, 1987], and cAMP and folic acid increase binding of GTP to membranes [Janssens and Van Haastert, 1987; Snaar-Jagalska et al., 1988]. In the relay pathway, GTP modulates activity of the adenylate cyclase [Thiebert et al., 1984; Van Haastert et al., 1987a,b]. In the chemotaxis pathways, GTP and analogs stimulate IP$_3$ formation, as does the stimulus cAMP [European-Finner and Newell, 1987a,b]. Synchronized cells exhibit oscillations of IP$_3$ and cGMP with cAMP stimulation and likewise with GTPyS treatment.

An interesting study of the *D. discoideum* ras protein (an oncogene product member of the family of smaller GTP-binding protein) implicates separate G proteins in the chemotaxis and relay sensory transductions [European-Finner et al., 1988; Van Haastert et al., 1987a,b]. *Dictyostelium* cells transformed with the homolog of the normal, human protooncogene (gly-12) aggregated normally, while those transformed with the homolog of the human activated oncogene (thr-12) showed abnormal chemotaxis, probably because of IP$_3$ levels elevated two to three times over basal levels. The oscillation in IP$_3$ normally induced by cAMP was aberrant and started from a higher basal level in transformants. In the same cells, the adenylate cyclase relay was not affected.

A modification of the G protein–receptor interaction appears to be responsible for adaptation, i.e., the unresponsiveness of cells to a second pulse of cAMP given within 30 sec of the first. There is no adaptation apparent if GTPyS, IP$_3$, or calcium is used to bypass the receptor and start the transduction pathway downstream from the ligand–receptor interaction [Small et al., 1987]. However, GTPyS cannot circumvent adaptation produced by cAMP binding to receptor. Therefore, adaptation must somehow involve the G protein–receptor interaction.

An interesting question for both neutrophils and *D. discoideum* regards the number of G proteins associated with receptors. There are separate G proteins coupled to the cAMP and folate receptors [Keskebe et al., 1990]. Are there two or more classes of G proteins to account for the divergent pathways? If, as Sklar argues for neutrophils, all receptors are functionally coupled, how can the number of receptors occupied translate into chemotaxis vs. oxidative burst and degranulation responses? Alternatively, are receptor populations different? If so, are the G proteins associated with them different? The availability of mutants has contributed significantly to the dissection of G protein function in *Dictyostelium*. Synag mutants are defective in interactions between G protein and the relay adenylate cyclase, but the defects probably do not lie in the G protein itself [Snaar-Jagalska and Van Haastert, 1988], while $fgdA$, mutants in a different gene, are no longer able to couple receptor to G protein properly yet GTP can activate their adenylate cyclase [Keskebe et al., 1988]. Therefore, the two transduction pathways (Fig. 5) appear to be mediated by separate G proteins, and the receptor-coupled G protein α-subunit has been identified among several cDNA clones.
for G proteins as G₂ₐ [Johnson et al., 1989]. Small ras-like G proteins are present in Dictostelium, and their overexpression or alteration affects chemosensory transduction possibly through the activation of protein kinase C [Robbins et al., 1989; Luderus et al., 1988]. However, it is not yet clear how these ras G proteins fit into the normal transductions pathways, if at all.

In both olfaction and taste there are two effector enzymes (adenylate cyclase and PLC) that are coupled to G proteins. Either or both are activated in a stimulated cell; both enzymes may function in sensory transduction and perhaps adenylate cyclase adaptation [Anholt, 1987]. It is clear, however, that G proteins play major roles in sensory transduction in olfaction and taste tissues. The lines of evidence for G proteins in olfaction are 1) GTP dependence of adenylate cyclase and PLC, 2) presence of G proteins in appropriate tissues, and 3) influence of GTP on stimulus binding. Only GTP dependence of adenylate cyclase and PLC has been established for taste [see Bruch, 1990a,b, for reviews].

1. In olfactory epithelium, from all sources, the adenylate cyclase is GTP dependent, and in frogs and mammals in particular the activation of adenylate cyclase by odorants requires GTP [Sklar et al., 1986; Lancet and Pace, 1987; Pace and Lancet, 1986]. The adenylate cyclase of catfish olfactory cilia also is activated by GTP, but Bruch and Teeter [1988] have found that odorant stimulation is not likely to work through the classic G₁ mechanism, despite the presence of G₁ in both catfish and other olfactory cilia [Bruch et al., 1987]. In pseudoparathyroid patients, the G₁ is defective, making modulation of adenylate cyclase by some hormones impossible [Carter et al., 1987; Weinstock et al., 1986]. Because such patients have deficits in olfactory function, there may be a role for G₁ in mammalian and perhaps frog adenylate cyclase, while a different mechanism is at work in the catfish.

In taste tissues from catfish, the adenylate cyclase is GTP dependent and rapidly activated by taster [Kalinowski et al., 1989a; Bruch et al., 1987]. Likewise, in rat the adenylate cyclase is activated by sugar in a GTP-dependent manner [Lancet et al., 1987].

The alternative transduction pathway in taste and smell is similarly dependent on GTP. The PLC of both olfactory and taste tissues of the catfish are both stimulus and GTP dependent [Bruch et al., 1987, 1989; Huque and Bruch, 1986, Huque et al., 1987].

2. Five cDNA clones for different GTP-binding proteins and the protein counterparts of G₁, G₅, and G₆ have been identified with molecular genetic and immunological probes in rat and frog olfactory epithelium [Jones and Reed, 1987; Anholt et al., 1987]. A sixth cDNA clone was found to code for a G protein specific to the olfactory epithelium (G₅₆) [Jones and Reed, 1989]. In a comparison of three species, Pace and Lancet [1986] isolated a 42 kD G protein and demonstrated that this G protein activates the adenylate cyclase and can be ADP-ribosylated by cholera toxin. Similarly, in catfish olfactory cilia, a G₅ α-subunit cholera toxin substrate (45 kD) and a pertussis toxin substrate of 40
kD have been identified [Bruch et al., 1987]. However, the only G protein that has been identified in taste tissues are the catfish α- and β-subunits of G, and the 41 kD form of G3 [Bruch and Kalinoski, 1990; Bruch, 1990a].

3. Bruch and Kalinoski [1987] measured binding of L-arginine and L-alanine that serve as both odorants and tastants to catfish and found that GTP decreases affinity of binding to the separate receptors by 1 order of magnitude in olfactory but not taste membranes. L-arginine opens channels from taste membranes directly [Teeter and Brand, 1987a; Teeter et al., 1987b], thereby obviating the need for a G-coupled receptor. However, the mechanism of the L-alanine stimulation of adenylate cyclase and PLC in a GTP-dependent manner in the apparent absence of a G-coupled taste receptor remains unclear.

The two lines of evidence for G protein interaction in the yeast mating type system are indirect yet compelling. First, the receptors resemble rhodopsin in their structure and therefore have been assigned to the family of receptors that are G protein coupled (Fig. 10). Second, two investigative groups have cloned the yeast gene for a protein homologous to the G, α-subunit. Disruption of this gene leads to growth arrest in Gt, which is characteristic of haploid cells.

![Fig. 10. Schematic of the response pathway in a cells to α-factor. The genes shown are involved in the response to pheromone that leads to transcriptional induction. The pathway apart from pheromone and receptor appears to be the same in α-cells. (Reproduced from Fields, 1990, with permission of the publisher.)](image-url)
exposed to mating pheromone [Dietzel and Kurjan, 1987; Miyajima et al., 1987]. Diploid cells are unaffected by mating pheromones and are also not growth arrested by the disruption of the G protein gene. No second messenger such as calcium or cAMP seems to evolve from interaction of G protein with effector enzyme [Herskowitz and Marsh, 1987]. Instead, it has been proposed that, in the absence of mating factor, the G protein is in the trimeric GDP-bound form and upon mating factor stimulation it is bound with GTP [Dietzel and Kurjan, 1987; Miyajima et al., 1987]. Dietzel and Kurjan [1987] proposed that the GDP-bound form of the G protein acts as an inhibitor of some effector, allowing the cell to traverse the cell cycle. When mating factor is bound to receptor, and the GTP-bound form G protein predominates, the affinity for effector is reduced and the effector is free to produce the cell cycle arrest; and the effector could be the β- and γ-subunits. Additional genetic and biochemical information is necessary to sort through the multiple models that are proposed for G protein function in yeast mating response [see Kurjan, 1990, for review].

C. Cyclic Nucleotides and Membrane Potential

The response of paramecia to organic, chemical stimuli includes an immediate hyperpolarization and a change to smoother, faster swimming [Van Houten, 1978, 1979]. Presumably the cilia are beating faster and at an angle that facilitates movement. Both membrane potential and cyclic nucleotides are implicated in the control of ciliary beating [Bonini et al., 1986; Gustin and Nelson, 1987; Bonini and Nelson, 1988; Nakoaka and Ooi, 1985; Majima et al., 1986; Klumpp et al., 1984; Schultz et al., 1984]. The induction of faster ciliary beating by hyperpolarization (without the complication of altered surface charge) or by cAMP is not in dispute; it is the causal relationship between the two that is complex and not yet clear.

cAMP injected into paramecia hyperpolarizes them, and their cilia increase beating frequency [Hennessey et al., 1985]. Voltage-clamping of the membrane prevents this increased frequency, and yet experiments with permeabilized cells (hence with no means of altering their membrane potential) show that cyclic nucleotides can increase the frequency of ciliary beating directly [Bonini et al., 1986; Bonini and Nelson, 1988; Majima et al., 1986]. It is significant that not all the modulation of speed can be attributed to internal cAMP levels; in an intact cell where membrane potential was regulated by external K+ levels, increases in internal cAMP (attained by incubation in permeant analogs of cAMP) did increase swimming speed, but the absolute speed was a function of both the Vm and cAMP levels [Bonini et al., 1986]. Therefore, it will be important to sort out the contributions of nucleotide-dependent phosphorylation of axonemal proteins and the effects of both hyper- and depolarizing membrane potential changes on the function of the cillum, both as the motor end of


the *Paramecium* chemosensory response pathway and for its relevance to yet other systems including sperm [Tash et al., 1986] and ciliated epithelium [Nelson and Wright, 1974; Satir, 1985; Brokaw, 1987], and perhaps ciliated olfactory epithelium.

D. Cyclic Nucleotide- and Ligand-Gating of Ion Channels

There are examples in the literature of ligands that activate one of the family of G protein–coupled receptors that activate ion channels indirectly [Dohlman et al., 1987] and of other ligands that activate channels directly [Stevens, 1987; Barnard et al., 1987]. Likewise, the second messengers that are generated in the G protein–associated systems can activate channels directly [Hockberger and Swandulla, 1987; Kuo and Gardner, 1987] or indirectly through protein kinase activities [Edelman et al., 1987; Hanks et al., 1988; Kikkawa and Nishizuka, 1986]. In olfactory systems and taste, there are observations of stimulus activation of G protein–dependent adenylate cyclase and PLC, cyclic nucleotide– and IP₃–activated conductances, direct odorant activation of conductances, and even direct odorant activation of adenylate cyclase, presumably through perturbation of lipid environment around the enzyme, in melanocytes that ought not to have odorant receptors [Lerner et al., 1988]. Therefore it remains to be seen which of these mechanisms is at work in olfaction and taste or, more aptly perhaps, how they are distributed across receptor cell types and whether they are associated with specific stimuli.

E. Bacterial CheY Protein

The bacterial chemoresponse systems seem to function without the internal messengers and components of the eukaryotes: cyclic nucleotides, calcium, Vm, IP₃, DAG, or G proteins. Indeed, no prokaryotic G proteins that control enzyme activity have been identified [Janssens, 1987]. Instead, the bacterial pathways seem to pass on the signal from protein to protein until it reaches the CheY protein that can interact directly with the switch to promote clockwise rotation (Fig. 3) [Ravid et al., 1986; Eisenbach and Matsumura, 1988]. The information passes from receptor–MCP to CheA as a phosphorylation, possibly mediated by CheW (Fig. 3) [Parkinson, 1988; Hess et al., 1988; Oosawa et al., 1988; Gegner and Dahlquist, 1991]. CheA in turn can be found in a complex with CheZ and CheY, and the CheA phosphate is rapidly passed on to CheY (and also to CheB). Upon phosphorylation of CheY, the phosphate has reached the protein that interacts directly with the flagellar switch. CheY can directly promote clockwise rotation in bacterial envelopes with flagella attached. CheZ acts as an antagonist to CheY and promotes counterclockwise rotation, but the flagella of envelopes will rotate counterclockwise without the presence of CheZ, implying that CheZ interacts with CheY to achieve the antagonism and does not interact directly with the flagellar switch. Presumably the phosphorylated form of
CheY is active in the switching function; the phosphate of CheY is rapidly transferred to CheZ, which accounts for the antagonism by CheZ. An important aspect yet being clarified is the nature of the CheA and MCP interaction that starts this phosphorylation cascade.

A common signalling motif is emerging for prokaryotes [Parkinson, 1988; Stock et al., 1989; Stock, 1987; Ronson et al., 1987]. Bacterial proteins with known "transmitter" modes and those with known "receiver" modes in signal transduction are being compared among themselves. It appears that CheA is one of the transmitter family, with CheY receiving its signal in the form of a phosphorylation. The transmitters have kinase domains, and the receivers have phosphate acceptor sites. Phosphorylation has been developed to a high degree in prokaryotes to serve in sensory transduction processes.

V. FUTURE DIRECTIONS

One challenge for future research in chemosensory transduction is the isolation and characterization of receptors. To date only four receptors from unicellular organisms have been purified and cloned. Peripheral receptors have remained technically difficult to isolate, but their isolation and characterization will be important in understanding their structure and function. Molecular genetics, which has been used successfully to clone neurohormone receptors [Koblika et al., 1988; Kerlavage et al., 1987; Boulter et al., 1986; Barnard et al., 1987], will be employed in this task, as will more immunological techniques [Fraser and Lindstrom, 1984; Bryant et al., 1987; Kalinoski et al., 1987; Price and Willey, 1988]. Because many receptors appear to fall into classes based on amino acid sequence (e.g., G-coupled vs. direct ion channel-gating), the gene sequence of a chemosensory receptor could give clues about its regions of potential G protein interactions or functions as ion channels [Koblika et al., 1988; Barnard et al., 1987] and uncover some consensus features unique to peripheral chemoreceptors.

A necessary process that is currently undereway is the cataloging of stimulus-related G proteins, protein kinases [Meier and Klein, 1988], and protein kinase substrates that first must be identified before their roles in sensory transduction can be elucidated. Likewise, second messenger- and ligand-activated conductances of receptor neurons are being sorted out prior to understanding where they fit into sensory transduction.

Another challenge for the future will be to untangle the relationships of the messengers in these overlapping and interacting pathways using pharmacological agents and, when possible, mutants. A catchword for future transduction studies is "cross-talk," and it is just this cross-talk between pathways and interactions between the internal messengers that allows chemosensory cells such a rich repertoire of responses and makes chemosensory transduction so complex in its control.
VI. NOTE ADDED IN PROOF

This chapter is like a snapshot of the field of chemical sensing, but the subject is moving rapidly and the picture is necessarily blurry. Since the writing of this chapter, more components of vertebrate olfactory transduction pathways can be studied at the gene level with the cloning of an olfactory specific adenylate cyclase [Bakalyar and Reed, 1990] and nucleotide gated channels [Ludwig et al., 1990; Dhallan et al., 1990]. These genes, along with those for a G protein (G_{olf}) [Jones and Reed, 1989] and degradative enzymes [Lazard et al., 1990, 1991; Ding et al., 1991] specific to olfactory epithelium almost complete the picture of major sensory transduction components. The receptor proteins have remained elusive, but now some of a family of genes may have been cloned [Buck and Axel, 1991; Nef and Dionne, personal communication]. These genes appear to comprise a large branch of the superfamily of rhodopsin-like proteins and await functional assays to verify their identity. The oocyte systems that have been patiently developed [Getchell et al., 1990] can serve as an important technique for screening genes and providing functional assays of odorant responsiveness of gene products. While the olfactory binding protein or its counterpart are being identified and cloned in more species [Krieger et al., 1991; Vandenberg and Zeilgelerberger, 1991; Vogt et al., 1990a], its functions still are not agreed upon [see Vogt et al., 1990b, for discussion].

Olfactory cells in culture from vertebrates, insects, and lobsters are a welcome development that will greatly facilitate the biochemical approaches to second messengers [Ronnert et al., 1990; Zufall et al., 1991; Fadool et al., 1991]. For example, while it was established that the lobster olfactory receptor cells do not utilize the cAMP transduction system for excitatory amino acids [McClimont et al., 1989], patch clamping of the cultured cells made it possible to identify IP_{3} as the second messenger [Fadool et al., 1991]. Similarly, the second messengers for the inhibitory stimuli that activate K conductances [Michel et al., 1991] can now be deciphered.

On other fronts, the purification and cloning of the olfactory IP_{3} gated calcium channel is in progress [Kalinoski, Restrepo, and Teeter, personal communication] and the electrophysiology of the olfactory receptor cells continues to develop [Firestein et al., 1990; Kleene and Gesteland, 1990; Lowe and Gold, 1990; O'Connell et al., 1990; Kaisling et al., 1991]. Additionally, the temporal nature of the stimulus at the receptor cells is being described in unprecedented detail [Moore et al., 1989].

Perhaps the greatest advances in olfaction have come from improved technology. Whole cell patch clamping, rapid kinetic systems [Breer and Boekhoff, 1991] for the analysis of second messengers, and molecular genetics (polymerase chain reaction, in particular) have greatly clarified the roles of second messengers and advanced the identification of the elusive receptors. As techniques continue to evolve, the molecular genetic approach that began with olfactory marker protein [Danciger et al., 1989] will accelerate. The ability to
measure second messengers on millisecond time scales has helped to sort out fast and slow responses to stimuli and, hence, potential for participation in transduction or slower processes like adaptation. From the second messenger biochemistry, it appears that "diversity" will be the operative word as it is in taste systems and that there will be multiple, cross-talking transduction pathways to unravel.

Taste cells, likewise, are yielding new information, particularly through voltage and patch clamping [Sugimoto and Teeter, 1990; Bigiani and Roper, 1991; among others] and cytochemistry [Dockstader et al., 1991; Oakley et al., 1990; Finger and Bottjer, 1990; among others]. Even questions of integration at the periphery can now be approached [Ewald and Roper, 1990]. Kinnamon [1988] brought the diversity of taste transduction mechanisms to our attention and the diverse second messenger systems involved in sweet and bitter taste can now be pursued through a combination of electrophysiology [Cummings et al., 1991; Avenet et al., 1991; Behe et al., 1990; Hernes, 1990] and fast kinetic biochemistry. The catfish taste system is providing a set of contrasting mechanisms of taste transduction including ligand gated channels with valuable behavioral correlates [Kohbara et al., 1990; Kumazawa et al., 1990].

On the unicellular side, the yeast story continues to evolve [Jackson and Hartwell, 1990; Stone and Reed, 1990; Cartwright and Tipper, 1991], and the members of the receptor guanylate cyclase and Dictyostelium cAMP receptor family continue to grow in number [Schulz et al., 1990; Saxe et al., 1991]. Along with the receptors, the other components of Dictyostelium pathway are being teased apart [Tao and Klein, 1990a,b]. The cloning of leukocyte receptors has provided a major advance that will provide sequences, derived protein structures, and an opportunity for producing and systematically altering the proteins [Thomas et al., 1990; Murphy et al., 1990; Coats and Navarro, 1990; Gerard and Gerard, 1991]. The ciliates, similarly, are amenable to the cloning of component parts of their transduction pathways, and such cloning offers new hope to sort out the mechanisms of mating type recognition among other processes [Meyer et al., 1991; Anderson et al., 1990; Ortenzi et al., 1990]. Progress continues in the bacterial systems, particularly in the non-methylating chemosensory pathways [Armitage et al., 1990] and B. subtilis [Zuberi et al., 1991]. For the bacteria, as for several of the other unicellular eukaryotic systems, the reader is directed to the volume by Armitage and Lackie [1990] for overviews.

The field of chemical sensing as a whole is experiencing a time of rapid advances and longstanding questions about peripheral and central systems should be resolved. The scope of this chapter is limited to transduction pathways of peripheral receptor cells and unicellular organisms and, even with this limitation, the chapter cannot touch upon structural, developmental, and coding aspects of olfactory and gustatory receptor cells, for example [see Farbman, 1990; Hill and Mistretta, 1990; Scott and Giza, 1990]. Therefore,
ologies for the necessary oversights) for more of an update on olfaction and *Dictyostelium* and to discover some of the excitement surrounding the recent developments in chemical sensing, the reader should consult Lewis [1991], Taylor [1991], and Lancet [1991].

**VII. REFERENCES**


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