Sensory Receptors and Signal Transduction: 65–136
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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

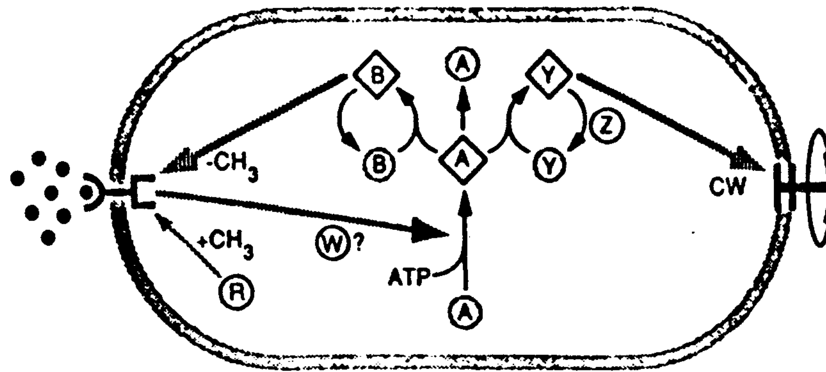

Fig. 1. Schematic of information transfer in bacterial chemoresponse. Ligand (•) binds to receptor, which signals the presence of ligand by way of *cheW* to *cheA* by enhancing the autophosphorylation of A (nonphosphorylated states are ○ and phosphorylated states are ◇). The phosphates on A proteins 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-

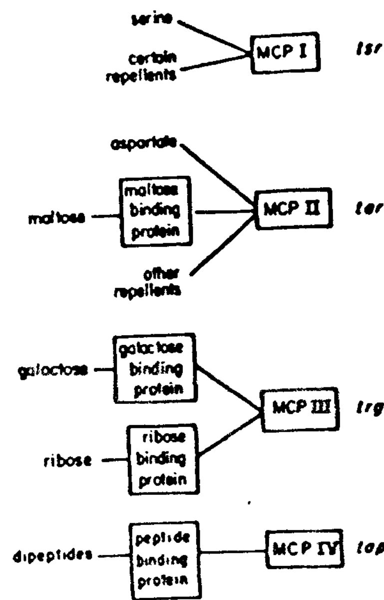

Fig. 2. The receptors and membrane signal transducing proteins of *E. coli*. The gene names for the methyl-accepting chemotaxis proteins (MCPs) are given in italics. (The sensory transduction pathway for phosphotransferase sugars joins with these transduction pathways downstream of the MCPs). [Reproduced 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

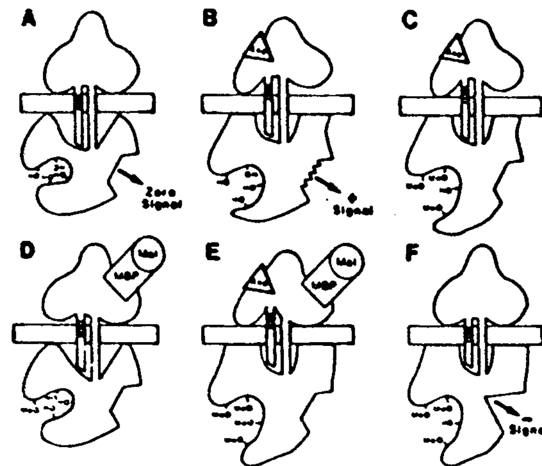

Fig. 3. Schematic of signaling and adaptation with maltose and aspartate: a vertical slide model. A: The unstimulated receptor is shown with an external domain that is responsible for ligand binding, two transmembrane segments, and a cytoplasmic domain that can send a signal and be methylated. The glutamic 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 [Thoekle 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 [Thoelke 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; Sockett 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 pH_i, 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_r, respectively

[Niedel and Cuatrecasas, 1980a,b; Rollins and Springer, 1985; see Allen et al., 1988, and Painter et al., 1984, for reviews]. There are ~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_r (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 radioiodinated FMLP characteristically results in a broad band of labeled protein from 50,000 to 60,000 M_r 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,"

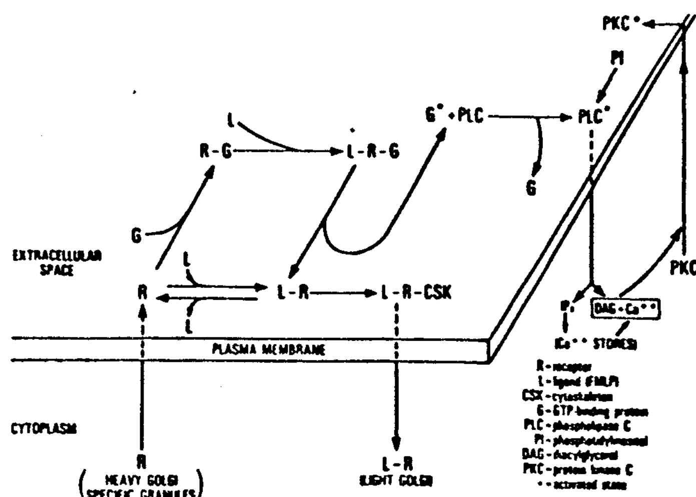

Fig. 4. A schematic of chemoattractant (e.g., FMLP) stimulation of neutrophils. Note the multiple states of receptors, including association with the cytoskeleton, G protein interactions with PLC and generation of multiple second messengers, many of which are not shown here as less than essential for activation. (From Allen et al., 1988, with permission.)

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 [Sarndahl 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 [Jesaitiset 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 M_r 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 cylase [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 sequelae: 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 K_d of 1 nM curiously correlates with the ED_{50} of chemotaxis, while the K_d of cytoskeleton-free receptors correlates with the ED_{50} 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 chemoresponse 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 [Zigmond and Sullivan, 1979; Sullivan and Zigmond, 1982]. (As Zigmond [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 Janssens 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

Receptor form	Apparent K_d (nM)	Dissociation rate constant (k^{-1}, s^{-7}; 20°C)	No. of sites per cell	Effect of guanine nucleotides on abundancy
A^H	60	4×10^{-1}	77,000	Decrease
A^L	450	10×10^{-1}		Increase
B^S	6–13	4.3×10^{-2}	2,300	Decrease
B^{SS}	6–13	4.7×10^{-3}	1,100	Decrease

Reproduced from Janssens and Van Haastert [1987], with permission of the publisher.

I). As with the neutrophil chemoattractant receptor [Sklar 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 ^{32}P-N_3-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,

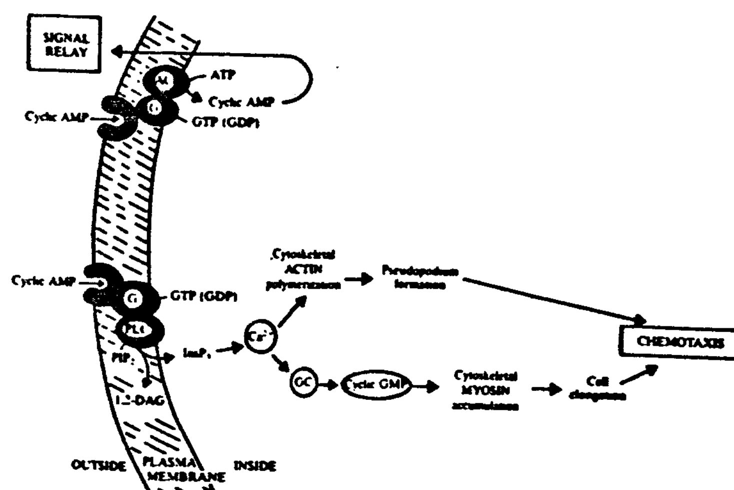

Fig. 5. Signal transduction pathways in *Dictyostelium discoideum* leading from cell surface cAMP receptors to the regeneration of the cAMP signal (signal relay) and to events of chemotaxis. More recent findings could add action of *synag 7* gene product at the G protein (G) and adenylate cyclase (AC) interaction and the *frgA* gene product as the G protein for chemotaxis [Snaar-Jaglaska and Van Haastert, 1988; Kesbeke 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 "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 Sherring, 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 ($>10^3$ kD, 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 M_r (48,000) can be labeled by ^{32}P-N_3-cAMP photolysis of whole cells. Covalent linking of N_3-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 blepharmone (gamone I, a glycoprotein of 20,000 M_r), and type II cells put out blepharisomone (gamone II, a tryptophan derivative) [see Van Houten and Preston, 1987, and Nobili, 1987, for reviews]. Blepharisomone is a chemoattractant to cells of mating type I and is a common gamone to all *Blepharisma* species. In contrast, gamone 1 is species specific. Binding studies using ^{125}I-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, *Euplotes*, 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 *Euplotes 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 ~28 kD in molecular mass, which is compatible with this model [Beale, 1990].

A different model for *Euplotes 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 chemoresponse].

4. Yeast. There are two mating types, *a* and α, 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 α are small peptides of 12 and 13 amino acids each [Thorner, 1981]. The α-factor binds to approximately 8,000 sites on an *MATa* cell with a K_d of 6×10^{-9} [Jenness et al., 1986, 1987]. A haploid cell will express the *a* or α-receptor gene, but not both, and likewise secretes only the pheromone to which it will not respond. The mechanism by which the *a*-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 α-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/β-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].

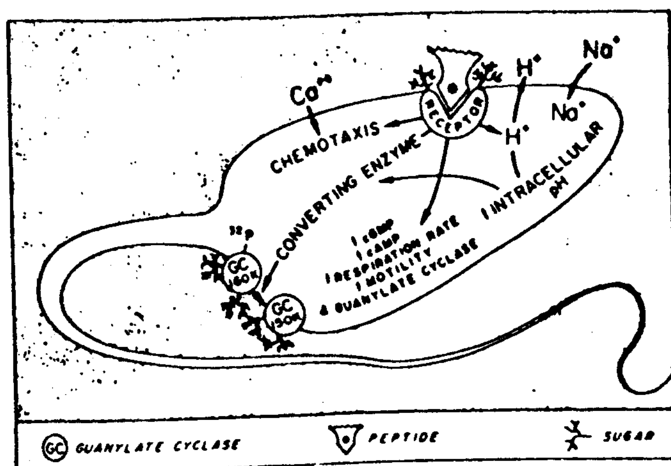

Fig. 6. Summary of events that occur upon an egg peptide interacting with its receptor on the sea urchin sperm cell. [Reproduced from Garbers et al., 1986, with permission of the publisher.]

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 spermatozoan resact receptor 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 phyla [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; Kaissling, 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; Kaissling, 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.

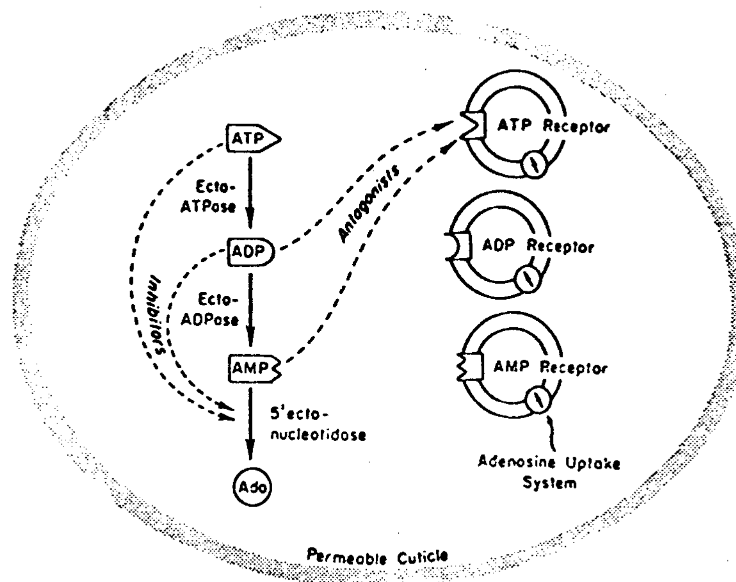

Fig. 7. Multiple receptors and signal degrading enzymes of the lobster olfactory sensilla. The cross section through the sensillum shows lymph surrounding dendrites, on which are separate receptors for adenine nucleotides and a ubiquitous adenosine uptake system. ATP, ADP, and AMP are stimulatory while 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 [Kaissling, 1987; Ache, 1987]. It is estimated that one molecule of pheromone can elicit an action potential [Kaissling, 1987]. The action potentials originate in either dendrite or soma [Ache, 1987; Kaissling, 1987], and their onic 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-^{3}H-hexadecaniehyl diazoacetate 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 $\simeq$50,000 daltons.

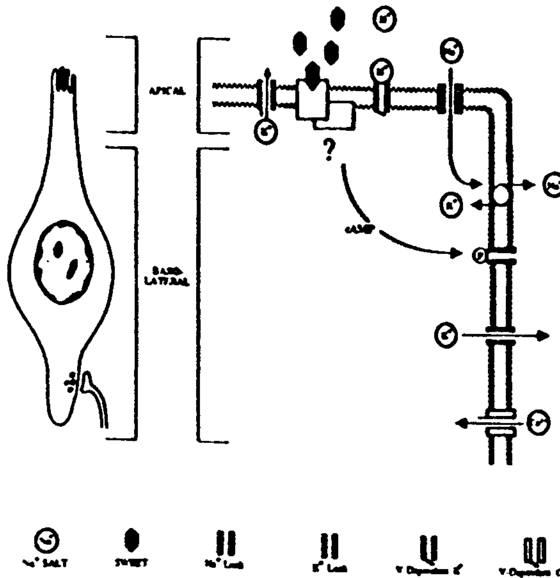

Fig. 8. Diagrammatic representation of taste transduction mechanisms. Sour transduction involves acid block of voltage-dependent K^+ channels, which are restricted to the apical membrane. Na^+ transduction involves the passage of Na^+ into taste cells through passive, amiloride-blockable Na^+ channels on the apical membrane of taste cells; Na^+ is then pumped out by an Na^+,K^+-ATPase on the basolateral membrane. Sweet transduction involves receptor-mediated stimulation of adenylate cyclase; voltage-independent K^+ channels on the basolateral membrane are then closed in response to cAMP-dependent phosphorylation. However, the link between increased adenylate cyclase activity in response to sweet stimuli and closure of K^+ channels by cAMP-dependent phosphorylation has not yet been shown in the same taste receptor cells. Transduction by all these pathways involves one final common pathway: depolarization and influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. Transduction mechanisms for other taste modalities have not been illustrated and include ligand or cyclic nucleotide activated cation channels. It is not yet clear if all these mechanisms are present on a single taste cell, as illustrated here, or if different taste cells are specialized to detect particular taste modalities. [Reproduced from Kinnamon, 1988, with permission of the publisher.]

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

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 10^4 [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,

1987, 1988]. 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_r (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 kD (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 ^{3}H-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 chemoreception. However, there are essential events that occur prior to and after ' inding, 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. Stengl 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

trough 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 OBP belongs, suggesting that this salivary protein might function in the concentration or delivery of sapid 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 [Kaissling, 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}–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 dibutyrl-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-Finner 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 orientated 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 $G_{2\alpha}$ to phospholipase C (PLC) can be examined separately from the "A"-mediated relay pathway that is coupled through the G_s protein to the adenylate cyclase, and it appears that $G_{2\alpha}$ 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 phosphoinositol 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

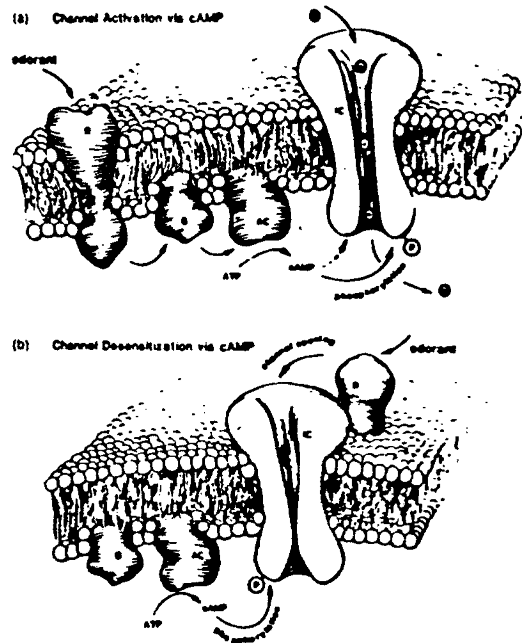

Fig. 9. Schematic of two alternative working hypotheses for signal transduction at the olfactory membrane. **a:** Linkage of an odorant receptor (R) via a G protein (G) to adenylate cyclase (AC) results in the generation of cAMP, which, either directly or via phosphorylation, activated an ion channel (IC). **b:** Linkage of an odorant recognition site to a channel causes opening of the channel directly. In this model, cAMP-dependent phosphorylation would lead to inactivation (desensitization) of the channel. Activation by the odorant of the olfactory adenylate cyclase may either be mediated via a distinct receptor protein or via direct activation (i.e., partial dissociation) of the G protein as a result of partitioning of the odorant in the membrane. There now is evidence that in some systems cyclic nucleotides can activate conductances as part of the sensory transduction pathway. [Reproduced from Anholt, 1987, with permission of the publisher.]

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 adenylate cyclase and that, at high concentrations of odorants, the olfactory specific G protein (G_{olf}) is activated, resulting in further stimulation of adenylate 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 adenylate cyclase may be sufficiently fast to allow cAMP levels to participate in transduction [Kalinoski et al., 1988,1989], but not all tastants in catfish activate adenylate 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 adenylate cyclase, but probably through inhibition of the phosphodiesterase [Jackowski and Sha'afi, 1979; Sha'afi 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'afi and Molski, 1988, for an overview].

Interestingly, dibutyrl-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. Phosphoinositol Lipid Hydrolysis and Sequelae

There is a renewed appreciation of the role of lipids and lipid metabolites in receptor functioning. In particular, arachiodonic acic (AA) and the inositol phospholipid hydrolysis products 1,4,5-IP_3 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-IP_3 and DAG are generated by the action of phospholipase C (PLC) on PIP_2, although DAG can be derived from other sources as well. AA is generated by the action of DAG lipase on DAG or phospholipase A_2 on membrane lipids. Therefore, AA synthesis can be linked to or separate from the PIP_2 metabolism. The functions of IP_3 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 IP_3, 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 O_2^- and H_2O_2.

Receptor–FMLP binding through the intervention of a G protein stimulates PLC activity and thereby increases the cellular content of 1,4,5-IP_3 and DAG [Dillon et al., 1987a]. 1,4,5-IP_3 affects internal calcium stores and also serves as a precursor to 1,3,4,5-IP (IP_4), a potential stimulus for opening calcium channels [Irvine and Moor, 1986; Houslay, 1987]. (External Ca^{2+} 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 IP_3 production is a matter of debate [Putney, 1987; Petersen, 1989; Schulz et al., 1989]. Additionally, the source of this secondary Ca^{2+} for sustained response need not be external [Krause et al., 1989].

The metabolism of 1,4,5-IP_3 follows two separate routes: 1) At ambient intracellular Ca, it is dephosphorylated to 1,4-IP_2, 4-IP, and inositol; and 2) at increased calcium levels, it is phosphorylated to 1,3,4,5-IP_4, 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 A_2 can produce AA from sources other than DAG, and activation of phospholipase A_2 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 lamellepodia 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*, PIP_3 and not PIP_2 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 [Wright 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-

torily mimic the pertussis toxin–sensitive, presumably gelsolin-mediated [Yin, 1987] changes in F-actin stimulated by FMLP [Sha'afi and Molski, 1987; Howard and Wang, 1987]. The pertussis toxin sensitivity may come in part from the G protein–mediated activation of phospholipase A_2 [Burgoyne et al., 1987]. The participation of AA generated by phospholipase A_2 in early stimulus-induced changes such as actin polymerization could account for some of the failures of phorbol esters to mimic more of the FMLP response [Lackie, 1988].

The oxidative burst, as chemotaxis, appears to involve multiple pathways [see Bagglioni and Wymann, 1990, for review] and is initiated through the receptor-mediated stimulation of PIP_2 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 IP_3 and possibly IP_4. 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 aand Furuya, 1988; Sha'afi and Molski, 1988]. Additionally, PMA pretreatment can circumvent this inhibition of the oxidative burst, PLC activation, and production of IP_3 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, IP_3, IP_4, and DAG (<30 sec), but FMLP sustained the increased calcium and IP_3 levels and elicited a second peak of DAG (at ~ 120 sec). This second peak of DAG was not derived from phosphoinositol 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 [Bagglioni 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 twofold, 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, ^3H-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 IP_3 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. disoideum* 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 PIP_2 hydrolysis in olfactory epithelium has been scrutinized. GTP and odorants stimulate IP_3 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 IP_3 in a GTP-dependent manner [Bruch et al., 1989]. (The catfish olfactory PLC that is responsible for generating IP_3 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 Ca^{2+}, and Ca^{2+} channel blockers and patch-clamping of the receptor cell membranes reveal an IP_3-gated calcium channel [Restrepo et al., 1990]. Therefore in catfish two parallel receptor–G protein pathways exist, one coupled to PLC that increases IP_3 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 IP_3 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 IP_3 [Breer et al., 1990a,b], qualifying IP_3 as a second messenger. However, unlike in catfish, the set of odorants that stimulate IP_3 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 Buekoff, 1991; see Restrepo et al., 1990, and Bruch and Teeter, 1989].

Similarly, in catfish *taste tissues*, the tastant L-alanine rapidly stimulates IP_3 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 IP_3 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 Ca^{2+} during chemotaxis [Sawyer et al., 1985; Jaconi et al., 1988]. However, calcium-sensitive dyes over very short sampling times (0.5 sec) show no consistent localization of Ca^{2+} 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^{-9}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 alkalinization 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 and not 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 alkalinization 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.

Paramecia 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 folic 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 Ca^{2+}-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 Ca^{2+}-ATPase to generate the hyperpolarization elicit a lithium-sensitive response and are not attractants to mutant *K-shy*, while NH_4Cl, for example, that is thought to affect internal pH and not affect Ca^{2+}-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 Ca^{2+}-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-

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 chemoresponse. 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 chemoresponse 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 O_2 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 PIP_2 to produce IP_3 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. G_s) that stimulate PLC and adenylate cyclase, respectively, along two separate sensory transduction pathways. Within the PLC pathway, IP_3, 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 A_2 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 trransfer 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 (G_s) and inhibitory (G_i) 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 PIP_2 metabolism, calcium by IP_3, IP_4, and AA, and protein kinase C by DAG and calcium. Therefore the major second messengers are IP_3 and DAG and third messengers are calcium, AA, and possibly IP_4. 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 PIP_2 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 G_i or G_o, but instead is a smaller G_c 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 G_c α-subunit.

Neutrophil phospholipase A_2 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 Ca^{2+}; actin polymerization; PI, PIP, and PIP_2 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, IP_2, and IP_3.

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-Jaglaska 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 [Europe-Finner and Newell, 1987a,b]. Synchronized cells exhibit oscillations of IP_3 and cGMP with cAMP stimulation and likewise with GTPγS 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 [Europe-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 GTPγS, 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, GTP γS 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 [Kesbeke 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-Jaglaska 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 [Kesbeke 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 $G_{2\alpha}$ [Johnson et al., 1989]. Small *ras*-like G proteins are present in *Dictyostelium*, 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 G_s mechanism, despite the presence of G_s in both catfish and other olfactory cilia [Bruch et al., 1987]. In pseudoparathyroidism patients, the G_s 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 G_s 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 tastant [Kalinoski 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 G_s, G_i, and G_o 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 (G_{olf}) [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 G_s 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_s and the 41 kD form of G_i [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_s α-subunit. Disruption of this gene leads to growth arrest in G_1, which is characteristic of haploid cells

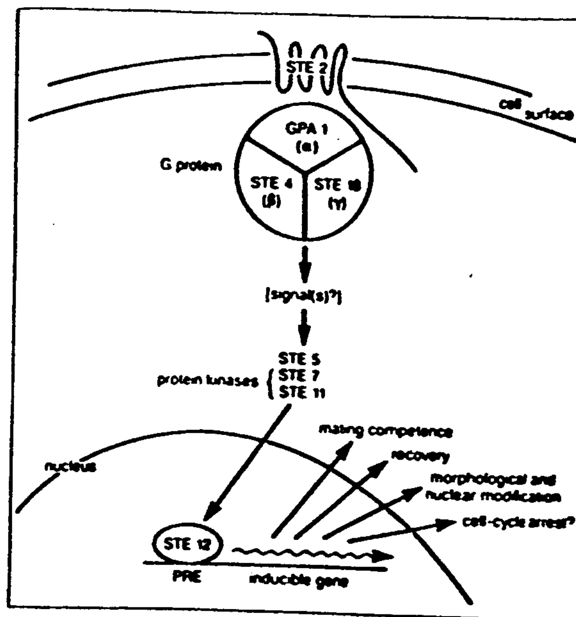

Fig. 10. Schematic of the response pathway in a cells to α-factor. The genes shown are involved in the response to pheromone that leads to transcriptional induction. The pathway apart from pheromone and receptor appears to be the same in α-cells. [Reproduced from Fields, 1990, with permission of the publisher.]

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 cilium,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 IP_3-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, IP_3, 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 underway 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 Zeilgelberger, 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 [Ronnett 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 [McClintock 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; Kaissling 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 combinatin of electrophysiology [Cummings et al., 1991; Avenet et al., 1991; Behe et al., 1990; Herness, 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 now 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 chemoresponse 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, (with apol-

ogies 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

Ache B (1987) Chemoreception in invertebrates. In Finger T, Silver W (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 39–64.

Ache BW (1989) Central and peripheral bases for mixture suppression in olfaction: A crustacean model. In Laing DG, Cain WS, McBride RL, Ache BW (eds): Perception of Complex Smells. Sydney: Academic Press, pp 101–114.

Adair WS, Hwang C, Goodenough U (1983) Identification and visualization of the sexual agglutinin from the mating-type plus flagellar membrane of *Chlamydomonas*. Cell 33:183–193.

Adamek GD, Gesteland RC, Mair RG, Oakley B (1984) Transduction physiology of olfactory receptor cilia. Brain Res 310:87–97.

Adler J (1987) How motile bacteria are attracted and repelled by chemicals: An approach to neurobiology. Biol Chem Hoppe-Seyler 368:163–173.

Akabas MH, Dodd J, Al-Awqati Q (1987) Mechanism of transduction of bitter taste in rat taste bud cells. Soc Neurosci Abstr 13:361.

Akabas MJ, Dodd J, Al-Awqati Q (1988) A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. Science 242:1047–1050.

Alkon DL, Rasmussen H (1988) A spatial–temporal model of cell activation. Science 239: 998–1004.

Allen RA, Traynor AE, Omann GM, Jesaitis AJ (1988) The chemotactic peptide receptor: A model for future understanding of chemotactic disorders. In Curnutte JT (ed): Phagocytic Defects: Abnormalities Outside of the Respiratory Burst. Philadelphia: WB Saunders, pp 33–59.

Anderson D, Raffioni S, Luporini P, Bradshaw RA, Eisenberg D (1990) Crystallization of the *Euplotes raikovi* mating pheromone Er-1. J Molec Biol 216:1–2.

Anholt R (1987) Primary events in olfactory reception. TIBS 12:58–62.

Anholt RH, Mumby SM, Stoffers DA, Girard PR, Kuo JF, Snyder SH (1987) Transduction proteins of olfactory receptor cells: Identification of guanine nucleotide binding proteins and protein kinase C. Biochemistry 26:788–795.

Anholt RH, Rivers AM (1990) Olfactory transduction: Cross-talk between second-messenger systems. Biochemistry 1990:4049–4054.

Armitage A, Havelka WA, Sockett RE (1990) Methylation-independent taxis in bacteria. In Armitage JP, Lackie JM (eds): Biology of the Chemotactic Response. Cambridge: Cambridge University Press, pp 177–198.

Armitage J (1988) Tactic responses in photosynthetic bacteria. Can J Microbiol 34:475–481.

Armitage J (1990) Sensory signalling in *Rhodobacter sphaeroides*. In Drews G (ed): Molecular Biology of Membrane Protein Complexes in Phototrophic Bacteria. New York: Plenum Press (in press).

Armitage J, Ingham C, Evans MCW (1985) Role of proton motive force in phototactic and aerotactic responses of *Rhodopseudomonas sphaeroides*. J Bacteriol 161:967–972.

Armitage JP, Lackie JM (eds) (1990) Biology of the Chemotactic Response. Cambridge: Cambridge University Press, pp 1–404.

Atema J (1985) Chemoreception in the sea: Adaptations of chemoreceptors and behavior to aquatic stimulus conditions. In Laverack AS (ed): Physiological Adaptations of Marine Animals. Society of Experimental Biology, Great Britain, pp 387–423.

Atema J (1987) Aquatic and terrestrial chemoreceptor organs: Morphological and physiological designs for interfacing with chemical stimuli. In Dejours P, Bolis L, Taylor CR, Weibel ER (eds): Comparative Physiology: Life in Water and on Land. Fidia Research Series, Padova: IX Liviana Press, pp 303–316.
Avenet P, Hofmann F, Lindemann B (1988) Patch-clamp study of isolated taste receptor cells of the frog. J Membr Biol 97:223–240.
Avenet P, Kinnamon S, Roper S (1991) In situ recording from hamster taste cells: Responses to salt, sweet and sour. Chem Senses 16 (in press).
Axelrod J, Burch RM, Jelsma CL (1988) Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. TINS 11:117–122.
Bagglioni M, and Wymann MP (1990) Turning on the respiratory burst. Trends Boichem Sci 15:69–72.
Bakalyar H, Reed R (1990) Identification of a specialized adenylate cyclase that may mediate odorant detection. Science 250:1403–1405.
Balsinde J, Diez E, Schüller A, Mollinedo F (1988) Phospholipase A_2 activity in resting and activated human neutrophils. J Biol Chem 263:1929–1936.
Barnard EA, Darlison MG, Seeburg P (1987) Molecular biology of the $GABA_A$ receptor: The receptor/channel superfamily. TINS 10:502–509.
Bass DA, Gerard C, Olbrantz P, Wilson J, McCall CE, McPhail LC (1987) Priming of the respiratory burst of neutrophils by diacylglycerol. J Biol Chem 262:6643–6649.
Beale G (1990) Self- and nonself recognition in the ciliate protozoan *Euplotes*. Trends in Genet 6:137–139.
Beaumier L, Faucher N, Naccache PH (1987) Arachidonic acid–induced release of calcium in permeabilized human neutrophils. FEBS Lett 221:289–292.
Bedale WA, Nettleton DO, Sopata CS, Thoekle MS, Ordal GW (1988) Evidence for methyl-group transfer between the methyl-accepting chemotaxis proteins in *Bacillus subtilis*. J Bacteriol 170:223–227.
Behe P, DeSimone JA, Avenet P, Lindemann (1990) Membrane currents in taste cells of the rat fungiform papilla—Evidence for two types of Ca currents and inhibition of K- currents by saccharin. J Gen Physiol 96:1061–1084.
Belardetti F, Siegelbaum SA (1988) Up- and down-modulation of single K^+ channel function by distinct second messengers. TINS 11:232–238.
Bentley JK, Khatra AS, Garbers DL (1987) Receptor-mediated phosphorylation of spermatozoa proteins. J Biol Chem 262:15708–15713.
Bentley JK, Shimomura H, Garbers DL (1986a) Retention of a functional resact receptor in isolated sperm plasma membranes. Cell 45:281–288.
Bentley JK, Tubb DJ, Garbers DL (1986b) Receptor-mediated activation of spermatozoan guanylate cyclase. J Biol Chem 261:14859–14862.
Berridge MJ (1987) Inositol triphosphate and diacylglycerol: Two interacting second messengers. Annu Rev Biochem 56:159–194.
Bigiani A, Roper S (1991) Mediation of responses to calcium in taste cells by modulation of a K conductance. Science 252:126–128.
Bignetti E, Cavaggioni A, Pelosi P, Persaud KL, Sorbi RT, Tirindelli R (1985) Purification and characterization of an odorant binding protein from cow nasal tissue. Eur J Biochem 149:227–231.
Böhme R, Bumann J, Aeckerle S, Malchow D (1987) A high-affinity plasma membrane Ca^{2+}-ATPase in *Dictyostelium discoideum*: Its relation to cAMP-induced Ca^{2+} fluxes. Biochim Biophys Acta 904:125–130.
Bonini NM, Gustin M, Nelson DL (1986) Regulation of ciliary motility by membrane potential in *Paramecium*: A role for cAMP. Cell Motil Cytoskel 6:256–272.

Bonini NM, Nelson DL (1988) Differential regulation of *Paramecium* ciliary motility by cAMP and cGMP. J Cell Biol 106:1615–1623.

Borroni PF, Atema J (1987) Self- and cross-adaptation of single chemoreceptor cells in the taste organs of the lobster, *Homarus americanus*. In Roper S, Atema J (eds): "Olfaction and Taste IX." New York: New York Academy of Sciences, pp 184–186.

Boulter J, Evans K, Goldman D, Martin G, Treco D, Heinemann S, Patrick S (1986) Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor α-subunit. Nature 319:368–374.

Boyd A, Simon M (1982) Bacterial chemotaxis. Annu Rev Physiol 44:501–517.

Boyle AG, Park YS, Huque T, Bruch RC (1987) Properties of phospholipase C in isolated olfactory cilia from the channel catfish (*Ictalurus punctatus*). Comp Biochem Physiol 88B: 767–775.

Brass JM (1986) The cell envelope of gram-negative bacteria: New aspects of its function in transport and chemotaxis. Curr Top Microbiol Immunol 129:1–92.

Breer H, Boekoff I (1991) Odorants of the same odor class activate different second messenger pathways. Chem Senses 16:16–29.

Breer H, Boekhoff I, Tareilus E (1990a) Rapid kinetics of second-messenger signalling in olfaction. Chem Senses 15:553.

Breer H, Boekhoff I, Tareilus E (1990b) Rapid kinetics of second-messenger formation in olfactory transduction. Nature 345:65–68.

Brokaw C (1987) A lithium sensitive regulator of sperm flagellar oscillations is activated by cyclic AMP-dependent phosphorylation. J Cell Biol 105:1789–1798.

Bruch RC (1990a) Signal transduction in olfaction and taste. In Inyegar R, Birnbaumer L (eds): G-Proteins. New York: Academic Press, pp 411–428.

Bruch RC (1990b) Signal transducing GTP-binding proteins in olfaction. Comp Biochem Physiol 95A:27–29.

Bruch RC, Huque T (1987) Odorant- and guanine nucleotide–stimulated phosphoinositide turnover in olfactory cilia. In Roper S, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 205–207.

Bruch RC, Kalinoski DL (1987) Interaction of GTP-binding regulatory proteins with chemosensory receptors. J Biol Chem 262:2401–2404.

Bruch RC, Kalinoski DL, Huque T (1987a) Role of GTP-binding regulatory proteins in receptor-mediated phosphoinositide turnover in olfactory cilia. Chem Senses 12:173.

Bruch RC, Rulli RD, Boyle AG (1987b) Olfactory L-amino acid receptor specificity and stimulation of potential second messengers. Chem Senses 12:642–643.

Bruch RC, Teeter JH (1989) Second-messenger signalling mechanisms in olfaction. In Brand JG, Teeter JH, Cagan RH, Kare MR (eds): Chemical Senses, Vol 1. New York: Marcel Dekker, pp 283–298.

Bruch RC, Teeter JH (1990) Cyclic AMP links amino acid chemoreceptors to ion channels in olfactory cilia. Chem Senses 15:419–430.

Burchell B (1991) Turning on and turning off the sense of smell. Nature 350:16–17.

Bryant BP, Brand JG, Kalinoski DL (1989) Receptor site specificity in taste. In Brand JG, Teeter JH, Cagan RH, Kare MR (eds): Chemical Senses, Vol 1. New York: Marcel Dekker, pp 35–53.

Bryant B, Brand JG, Kalinoski DL, Bruch RC, Cagan RH (1987) Use of monoclonal antibodies to characterize amino acid taste receptors in catfish: Effects on binding and neural responses. In Roper R, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 208–209.

Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65:175–187.

Burgoyne RD, Cheek TR, O'Sullivan AJ (1987) Receptor-activation of phospholipase A_2 in cellular signalling. Trends Bioch 12:332–333.

Burkholder AC, Hartwell LH (1985) The yeast *a*-factor receptor: Structural properties deduced from the sequence of the STE2 gene. Nucleic Acids Res 13:8463–8473.

Cagan RH (1981) Recognition of taste stimuli at the initial binding interaction. In Cagan RH, Kare MR (eds): Biochemistry of Taste and Olfaction. New York: Academic Press, pp 175–204.

Cagan RH, Boyle AG (1984) Biochemical studies of taste sensation XI. Isolation, characterization and taste ligand binding activity of plasma membranes from catfish taste tissue. Biochim Biophys Acta 799:230–237.

Caprio J (1987a) Olfactory receptor responses to binary mixtures of amino acids in the channel catfish, *Ictalurus punctatus*. In Miller IS (ed): LM Beidler Symposium on Taste and Smell (in press).

Caprio J (1987b) Peripheral filters and chemoreceptor cells in fishes. In Atema J, Fay R, Popper A, Tavolga W (eds): Sensory Biology of Aquatic Animals. New York: Springer-Verlag, pp 311–336.

Caprio J (1988) Peripheral filters and chemoreceptor cells in fishes. In Atema J, Fay RR, Popper AN, Tavoiga WN (eds): Sensory Biology of Aquatic Animals. Berlin: Springer Verlag, pp 313–338.

Carr WES (1989) Chemical signaling systems in lower organisms. In Anderson PAV (ed): Evolution of the First Nervous System. New York: Plenun Press, pp. 81–94.

Carr WES, Ache BW, Gleeson RA (1987) Chemoreceptors of crustaceans: Similarities to receptors for neuroactive substances in internal tissues. Environ Health Perspect 71:31–46.

Carr WES, Netherton JC, Milstead ML (1984) Chemoattractants of the shrimp, *Palamemonetes pugio*. Comp Biochem Physiol 77A:469–474.

Carter A, Bardin C, Colins R, Simons C, Bray P, Spiegel A (1987) Reduced expression of multiple forms of the α-subunit of the stimulatory GTP-binding protein in pseudohypoparathyroidism type Ia. Proc Natl Acad Sci USA 84:7266–7269.

Cartwright CP, Tipper DJ (1991) In vivo topological analysis of Ste2, a yeast plasma membrane protein, by using beta-lactamase gene fusions. Mol Cell Biol 11:2620–2628.

Cassimeris H, McNeill H, Zigmond SH (1990) Chemoattractant-stimulated polymorphonuclear leukocytes contain two populations of actin filaments that differ in their spatial distributions and relative stabilities. J Cell Biol 110:1067–1075.

Chen Z, Pace U, Ronen D, Lancet D (1986) Polypeptide gp95, a unique glycoprotein of olfactory cilia with transmembrane receptor properties. J Biol Chem 261:1299–1305.

Coats WD, Navarro J (1990) Functional reconstitution of the f-met-leu-phe receptor in *Xenopus laevis* oocytes. J Biol Chem 265:5964–5966.

Cochrane CG (1984) Mechanisms coupling stimulation and function in leukocytes. Fed Proc 43:2729–2731.

Cockroft S (1987) Polyphosphoinositide phosphodiesterase: Regulation by a novel guanine nucleotide binding protein. G_p. TIBS 12:75–78.

Condeelis J, Hall A, Bresnick A, Warren V, Hock R, Bennett H, Ogihara S (1988) Actin polymerization and pseudopod extension during amoeboid chemotaxis. Cell Motil Cystoskel 10:77–90.

Costa-Casnellie MR, Segel GB, Lichtman MA (1986) Signal transduction in human monocytes: Relationship between superoxide production and the level of kinase C in the membrane. J Cell Physiol 129:336–342.

Crabbendam KJ, Klis FM, Musgrave A, van den Ende H (1986) Ultrastructure of the plus and minus mating-type sexual agglutinins of *Chlamydomonas eugametos*, as visualized by negative staining. J Ultrastruct Mol Struct Res 96:151–159.

Cummings TA, Avenet P, Roper SD, Kinnamon SC (1991) Patch-clamp recordings of hamster taste cells: Effects of saccharin and cAMP. Chem Senses (in press).

Danciger E, Mettling C, Vidal M, Morris R, Margolis F (1989) Olfactory marker protein gene: Its structure and olfactory neuron-specific expression in transgenic mice. Proc Natl Acad Sci USA 86:8565–8569.

Dangott LJ, Garbers DL (1984) Identification and partial purification of the receptor for speract. J Biol Chem 259:13712–13716.

Della Bianca V, Grzeskowiak M, Cassatella MA, Zeni L, Rossi F (1986) Inhibition by verapamil of neutrophil responses to f-met-leu-phe and phorbol myristate acetate. Biochem Biophys Res Commun. 135:556–567.

DeLozanne A, Spudich JA (1987) Disruption of the *Dictyostelium* myosin heavy chain gene by homolgous recombination. Science 236:1086–1091.

Derby CD, Ache BW (1984) Quality coding of a complex odorant in an invertebrate. J Neurophys 51:906–924.

Derby CD, Atema J (1987) Chemoreceptor cells in aquatic invertebrates: Peripheral mechanisms of chemical signal processing in decapod crustaceans. In Atema J, Fay RR, Popper AN, Tavolga WN (eds): Sensory Biology of Aquatic Animals. New York: Springer-Verlag, pp 365–385.

Derby CD, Giraradot M-N, Daniel P, Fine-Levy JB (1989) Olfactory discrimination of mixtures: Behavioral electrophysiological and theoretical studies using the spiny lobster *Panulirus argus*. In Laing DG, Cain WS, McBride RL, Ache BW (eds): Perception of Complex Smells. Sydney: Academic Press, pp 65–82.

Derby CD, Hamilton KA, Ache BW (1984) Processing of olfactory information at three neuronal levels in the spiny lobster. Brain Res 300:311–319.

DeSimone JA, Ferrell F (1985) Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. J Physiol 369:R52–R61.

DeSimone JA, Heck GL, DeSimone SK (1981) Active ion transport in dog tongue: A possible role in taste. Science 214:1039–1041.

Dethier V (1978) Other tastes, other worlds. Sci 201:224–228.

Devreotes PN, Sherring JA (1985) Kinetics and concentration dependence of reversible cAMP-induced modification of the surface cAMP receptor in *Dictyostelium*. J Biol Chem 260:6378–6384.

Devreotes PN, Zigmond SH (1988) Chemotaxis in eukaryotic cells. Annu Rev Cell Biol 4:649–686.

Dhallan RS, Yau K, Schrader KA, Reed RR (1990) Primary structure and function expression of a cyclic nucleotide-activated channel from olfactory neurons. Nature 347:184–186.

Dietzel C, Kurjan J (1987) The yeast SCG1 gene: A G_α-like protein implicated in the a and a-factor response pathway. Cell 50:1001–1010.

Dillon SB, Murray JJ, Uhing RJ, Snyderman R (1987a) Regulation of inositol phospholipid and inositol phosphate metabolism in chemoattractant-activated human polymorphonuclear leukocytes. J Cell Biochem 35:345–359.

Dillon SB, Murray JJ, Verghese MW, Snyderman R (1987b) Regulation of inositol phosphate metabolism in chemoattractant-stimulated human polymorphonuclear leukocytes. J Biol Chem 262:11546–11552.

DiNallo M, Wohlford M, Van Houten J (1982) Mutants of *Paramecium* defective in chemoreception of folate. Genetics 103:453–468.

Ding XX, Porter TD, Peng HM, Coon MJ, (1991) cDNA and derived amino acid sequence of rabbit nasal cytochrome P450NMb, a unique isozyme possibly involved in olfaction. Arch Biochem Biophys 285:120–125.

Dionne V (1988) How do you smell? Principle in question. Trends Neurol Sci 11:188–189.

Dionne V (1990) Excitatory and inhibitory responses induced by amino acids in isolated mudpuppy olfactory receptor neurons. Chem Senses 15:566.

Dockstader KC, Dunwiddie TV, Finger TE (1991) Glutamate in NOT the neurotransmitter of primary gustatory afferent fibers. Chem Senses 16 (in press).

Dohlman HG, Caron MG, Lefkowitz R (1987) A family of receptors coupled to guanine nucleotide regulatory proteins. Biochemistry 26:2657–2664.

Dunlap K, Holz GG, Rane SG (1987) G proteins as regulators of ion channel function. TINS 10:241–244.

Eberle M, Traynorcaplan AE, Sklar L, Norgauer J (1990) Is there a relationship between phosphatidylinositol trisphosphate and F-actin polymerization in human neutrophils? J Biol Chem 265:16725–16728.

Edelman AM, Blumenthal DK, Krebs EG (1987) Protein serine/threonine kinases. Annu Rev Biochem 56:567–614.

Edwards DA, Mather RA, Dodd GH (1988) Spatial variation in response to odorants on the rat olfactory epithelium. Experientia 44:208–211.

Eisenbach M, Matsumura P (1988) In vitro approach to bacterial chemotaxis. Botan Acta 101:93–99.

Europe-Finner GN, Luderus MEE, Small NV, Van Driel R, Reymond CD, Firtel RA, Newell PC (1988) Mutant *ras* gene induces elevated levels of inositol *tris*- and hexakisphosphates in *Distyostelium*. J Cell Sci 89:13–20.

Europe-Finner GN, Newell PC (1985) Inositol 1,4,5-trisphosphate induces cGMP formation in *Dictyostelium discoideum*. Biochem Biophys Res Commun 130:1115–1122.

Europe-Finner GN, Newell PC (1986) Inositol 1,4,5-trisphosphate induces calcium release from a non-mitochondrial pool in amoebae of *Dictyostelium*. Biochim Biophys Acta 887:335–340.

Europe-Finner GN, Newell PC (1987a) GTP analogues stimulate inositol trisphosphate formation transiently in *Dictyostelium*. J Cell Sci 87:513–518.

Europe-Finner GN, Newell PC (1987b) Cyclic AMP stimulates accumulation of inositol trisphosphate in *Dictyostelium*. J Cell Sci 87:221–229.

Evans TC, Hennessey T, Nelson DL (1987) Electrophysiological evidence suggests a defective Ca^{2+} control mechanism in a new *Paramecium* mutant. J Membr Biol 98:275–283.

Ewald DA, Roper SD (1990) Delayed depolarizing responses of *Necturus* taste cells to chemical and electrical stimuli at the apical pore of taste buds. Soc Neurosci Abstr 16:25.

Fadool DA, Michel WC, Ache BW (1991) G-proteins and inositol-phospholipid metabolism implicated in odor response of cultured lobster olfactory neurons. Chem Senses 16 (in press).

Falke JJ, Koshland DE (1987) Global flexibility in a sensory receptor: A site-directed cross-linking approach. Science 237:1596–1600.

Farbman AI (1990) Olfactory neurogenesis—Genetic or environmental controls. Trends in Neurosci 13:362–365.

Featherstone C (1990) An ATP-driven pump for secretion of yeast mating factor. Trends Biochem Sci 15:169–170.

Fesenko EE, Novoselov VI, Bystrova MF (1987) The subunits of specific odor-binding glycoproteins from rat olfactory epithelium. FEB Lett 219:224–226.

Fields S (1990) Pheromone response in yeast. Trends Biochem Sci 15:270–273.

Finger TE, Bottjer B (1990) Transcellular labeling of taste bud cells by carbocyanine dye (DiI) applied to peripheral nerves in the barbels of the catfish, Ictalurus-punctatus. J Compar Neurol 302:884–892.

Firestein S, Shepherd GM (1989) Olfactory transduction is mediated by the action of cAMP. Soc Neurosci Abstracts 19:749.

Firestein S, Shepherd GM (1990) The role of cAMP as a second messenger in vertebrate olfactory transduction. Chem Senses 15:574.

Firestein S, Shepherd G, Werblin FS (1990) Time course of the membrane current underlying sensory transduction in salamander olfactory receptor neurones. J Physiol (London) 430:135–158.

Firestein S, Werblin F (1987) Electrophysiological basis of the response of olfactory receptors to

odorant and current stimuli. In Roper S, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 287–289.

Fletcher MP, Seligmann BE (1986) PMN heterogeneity: Long-term stability of fluorescent membrane potential responses to the chemoattractant *N*-formyl-methionyl-leucyl-phenylalanine in healthy adults and correlation with respiratory burst activity. Blood 68:611–618.

Foder B, Scharff O, Thastrup O (1989) Ca^{2+}-transcients and Mn^{2+} entry in human neutrophils induced by thapsigargin. Cell Calcium 10:477–490.

Fraser CM, Lindstrom J (1984) The use of monoclonal antibodies in receptor characterization and purification. In Venter JC, Harrison LC (eds): Molecular and Chemical Characterization of Membrane Receptors. New York: Alan R. Liss, pp 1–30.

Garbers DL (1989a) Guanylate cyclase, a cell surface receptor. J Biol Chem 264:9103–9106.

Garbers DL (1989b) Molecular basis of signalling in the spermatozoon. J Androl 10:99–107.

Garbers DL, Kopf GS (1980) The regulation of spermatozoa by calcium and cyclic nucleotides. Adv Cyclic Nucleotide Res 13:251–306.

Garbers DL, Noland TD, Dangott LJ, Ramarao CS, Bentley JK (1986) The interaction of egg peptides with spermatozoa. In Dhindsa DS, Bahl Om P (eds): Molecular and Cellular Aspects of Reproduction. New York: Plenum, pp 145–163.

Gegner JA, Dahlquist FW (1991) Signal transduction in bacteria—CheW forms a reversible complex with protein kinase CheA. Proc Natl Acad Sci USA 88:750–754.

Gerard N, Gerard C (1991) The chemotactic receptor for human C5a and phylatoxin. Nature 349:614–617.

Gesteland RC (1986) Speculation on receptor cells as analyzers and filters. Experientia 42:287–291.

Getchell TV, Getchell ML (1987) Peripheral mechanisms of olfaction: Biochemistry and neurophysiology. In Finger TE, Siver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 91–123.

Getchell TV, Grillo M, Tate SS, Urade R, Teeter J, Margolis FL (1990) Expression of catfish amino acid taste receptors in *Xenopus* oocytes. Neurochem Res 15:449–456.

Getchell TV, Margolis FL, Getchell ML (1984) Perireceptor and receptor events in vertebrate olfaction. Prog Neurobiol 23:317–345.

Gilman AG (1987) G proteins: Transducers of receptor-generated signals. Annu Rev Biochem 56:615–650.

Golman DW, Goetzl EJ (1982) Specific binding of leukotriene B_4 to receptors on human polymorphonuclear leukocytes. J Immunol 129:1600.

Goodenough U, Adair WS, Collin-Osdoby P, Heuser JE (1985) Structure of the *Chlamydomonas* agglutinin and related flagellar surface proteins in vitro and in situ. J Cell Biol 101:924–941.

Grinstein S, Furuya W (1988) Receptor-mediated activation of electropermeabilized neutrophils. J Biol Chem 263:1779–1783.

Grob PM, David E, Warren TC, DeLeon RP, Farina PR, Homon CA (1990) Characterization of a receptor for human monocyte-derived neutrophil chemotactic factor/interleukin-8. J Biol Chem 265:8311–8316.

Gustin MC, Nelson DL (1987) Regulation of ciliary adenylate cyclase by Ca^{2+} in *Paramecium*. Biochem J 246:337–345.

Hagan DC, McCafrey G, Sprague GF (1986) Evidence the yeast STE3 gene encodes a receptor for the peptide pheromone a factor: Gene sequence and implications for the structure of the presumed receptor. Proc Natl Acad Sci USA 83:1418–1422.

Hall AL, Watten V, Condeelis J (1989) Transduction of the chemotactic signal to the actin cytoskeleton of *Dictyostelium discoideum*. Dev Biol 136:517–525.

Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: Conserved features and deduced phylogeny of the catalytic domains. Science 241:42–52.

Hanley MR, Jackson T (1987) Return of the magnificent seven. Nature 329:766-767.

Harvath L (1989) Regulation of neutrophil chemotaxis. Ann Reports Med Chem 24:233-241.

Heckmann K, Kuhlmann H-W (1986) Mating types and mating substances in *Euplotes octocarinatus*. J Exp Zool 237:87-96.

Hennessey T, Machemer H, Nelson DL (1985) Injected cAMP increases ciliary beat frequency in conjunction with membrane hyperpolarization. Eur J Cell Biol 36:153-156.

Herness MS (1990) Sucrose stimulation of rat taste cells can increase membrane potassium conductance. Soc Neurosci Abstr 16:25.

Herskowitz I, Marsh L (1987) Conservation of a receptor/signal transduction system. Cell 50:995-996.

Hess JF, Oosawa K, Kaplan N, Simon M (1988) Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. Cell 53:79-87.

Hill DL, Mistretta CM (1990) Developmental neurobiology of salt taste sensation. Trends in Neurosci 13:188-195.

Hockberger PE, Swandulla D (1987) Direct ion channel gating: A new function for intracellular messengers. Cell Mol Neurobiol 7:229-236.

Homan W, Sigon C, van den Briel W, Wagter R, de Nobel H, Mesland D, Musgrave A, van den Ende H (1987) Transport of membrane receptors and the mechanics of sexual cell fusion in *Chlamydomonas eugametos*. FEBS Lett 215:323-326.

Houslay MD (1987) Egg activation unscrambles a potential role for IP_4. TIBS 12:1-2.

Howard TH, Wang D (1987) Calcium ionophore, phorbol ester, and chemotactic peptide-induced cytoskeleton reorganization in human neutrophils. J Clin Invest 79:1359-1364.

Huque T, Brand JG (1988) Phosphatidylinositol-4,5-bisphosphate phosphodiesterase (PIP_2-PDE) activity of catfish taste tissue. Chem Senses 13:698.

Huque T, Brand JG, Rabinowitz JL, Bayley DL (1987) Phospholipid turnover is catfish barbel (taste) epithelium with special reference to phosphatidylinositol-4,5-bisphosphate. Chem Senses 12:666-667.

Huque T, Bruch RC (1986) Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia. Biochem Biophys Res Commun 137:36-42.

Ingham CJ, Armitage JP (1987) Involvement of transport in *Rhodobacter shpaeroides* chemotaxis. J Bacteriol 169:5801-5807.

Imagawa M, Chium R, Karin M (1987) Transcription factor AP-2 mediates induction by two different signal transduction pathways: Protein kinase C and cAMP. Cell 51:251-260.

Irvine RF, Moor RM (1986) Microinjection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on extenal Ca^{2+}. Biochem J 240:917-920.

Jackowski S, Sha'afi RI (1979) Response of adenosine cyclic-3' 5' monophosphate level in rabbit neutrophils to the chemotactic peptide formyl methionyl-leucyl-phenyl alanine. Mol Pharmacol 16:473-481.

Jackson C, Hartwell L (1990) Courtship in *Saccharomyces cerivisiae:* Both cell types choose mating partners by responding to the strongest pheromone signal. Cell 63:1039-1051.

Jaconi MEE, Rivest RW, Schlegel W, Wollheim CG, Pittet D, Lew PD (1988) Spontaneous and chemoattractant-induced oscillations of cytosolic free calcium in single adherent human neutrophils. J Biol Chem 263:10557-10560.

Janssens PMW (1987) Did vertebrate signal transduction mechanisms originate in eukaryotic microbes? TIBS 12:456-459.

Janssens PMW, Van Haastert PJM (1987) Molecular basis of transmembrane signal transduction in *Dictyostelium discoideum*. Microbiol Rev 51:396-418.

Jenness DD, Burkholder AC, Hartwell LH (1986) Binding of a-factor pheromone to *Saccharomyces cerevisiae* a cells: Dissociation constant and number of binding sites. Mol Cell Biol 6:318-320.

Jenness DD, Goldman BS, Hartwell LH (1987) *Saccharomyces cerevisiae* mutants unresponsive to a-factor pheromone: *a*-Factor binding and extragenic suppression. Mol Cell Biol 7:1311–1319.

Jesaitis AJ, Bokoch GM, Allen RA (1988a) Regulation of signal transduction in human neutrophils by cytoskeleton-mediated reorganization of the plasma membrane chemoattractant receptors. In Condeelis J, Satir P, Lazarides E (eds): Signal Transduction in Cytoplasmic Organization and Cell Motility. New York: Alan R. Liss, pp 325–337.

Jesaitis AJ, Bokoch GM, Tolley JO, Allen RA (1988b) Lateral segregation of occupied chemotactic receptors into actin and fodrin-rich plasma membrane microdomains depleted in guanyl nucleotide proteins. J Cell Biol 107:921–928.

Jesaitis AJ, Naemura JR, Sklar LA, Cochrane CG, Painter RG (1984) Rapid modulation of *N*-formyl chemotactic peptide receptors on the surface of human granulocytes: Formation of high-affinity ligand–receptor complexes in transient association with cytoskeleton. J Cell Biol 98:1378–1387.

Jesaitis AJ, Tolley JO, Allen RA (1986) Receptor–cytoskeleton interactions and membrane traffic may regulate chemoattractant-induced superoxide production in human granulocytes. J Biol Chem 261:13662–13669.

Jesaitis AJ, Tolley JO, Bokoch GM, Allen RA (1989) Regulation of chemoattractant receptor interaction with transducing proteins by organizational control in the plasma membrane of human neutrophils. J Cell Biol 109:2783–2790.

Johnson BR, Voigt R, Atema J (1989) Response properties of lobster chemoreceptor cells: Response modulation by stimulus mixtures. Physiol Zool 62:559–579.

Johnson RL, Gundersen R, Lilly P, Pitt GS, Pupillo M, Sun TJ, Vaughan RA, Devreotes PN (1989) G-protein–linked signal transduction systems control development in *Dictyostelium*. Development S1989:75–80.

Jones DT, Reed RR (1987) Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium. J Biol Chem 262:14241–14249.

Jones DT, Reed RR (1989) G_{olf}: An olfactory neuron specific G-protein involved in odorant signal transduction. Science 244:790–795.

Juliani MH, Klein C (1981) Photoaffinity labeling of the cell surface adenosine 3′:5′-monophosphate receptor of *Dictyostelium discoideum* and its modification in down-regulated cells. J Biol Chem 256:613–619.

Kaissling K-E (1987) In Colbow K (ed): RH Wright Lectures on Insect Olfaction. Simon Fraser University, Burnaby, British Columbia.

Kaissling K-E, Keil TA, Williams JLD (1991) Pheromone stimulation in perfused sensory hairs of the moth *Antheraea polyphemus*. J Insect Physiol 37:71–78.

Kalinoski DL, Huque T, LaMorte VJ, Brand JG (1989a) Second-messenger events in taste. In Brand JG, Teeter JH, Cagan RH, Kare MR (eds): Chemical Senses, Vol 1. New York: Marcel Dekker, pp 85–101.

Kalinoski DL, La Morte VJ, Johnson LC, Brand JG (1988) L-amino acid stimulated adenylate cyclase in catfish barbel epithelium: Specificity and characterization. Chem Senses 13:700.

Kashiwayanagi M, Swenaga A, Enomoto S, Kurihara K (1990) Membrane fluidity changes of liposomes in response to various odorants. Biophys J 58:887–895.

Kato H, Ishitoya J, Takenawa T (1986) Inhibition of inositol phospholipids metabolism and calcium mobilization by cyclic AMP-increasing agents and phorbol ester in neutrophils. Biochem Biophys Res Commun 139:1272–1278.

Kauer JS (1987) Coding in the olfactory system. In Finger TE, Silver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 205–231.

Keller H, Gerisch G, Wissler JH (1979) A transient rise in cAMP levels following chemotactic stimulation of neutrophil granulocytes. Cell Biol Int Rep 3:759–765.

Kerlavage AR, Fraser CM, Venter JC (1987) Muscarinic cholinergic receptor structure: Molecular biological support for subtypes. TIPS 8:426–431.

Kesbeke F, Snaar-Jagalska BW, Van Haastert P (1988) Signal transduction in *Dictyostelium* fgd A mutants with a defective interaction between surface cAMP receptors and a GTP-binding regulatory protein. J Cell Biol 107:521–528.

Kesbeke F, Van Haastert PJM, DeWit RJW, Shaar-Jagalska BE (1990) Chemotaxis to cAMP and folic acid is mediated by different G-proteins in *Dictyostelium discoideum*. J Cell Sci 96:669–673.

Kikkawa U, Nishizuka Y (1986) The role of protein kinase C in transmembrane signalling. Annu Rev Cell Biol 2:149–178.

Kinnamon J (1987) Organization and innervation of taste buds. In Finger TE, Silver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 277–297.

Kinnamon S, Roper S (1988) Evidence for a role of voltage-sensitive apical K^+ channels in sour and salt taste transduction. Chem Senses 13:115–121.

Kinnamon SC (1988) Taste transduction: A diversity of mechanisms. Trends in Neurosci 11:491–496.

Kleene SJ, Gesteland RC (1990) Transmembrane currents in frog olfactory cilia. J Membr Biol 120:75–81.

Klein C, Lubs-Haukeness J, Simons S (1985a) cAMP induces a rapid and reversible modification of the chemotactic receptor in *Dictyostelium discoideum*. J Cell Biol 100:715–730.

Klein C, Sadeghi H, Simons S (1986) Immunological analysis of the chemotactic receptor of *Dictyostelium discoideum*. J Biol Chem 261:15192–15196.

Klein P, Knox B, Borleis J, Devreotes P (1987a) Purification of the surface cAMP receptor in *Dictyostelium*. J Biol Chem 262:352–357.

Klein PS, Sun TJ, Saxe CL, Kimmel AR, Johnson RL, Devreotes PN (1988) A chemoattractant receptor controls development in *Dictyostelium discoideum*. Science 241:1467–1472.

Klein P, Theibert A, Fontana D, Devreotes PN (1985b) Identification and cyclic AMP-induced modification of the cyclic AMP receptor in *Dictyostelium discoideum*. J Biol Chem 260:1757–1764.

Klein P, Vaughan R, Borleis J, Devreotes P (1987b) The surface cyclic AMP receptor in *Dictyostelium*. J Biol Chem 262:358–364.

Klumpp S, Gierlich D, Schultz JE (1984) Adenylate cyclase and guanylate cyclase in the excitable ciliary membrane from *Paramecium*: Separation and regulation. FEBS Lett 171:95–99.

Knecht DA, Loomis WF (1987) Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. Science 236:1081–1086.

Koblika BK, Koblika TS, Daniel K, Regan JW, Caron MG, Lefkowitz FJ (1988) Chimeric a_2-, β_2-adrenergic receptors: Delineation of domains involved in effector coupling and ligand binding specificity. Science 240:1310–1316.

Kohbara J, Wegert S, Caprio J (1990) Two types of arginine-best taste units in the channel catfish. Chem Senses 15:601.

Kolesnikov SS, Zhainazarov AB, Kosolapov AV (1990) Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane. FEBS Lett 266:96–98.

Korchak HM, Vienne K, Rutherford LE, Weissmann G (1984) Neutrophil stimulation: Receptor, membrane and metabolic events. Fed Proc 43:2749–2754.

Krause K-H, Pittet D, Volpe D, Pozzan T, Meldolesi J, Leco D (1989) Calciosome, a sarcoplasmic reticulum-like organelle involved in intracellular Ca^{2+}-handling by non-muscle cells: Studies in human neutrophils and HL-60 cells. Cell Calcium 10:351–361.

Krieger J, Raming K, Breer H (1991) Cloning of genomic and complementary DNA encoding insect pheromone binding proteins—Evidence for microdiversity. Biochim Biophys Acta 1088:277–284.

Kumazawa T, Teeter JH, Brand JG (1990) L-proline activates cation channels different from those activated by L-arginine in reconstituted taste epithelial membranes from channel catfish. Chem Senses 15:8603.

Kuo M, Gardner P (1987) Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. Nature 326:301–304.

Kurja J (1990) G proteins in yeast *Saccharomyces cerevisiae*. In Iyenar R, Birnbaumer L (eds): G-Proteins. New York: Academic Press, pp 571–599.

Labarca P, Simon SA, Anholt RH (1988) Activation by odorants of a multistate cation channel from olfactory cilia. Proc Natl Acad Sci USA 85:944–947.

Lackie JM (1988) The behavioural repertoire of neutrophils requires multiple signal transduction pathways. J Cell Sci 89:449–452.

Lancet D (1991) The strong scent of success. Nature 351:275–276.

Lancet D, Pace U (1987) The molecular bases of odor recognition. TIBS 12:63–66.

Lancet D, Helman J, Khen M, Lazard D, Lazarovits J, Margalit T, Poria Y, Aupko K (1989) Molecular cloning and localization of novel olfactory-specific enzymes possibly involved in signal termination: A new role for supporting cells. Soc Neurosci Abstr 15:749.

Lancet D, Striem BJ, Pace U, Zehavi U, Naim M (1987) Adenylate cyclase and GTP binding protein in rat sweet taste transduction. Soc Neurosci Abstr 13:361.

Laskin DL, Gardner CR, Laskin JD (1987) Induction of chemotaxis in mouse peritoneal macrophages by activators of protein kinase C. J Leukocyte Biol 41:474–480.

Lazard D, Tal N, Rubinstein M, Khen M, Lancet D, Zupko K (1990) Identification and biochemical analysis of novel olfactory-specific cytochrome-P-450IIa and UDP-glucuronosyl transferase. Biochem 29:7433–7440.

Lazard D, Zupko K, Poria Y, Nef P, Lazarovits J, Horn S, Khen M, Lancet D (1991) Odorant signal termination by olfactory UDP glucuronosyl transferase. Nature 349:790–793.

Lazzari KG, Proto P, Simons ER (1990) Neutrophil hyperpolarization in response to a chemotactic peptide. J Biol Chem 265:10959–10967.

Lee K-H, Wells RG, Reed RR (1987) Isolation of an olfactory cDNA: Similarity to retinol-binding protein suggests a role in olfaction. Science 235:1053–1056.

Lengeler J, Auburger AJ, Mayer R, Pecher A (1981) The phosphoenolpyruvate dependent carbohydrate: Phosphotransferase system enzymes II as chemoreceptors in chemotaxis of *Escherichia coli* K12. Mol Gen Genet 183:163–170.

Lerner MR, Reagan J, Gyorgyi T, Roby A (1988) Olfaction by melanophores: What does it mean? Proc Natl Acad Sci USA 85:261–264.

Lew D, Wollheim C, Waldvogel F, Pozzan T (1984) Modulation of cytosolic-free calcium transients by changes in intracellular buffering capacity: Correlation with exocytosis and O_2 production in human neutrophils. J Cell Biol 99:1208.

Lewis R (1991) A family of cAMP receptor subtypes helps solve a slime mold mystery. J NIH Research 3:59–62.

Linn CE, Campbell MG, Roelofs WL (1985) Male moth sensitivity to multicomponent pheromones: Critical role of female-released blend in determining the functional role of components and active space of the pheromone. J Chem Ecol 12:659–668.

Linn CE, Roelofs WL (1989) Response specificity of male moths to multicomponent pheromones. Chem Senses 14:421–437.

Liu G, Newell PC (1988) Evidence that cyclic GMP regulates myosin interaction with the cytoskeleton during chemotaxis of *Dictyostelium*. J Cell Sci 90:123–129.

Lohr KM, Snyderman R (1982) Amphotericin B alters the affinity and functional activity of the oligopeptide chemotactic factor receptor on human polymorphonuclear leukocytes. J Immunol 129:1594–1599.

Lowe DG, Chang M-S, Hellmiss R, Chen E, Singh S, Garbers DL, Goeddel DV (1989) Human

atrial natriuretic peptide receptor defines a new paradigm for second messenger signal transduction. EMBO J 8:1377–1384.

Lowe G, Gold GH (1990) Cilia are the site of olfactory transduction. Soc Neurosci Abstr 16:25.

Lowe G, Nakamura T, Gold GH (1988) EOG amplitude is correlated with odor-stimulated adenylate cyclase activity in the bullfrog olfactory epithelium. Chem Senses 13:710.

Lu DJ, Grinstein S (1990) ATP and guanine nucleotide dependence of neutrophil activation—Evidence for the involvement of two distinct GTP-binding proteins. J Biol Chem 265:13721–13729.

Luderus MEE, Reymond CD, Van Haastert PJM, Van Driel R (1988) Expression of a mutated *ras* gene in *Dictyostelium discoideum* alters the binding of cyclic AMP to its chemotactic receptor. J Cell Sci 90:701–706.

Ludwig J, Margalit T, Eismann E, Lancet D, Kaupp UB (1990) Primary structure of cAMP-gated channel from bovine olfactory epithelium. FEBS Lett 270:24–29.

Luporini P, Miceli C (1986) Mating pheromones. In Gall J (ed): Molecular Biology of Ciliated Protozoa. New York: Academic Press, pp 263–299.

Machemer H (1976) Interactions of membrane potential and cations in regulation of ciliary activity in *Paramecium*. J Exp Biol 65:427–448.

Machemer H (1989) Cellular behaviour modulated by ions: Electrophysiological implications. J Protozool 36:463–487.

Majima T, Hamasaki T, Arai T (1986) Increase in cellular cyclic GMP level by potassium stimulation and its relation to ciliary orientation in *Paramecium*. Experientia 42:62–64.

Margolis FL (1987) Chemical senses: Overview of the peripheral olfactory system and the molecular biology of the olfactory marker protein. Discussions in Neurosci 4:47–52.

Marks PW, Maxfield FR (1990a) Transient increases in cytosolic free calcium appear to be required for the migration of adherent human neutrophils. J Cell Biol 110:43–52.

Marks PW, Maxfield FR (1990b) Local and global changes in cytosolic free calcium in neutrophils during chemotaxis and phagocytosis. Cell Calcium 11:181–190.

Marsh L, Herskowitz I (1988) STE2 protein of *Saccharomyces kluyveri* is a member of the rhodopsin/β-adrenergic receptor family and is responsible for recognition of the peptide ligand a factor. Proc Natl Acad Sci USA 85:3844–3859.

Marx JL (1987) Receptor gene family is growing. Science 238:615–616.

Mato JM, Krens FA, van Haastert PJM, Konijn TM (1977) Cyclic AMP dependent cGMP accumulation in *Dictyostelium discoideum*. Proc Natl Acad Sci 74:2348–2351.

McClintock TS, Schutte K, Ache BW (1989) Failure to implicate cAMP in transduction in lobster olfactory receptor cells. Chem Senses 14:817–827.

McClintock TS, Ache B (1989a) Hyperpolarizing receptor potentials in lobster olfactory receptor cells. Chem Senses 14:637–648.

McClintock TS, Ache BW (1989b) Ionic currents and ion channels of lobster olfactory receptor neurons J Gen Physiol 94:1085–1099.

Meier K, Klein C (1988) An unusual protein kinase phosphorylates the chemotactic receptor of *Dictyostelium discoideum*. Proc Natl Acad Sci USA 85:2181–2185.

Menco B (1991) Ultrastructural localization of antibodies to olfactory epithelial-specific glycoprotein gp95, olfactomedin, and 90E and 50E antigens in the frog's olfactory epithelium. Chem Senses 16 [Abstr] (in press).

Meyer F, Schmidt HJ, Plümper E, Hasilik A, Mersmann G, Meyer H, Engström A, Heckmann K (1991) UGA is translated as cysteine in pheromone 3 of *Euplotes octocarinatus*. Proc Natl Acad Sci USA 88:3758–3761.

Meyers-Hutchins BL, Frazier WA (1984) Purification and characterization of a membrane associated cAMP-binding protein from developing *Dictyostelium discoideum*. J Biol Chem 256: 4379–4388.

Miceli C, LaTerza A, Melli M (1989) Isolation and structural characterization of cDNA clones encoding the mating pheromone Er-1 secreted by the ciliate *Euplotes raikovi*. Proc Natl Acad Sci USA 86:3016–3020.

Michel WC, Ache B (1990) Odor-activated conductance inhibits lobster olfactory receptor cells. Chem Senses 15:619–620.

Michel WC, McClintock TS, Ache BW (1991) Inhibition of lobster receptor cells by an odor-activated potassium conductance. J Neurophysiol (in press).

Mierson S, DeSimone SK, Heck GL, DeSimone JA (1988) Sugar-activated ion transport in canine lingual epithelium: Implications for sugar taste transduction. J Gen Physiol (in press).

Miller RJ (1988) G proteins flex their muscles. TINS 11:3–8.

Milligan DL, Koshland DE (1988) Site-directed crosslinking: Establishing the dimer structure of the aspartate receptor of bacterial chemotaxis. J Biol Chem 263:6268–6275.

Miyajima I, Nakafuku M, Nakayama N, Brenner C, Miyajima A, Kaibuchi K, Arai K-I, Kaziro Y, Matsumoto K (1987) GPA1, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. Cell 50:1011–1019.

Moore PA, Gerhardt GA, Atema J (1989) High resolution spatio-temporal analysis of aquatic chemical signals using microelectrochemical electrodes. Chem Senses 14:829–840.

Mowbray SL, Koshland DE (1987) Additive and independent responses in a single receptor: Aspartate and maltose stimuli on the Tar protein. Cell 50:171–180.

Murphy PM, Gallen EK, Tiffany HL, Malech HZ (1990) The formyl peptide chemoattractant receptor is encoded by a 2 kilobase mRNA. FEBS Lett 261:353–357.

Musgrave A, van den Ende H (1987) How *Chlamydomonas* court their partners. TIBS 12:469–473.

Nakamura T, Gold GH (1987) A cyclic nucleotide–gated conductance in olfactory receptor cilia. Nature 325:442–444.

Nakayama N, Miyajima A, Arai K (1985) Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J 4:2643–2648.

Nakoaka Y, Ooi H (1985) Regulation of ciliary reversal in Triton-extracted *Paramecium* by calcium and cyclic adenosine monophosphate. J Cell Sci 77:195–195.

Nasmith PE, Grinstein S (1987a) Phorbol ester-induced changes in cytoplasmic Ca^{2+} in human neutrophils. J Biol Chem 262:13558–13566.

Nasmith PE, Grinstein S (1987b) Are Ca^{2+} channels in neutrophils activated by a rise in cytosolic free Ca^{2+}? FEBS Lett 221:95–100.

Nasmyth K (1982) Molecular genetics of yeast mating types. Annu Rev Genet 16:439–450.

Nathan P, Hahnenberger A, Newton A, Shapiro L (1986) Differential localization of membrane receptor chemotaxis protein in the *Caulobacter* predivisional cell. J Mol Biol 191:433–440.

Neer EJ, Clapham DE (1988) Roles of G protein subunits in transmembrane signalling. Nature 333:129–139.

Nelson DJ, Wright EM (1974) The distribution, activity, and function of the cilia in the frog brain. J Physiol [Lond] 243:63–78.

Newell PC (1986) The role of actin polymerization in amoebal chemotaxis. BioEssays 5:208–211.

Newell PC, Europe-Finner GN, Small NV, Liu G (1988) Inositol phosphates, G-proteins, and *ras* genes involved in chemotactic signal transduction in *Distyostelium*. J Cell Sci 89:123–127.

Niedel JE, Cuatrecasas P (1980a) Covalent affinity labeling of formyl peptide chemotactic receptors. J Biol Chem 255:7063–7066.

Niedel JE, Cuatrecasas P (1980b) Formyl peptide chemotactic reception of leukocytes and macrophages. Curr Top Cell Regul 17:137–170.

Nishihara J, McPhail LC, O'Flaherty JT (1986) Stimulus-dependent mobilization of protein kinase C. Biochem Biophys Res Commun 134:587–594.

Nobili R, Luporini P, Esposito F (1987) Compatibility systems in ciliates. In Greenberk AH (ed): Invertebrate Models: Cell Receptors and Cell Communication. Basel: Karger, pp 1–28.

Novoselov VI, Krapivinskaya LD, Fesenko EE (1988) Amino acid binding glycoproteins from the olfactory epithelium of skate (*Dasyatis partinaca*). Chem Senses 13:267–279.

Oakley B, Lawton A, Wong L (1990) Rabbit gustatory epithelium expresses keratin 6-like immunoreactivity. Soc Neurosci Abstr 16:25.

O'Connell RJ (1986) Responses to pheromone blends in insect olfactory receptor neurons. J Comp Physiol 156:747–761.

O'Connell RJ, Costanzo RM, Hildebrandt JD (1990) Adenylyl cyclase activation and electrophysiological responses elicited in male hamster olfactory receptor neurons by components of female pheromones. Chem Senses 15:725–739.

Oosawa K, Hess J, Simon M (1988) Mutants defective in bacterial chemotaxis show modified protein phosphorylation. Cell 53:89–96.

Ortenzi C, Miceli C, Bradshaw RA, Luporini P (1990) Identification and initial characterization of an autocrine pheromone receptor in the protozoan ciliate *Euplotes raikovi*. J Cell Biol 111:607–614.

Pace U, Lancet D (1986) Olfactory GTP-binding protein: Signal-transducing polypeptide of vertebrate chemosensory neurons. Proc Natl Acad Sci USA 83:4947–4951.

Painter RG, Sklar LA, Jesaitis AJ, Schmitt M, Cochrane CG (1984) Activation of neutrophils by *N*-formyl chemotactic peptides. Fed Proc 43:2737–2742.

Painter RG, Zahler-Bentz K, Dukes RE (1987): Regulation of the affinity state of the *N*-formylated peptide receptor of neutrophils: Role of guanine nucleotide–binding proteins and the cytoskeleton. J Cell Biol 105:2959–2971.

Parkinson JA (1988) Protein phosphorylation in bacterial chemotaxis. Cell 53:1–2.

Parsons JT (1990) Closing the GAP in signal transduction pathway. Trends Genet 6:169–171.

Pasquale SM, Goodenough U (1987) Cyclic AMP functions as a primary sexual signal in gametes of *Chlamydomonas reinhardtii*. J Cell Biol 105:2279–2292.

Paul A, Marala RB, Jaiswal RK, Sharma RK (1987) Coexistence of guanylate cyclase and atrial natriuretic factor receptor in a 180-kD protein. Science 235:1224–1226.

Pelosi P, Baldaccini E, Pisanelli AM (1982) Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. Biochem J 201:245–248.

Pelosi P, Dal Monte M (1990) Purification and characterization of odorant binding proteins from nasal mucosa of pig and rabbit. Chem Senses 15:624.

Persaud KC, Chiavacci L, Pelosi P (1988) Binding proteins for sweet compounds from gustatory papillae of the cow, pig, and rat. Biochim Biophys Acta 967:65–75.

Persaud KC, DeSimone JA, Getchell ML, Heck GL, Getchell TV (1987) Ion transport across the frog olfactory mucosa: The basal and odorant-stimulated states. Biochim Biophys Acta 902:65–79.

Petersen OH (1989) Does inositol tetrakisphosphate play a role in the receptor-mediated control of calcium mobilization? Cell Calcium 10:375–383.

Pevsner J, Sklar PA, Snyder SH (1986) Odorant-binding protein: Localization to nasal glands and secretions. Proc Natl Acad Sci USA 83: 4942–4946.

Pevsner J, Trifiletti RR, Strittmatter SM, Snyder SH (1985) Isolation and characterization of an olfactory receptor protein for odorant pyrazines. Proc Natl Acad Sci USA 82:3050–3054.

Pike MC, DeMeester CA (1988) Inhibition of phosphoinositide metabolism in human polymorphonuclear leukocytes by *S*-adenosylhomocysteins. J Biol Chem 263:3592–3599.

Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH, Belardetti F (1987) Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cell. Nature 328:38–43.

Polakis PG, Uhing RJ, Snyderman R (1988) The formylpeptide chemoattractant receptor copurifies with a GTP-binding protein containing a distinct 40-kDa pertussis toxin substrate. J Biol Chem 263:4969–4976.

Postma PW, Lengeler JW (1985) Phosphoenolpyruvate: Carboxyhydrate phosphotransferase system of bacteria. Microbiol Rev 49:232–269.

Preston RR, Usherwood PNR (1988) L-glutamate-induced membrane hyperpolarization and behavorial responses in *Paramecium tetraurelia*. J Comp Physiol 164:75–82.

Preston RR, Van Houten JL (1987a) Localization of the chemoreceptive properties of the surface membrane of *Paramecium tetraurelia*. J Comp Physiol [A] 160:537–541.

Preston RR, Van Houten JL (1987b) Chemoreception in *Paramecium tetraurelia:* acetate- and folate-induced membrane hyperpolarization. J Comp Physiol [A] 160:525–535.

Price S (1987) Effects of odorant mixtures on olfactory receptor cells. In Roper S, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 55–60.

Price S, Willey A (1987) Benzaldehyde binding protein from dog olfactory epithelium. In Roper S, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 561–564.

Price S (1981) Receptor proteins in vertebrate olfaction. In Cagan RH, Kare MR (eds): Biochemistry of Taste and Olfaction. New York: Academic Press, pp 69–84.

Price S, Willey A (1988) Effects of antibodies against odorant binding proteins on electrophysiological responses to odorants. Biochim Biophys Acta 965:127–129.

Pun R, Gesteland R (1990) Whole cell patch recordings show frog and salamander olfactory neurons are different. Chem Senses 15:628.

Putney JW (1987) Formation and actions of calcium-mobilizing messenger inositol 1,4,5-trisphosphate. Am J Physiol 252:G149–G157.

Rasmussen H (1981) Calcium and Cyclic AMP as Synarchic Messengers. New York: John Wiley & Sons.

Ravid S, Matsumura P, Eisenbach M (1986) Restoration of flagellar clockwise rotation in bacterial envelopes by insertion of the chemotaxis protein CheY. Proc Natl Acad Sci USA 83:7157–7161.

Reibmann J, Korchak HM, Vosshall LB, Haines KA, Rich AM, Weissmann G (1988) Changes in diacylglycerol labeling, cell shape, and protein phosphorylation distinguish "triggering" from "activation" of human neutrophils. J Biol Chem 263:6322–6328.

Restrepo D, Miyamoto T, Bryant BP, Teeter JH (1990) Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. Science 249:1166–1168.

Rhein LD, Cagan RH (1981) Role of cilia in olfactory recognition. In Cagan RH, Kare MR (eds): Biochemistry of Taste and Olfaction. New York: Academic Press, pp 47–68.

Robbins SM, Williams JG, Jermyn KA, Spiegelman GB, Weeks G (1989) Growing and developing *Dictyostelium* cells express different *ras* genes. Proc Natl Acad Sci USA 86:938–942.

Rollins TE, Springer MS (1985) Identification of the polymorphonuclear leukocyte C5a receptor. J Biol Chem 260:7157–7160.

Ronnett GV, Hester LD, Snyder SH (1990) The effect of odorants of adenylate cycalse in primary cultures of rat olfactory neurons. Soc Neurosci Abstr 16:26.

Ronson CW, Nixon BT, Ausubel FM (1987) Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell 49:579–581.

Roos FJ, Zimmermann A, Keller HU (1987) Effect of phorbol myristate acetate and the chemotactic peptide fNLPNTL on shape and movement of human neutrophils. J Cell Sci 88:399–406.

Roper SD (1989) The cell biology of vertebrate taste receptors. Annu Rev Neurosci 12:329–353.

Ross FM, Newell PC (1981) Streamers: Chemotactic mutants of *Dictyostelium discoideum* with altered cyclic GMP metabolism. J Gen Microb 127:339–350.

Russo AF, Koshland DE (1983) Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. Science 220:1016–1020.

Sarndahl E, Lindroth M, Bengsteeon T, Fallman M, Gustavsson J, Stendahl O, Andersson T (1989) Association of ligand-receptor complexes with actin filaments in human neutrophils: A possible regulatory role for the G-protein. J Cell Biol 109:2791–2799.

Sasner JM, Van Houten JL (1989) Evidence for a folate chemoreceptor in *Paramecium*. Chem Senses 14:587–595.

Satir P (1985) Switching mechanisms in the control of ciliary motility. In Satir B (ed): Modern Cell Biology, Vol 4. New York: Alan R. Liss, pp 1–46.

Sato M (1987) Taste receptor proteins. Chem Senses 12:277–284.

Sato T, Okada Y, Miyamoto T (1987) Ionic mechanism of generation of receptor potential in frog taste cells. In Roper S, Atema J (eds): Olfaction and Taste IX. New York: New York Academy of Sciences, pp 23–26.

Sawyer DW, Sullivan JA, Mandeli GL (1985) Intracellular free calcium localization in neutrophils during phagocytosis. Science 230:663–666.

Saxe CL, Johnson RL, Devreotes PN, Kimmell AR (1991) Expression of a cAMP receptor gene of *Dictyostelium* and evidence for a multigene family. Genes Dev 5:129–135.

Saxe CL, Klein P, Sun TJ, Kimmel AR, Devreotes PN (1988) Structure and expression of the cAMP cell-surface receptor. Dev Genet 9:227–235.

Schiffmann S (1990) The role of sodium and potassium transport pathways in taste transduction: An overview. Chem Senses 15:366–329.

Schmale H, Holfgreve-Grez H, Heidje Christiansen J (1990) Possible role for salivary gland protein in taste reception indicated by homology to lipophilic-ligand carrier proteins. Nature 343:366–369.

Schmied el-Jakob I, Anderson PAV, Ache BW (1989) Whole cell recording from lobster olfactory receptor cells: Responses to current and odor stimulation. J Neurophys 61:994–1000.

Schmitt BC, Ache BW (1979) Olfaction: Response enhancement by flicking in a decapod crustacean. Sci 205:204–206.

Schultz JE, Grunemund R, von Hirschhausen R, Schönefeld U (1984) Ionic regulation of cyclic AMP levels in *paramecium tetraurelia* in vivo. FEBS Lett 167:113–116.

Schulz I, Thevenod F, Dehlinger-Kremer M (1989) Modulation of intracellular free Ca^{2+} concentration by IP_3-sensitive and IP_3-insensitive nonmitochondrial Ca^{2+} pools. Cell Calcium 10:325–336.

Schulz S, Chinkers M, Garbers DL (1989) The guanylate cyclase/receptor family of proteins. FASEB J 3:2026–2035.

Schulz S, Denaro M, Van Houten J (1984) Relationship of folate binding and uptake to chemoreception in *Paramecium*. J Comp Physiol [A] 155:113–119.

Schulz S, Green C, Yuen P, Garbers DL (1990) Guanylate cyclase is a heat-stable enterotoxin receptor. Cell 63:941–948.

Scott TR, Giza BK (1990) Coding channels in the taste system of the rat. Science 249:1585–1587.

Segall JE, Bominaar AA, Wallraff E, De Wit RJ (1988) Analysis of a *Dictyostelium* chemotaxis mutant with altered chemoattractant binding. J Cell Sci 91:479–489.

Segall JE, Fisher PR, Gerisch G (1987) Selection of chemotaxis mutants of *Dictyostelium discoideum*. J Cell Biol 104:151–161.

Segall JE, Gerisch G (1989) Genetic approaches to cytoskeleton function and the control of cell motility. Curr Opinion Cell Biol 1:44–50.

Seligmann BE, Fletcher MP, Gallin JI (1982) Adaptation of human neutrophil responsiveness to the chemoattractant *N*-formylmethionylleucylphenylalanine. J Biol Chem 257:6280–6286.

Sha'afi RI, Molski RFP (1987) Signalling for increased cytoskeletal actin in neutrophils. Biochem Biophys Res Commun 145:934–941.

Sha'afi RI, Molski RFP (1988) Activation of the neutrophil. Prog Allergy 42:1–64.

Shimomura H, Dangott LJ, Garbers DL (1986) Covalent coupling of a resact analogue to guanylate cyclase. J Biol Chem 261:15778–15782.

Shioi J, Dang CV, Taylor BL (1987) Oxygen as attractant and repellent in bacterial chemotaxis. J Bacteriol 169:3118–3123.

Shioi J, Taylor BL (1984) Oxygen taxis and proton motive force in *Salmonella typhimurium*. J Biol Chem 259:10983–10988.

Shirley SG, Robinson CJ, Dickinson K, Aujla R, Dodd GH (1986) Olfactory adenylate cyclase of the rat: Stimulation by odorants and inhibition by Ca. Biochem J 240:605–607.

Sibley DR, Benovic JL, Caron MG, Lefkowitz RJ (1987) Regulation of transmembrane signaling by receptor phosphorylation. Cell 48:913–922.

Sibley DR, Lefkowitz RJ (1985) Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317:124–129.

Sicard G, Holley A (1984) Receptor cell responses to odorants: Similarities and differences among odorants. Brain Res 292:283–296.

Silver WL (1987) The common chemical sense. In Finger TE, Silver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 65–88.

Sklar LA, Bokoch GM, Button D, Smolen JE (1987) Regulation of ligand-receptor dynamics by guanine nucleotides. J Biol Chem 262:135–139.

Sklar LA, Finney DA, Oades ZG, Jesaitis AJ, Painter RG, Cochrane CG (1984a) The dynamics of ligand-receptor interactions. J Biol Chem 259:5561–5569.

Sklar L, Jesaitis AJ, Painter RG (1984b) The neutrophil *N*-formyl peptide receptor: Dynamics of ligand–receptor interactions and their relationship to cellular responses. In Snyderman R (ed): Contemporary Topics in Immunobiology, Vol 14. New York: Plenum Press, pp 29–82.

Sklar LA, Mueller H, Omann G, Oades Z (1989) Three states for the formyl peptide receptor on intact cells. J Biol Chem 264:8483–8486.

Sklar PB, Anholt RH, Snyder SH (1986) The odorant-sensitive adenylate cyclase of olfactory receptor cells. J Biol Chem 261:15538–15543.

Small NV, Europe-Finner GN, Newell PC (1987) Adaptation to chemotactic cyclic AMP signals in *Dictyostelium* involves the G-protein. J Cell Sci 88:537–545.

Smith R, Preston RR, Schulz S, Van Houten J (1987) Correlation of cyclic adenosine monophosphate binding and chemoresponse in *Paramecium*. Biochim Biophys Acta 928:171–178.

Smollen JE, Korchak HM, Weissman G (1980) Increased levels of cyclic AMP in human polymorphonuclear leukocytes surface stimulation. J Clin Invest 65:1077–1085.

Snaar-Jagalski BE, DeWit RJW, Van Haastert PJM (1988) Pertussis toxin inhibits cAMP surface receptor-stimulated binding of [^{35}S]GTPgS to *Dictyostelium discoideum* membranes. FEBS Lett 232:148–152.

Snaar-Jaglaska BE, Van Haastert PJM (1988) *Dictyostelium discoideum* mutant *synag 7* with altered G-protein–adenylate cyclase interaction. J Cell Sci 91:287–294.

Snyder SH, Sklar PB, Pevsner J (1988) Molecular mechanisms of olfaction. J Biol Chem 263:13871–13974.

Snyderman R (1984) Regulatory mechanisms of a chemoattractant receptor on leukocytes. Fed Proc 43:2743–2748.

Snyderman R, Pike MC, Edge S, Lane B (1984) A chemoattractant receptor on macrophages exists in two affinity states regulated by guanine nucleotides. J Cell Biol 989:444–448.

Sockett RE, Armitage JP, Evans MCW (1987) Methylation-independent and methylation-dependent chemotaxis in *Rhodobacter sphaeroides* and *Rhodospirillum rubrum*. J Bacteriol 169: 5808–5814.

Stengl M, Zufall F, Hatt H, Dudel J, Hildebrand JG (1989) Patch clamp analysis of male *Manduca sexta* olfactory receptor neurons in primary cell culture. Soc Neurosci Abstr 15:365.

Stengl M, Zufall F, Hatt H, Hildebrand JG (1990) Olfactory receptor neurons from developing male *Manduca sexta* antennae respond to species-specific sex pheromone in vitro. Chem Senses 15:643–644.

Stevens C (1987) Channel families in the brain. Nature 328:198–199.

Stock A, Chen T, Welsh D, Stock J (1989) CheA protein, a central regulator of bacterial chemotaxis, belongs to a family of proteins that control gene expression in response to changing environmental conditions. Proc Natl Acad Sci USA 85:1403–1407.

Stock J (1987) Mechanisms of receptor function and the molecular biology of information processing in bacteria. BioEssays 6:199–203.

Stock J, Stock A (1987) What is the role of receptor methylation in bacterial chemotaxis? TIBS 12:371–374.

Stone DE, Reed SI (1990) G-protein mutations that alter the pheromone response in *Saccharomyces cerivisiae*. Mol Cell Biol 10:4439–4446.

Striem B, Pace U, Zehavi U, Naim M, Lancet D (1989) Sweet tastants stimulate adenylate cyclase coupled to GTP-binding proteins in rat tongue. Biochem J 260:121–126.

Stryer L, Bourne HR (1986) G proteins: A family of signal transducers. Annu Rev Cell Biol 2:391–419.

Sugimoto K, Teeter JH (1990) Voltage-dependent ionic currents in taste receptor cells of the larval tiger salamander. J Gen Physiol 96:809–834.

Sullivan SJ, Zigmond SH (1982) Asymmetric receptor distribution on PMNS. J Cell Biol 95:418a.

Suzuki K, Hazama M, Ota H, Oguro K, Uchiyama H, Fujikura T (1990) Phosphorylation of 64K-Da protein, enzyme release, and increase in intracellular cAMP level in human polymorphonuclear leukocytes induced by fmet-leu-phe stimulation. J Clin Bioch Nut 8:21–32.

Takenawa T, Ishitoya J, Nagai Y (1986) Inhibitory effect of PG EZ, forskolin and dibutyryl cAMP on arachidonic acid release and inositol phospholipid metabolism in guinea pig neutrophils. J Biol Chem 261:1092–1098.

Tao YP, Klein C (1990a) Properties of CAR-kinase—The enzyme that phosphorylates the cAMP chemotactic receptor of *D. discoideum*. J Prot Chem 9:565–572.

Tao YP, Klein C (1990b) Localization of functional domains of the cAMP chemotactic receptor of *Dictyostelium discoideum*. J Biol Chem 265:15584–15589.

Tash JS, Hidaka H, Means AR (1986) Axokinin phosphorylation by cAMP-dependent protein kinase is sufficient for activation of sperm flagellar motility. J Cell Biol 103:649–655.

Taylor BL, Johnson MS, Smith JM (1988) Signaling pathways in bacterial chemotaxis. Botan Acta 101:101–104.

Taylor R (1991) Whiff and poof: Adenylyl cyclase in olfaction. J NIH Research 3:49–53.

Teeter JH, Gold GH (1988) A taste of things to come. Nature 331:298–299.

Teeter JH, Brand JG (1987a) L-arginine–activated cation channels from the catfish taste epithelium. Soc Neurosci Abstr 13:361.

Teeter JH, Brand JG (1987b) Peripheral mechanisms of gustation: Physiology and biochemistry. In Finger TE, Silver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 299–329.

Teeter JH, Brand JG, Kalinoski DL, Bryant BP (1988) Cation channels in reconstituted catfish taste epithelial membrane preparations activated by arginine in an enantiomerically specific manner. Chem Senses 13:740.

Teeter JH, Brand JG, Kumazawa T (1990) A stimulus-activated conductance in isolated taste epithelial membranes. Biophys J 58:253–259.

Teeter J, Funakoshi M, Kurihara K, Roper S, Sato T, Tonosaki K (1987) Generation of the taste cell potential. Chem Senses 12:217–234.

Teeter JH, Sugimoto K, Brand JG (1989) Ionic currents in taste cells and reconstituted taste epithelial membranes. In Brnad JG, Teeter JH, Cagan RH, Kare MR (eds): Chemical Senses, Vol 1. New York: Marcel Dekker, pp 151–170.

Thiebert A, Klein P, Devreotes PN (1984) Specific photoaffinity labeling of the cAMP surface receptor in *Dictyostelium discoideum*. J Biol Chem 259:12318–12321.

Thoelke MS, Bedale WA, Nettleton DO, Ordal GW (1987) Evidence for an intermediate methyl-acceptor for chemotaxis in *Bacillus subtilis*. J Biol Chem 262:2811–2816.

Thoelke MS, Casper JM, Ordal GW (1990) Methyl transfer in chemotaxis toward sugars by *Bacillus subtilis*. J Bacteriol 172:1148–1150.

Thomas KM, Pyun HY, Navarro J (1990) Molecular cloning of the fmet-leu-phe receptor from neutrophils. J Biol Chem 265:20061–20064.

Thorner J (1981) Pheromonal regulation of development in *Saccharomyces cerevisiae*. In Strathern JN, Jones EW, Broach JR (eds): Molecular Biology of the Yeast *Saccharomyces*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 143–180.

Thorpe DS, Garbers DL (1989) The membrane form of guanylate cyclase. J Biol Chem 264:6545–6549.

Tonosaki K, Funakoshi M (1984) Intracellular taste cell responses of mouse. Comp Bioch Physiol 78A:651–656.

Trapido-Rosenthal HG, Carr WES, Gleeson RA (1987) Biochemistry of an olfactory purinergic system: Dephosphorylation of excitatory nucleotides and uptake of adenosine. J Neurochem 49:1174–1182.

Trapido-Rosenthal HG, Carr WES, Gleeson RA (1989) Biochemistry of purinergic olfaction. In Brand JG, Teeter J, Cagan RH, Kure MR (eds): Chemical Senses, Vol. 1. New York: Marcel Dekker, pp 243–261.

Trapido-Rosenthal HG, Carr WES, Gleeson RA (1990) Ectonucleotidase activities associated with the olfactory organ of the spiny lobster. J Neurochem 54:1–9.

Trimmer JS, Vacquier VD (1986) Activation of sea urchin gametes. Annu Rev Cell Biol 2:1–26.

Truett AP, Verghese MW, Dillon SB, Snyderman R (1988) Calcium influx stimulates a second pathway for sustained diacylglycerol production in leukocytes activated by chemoattractants. Proc Natl Acad Sci USA 85:1549–1553.

van den Ende H, Musgrave A, Klis FM (1990) The role of flagella in the sexual reproduction of *Chlamydomonas* gametes. In Bloodgood R (ed): Ciliary and Flagellar Membranes. New York: Plenum Press, pp 129–148.

Vandenberg MJ, Ziegelberger G (1991) On the function of the pheromone binding protein in the olfactory hairs of *Antheraea polyphemus*. J Insect Physiol 37:79–85.

Van Duijn B, Vogelzang SA, Ypey DL, Van Der Molen LB, Van Haastert PJM (1990) Normal chemotaxis in *Dictyostelium discoideum* cells with a depolarized plasma membrane potential. J Cell Sci 95:177–183.

Van Epps DE, Bender JG, Simpson SJ, Chenoweth DE (1990) Relationship of chemotactic receptors for formyl peptide and C5a, C1, CR3 and Fc receptors on human neutrophils. J Leukocyte Biol 47:519–527.

Van Haastert PJM, De Wit R (1984) Demonstration of receptor heterogeneity and negative cooperativity by nonequilibrium binding experiments. J Biol Chem 259:13321–13328.

Van Haastert PJM, Kesbeke F, Reymond CD, Firtel RD, Luderus E, Van Driel R (1987a) Aberrant transmembrane signal transduction in *Dictyostelium* cells expressing a mutated *ras* gene. Proc Natl Acad Sci USA 84:4905–4909.

Van Haastert PJM, Snaar-Jagalska BW, Janssens PMW (1987b) The regulation of adenylate cyclase by guanine nucleotides in *Dictyostelium membranes*. Eur J Biochem 162:251–258.

Van Haastert PJM, Van Lookeven, Campagne MM, Ross FM (1982) Altered cGMP-phosphodiesterase activity in chemotactic mutants of *Dictyostelium discoideum*. FEBS Lett 147:147–152.

Van Houten J (1978) Two mechanisms of chemotaxis in *Paramecium*. J Comp Physiol [A] 127:167–174.

Van Houten J (1979) Membrane potential changes during chemokinesis in *Paramecium*. Science 249:167–174.

Van Houten J (1990) Chemosensory transduction in *Paramecium*. In Armitage J, Lackie J (eds): Motility and Taxis. Cambridge: Cambridge University Press, pp 297–322.

Van Houten J, Cote B, Zhang J, Baez J, Gagnon ML (1990) Studies of the cyclic adenosine monophosphate chemoreceptor of *Paramecium*. J Membr Biol 119:15–24.

Van Houten J, Hauser DCR, Levandowsky M (1981) Chemosensory behavior in protozoa. In Hutner SH, Levandowsky ML (eds): Biochemistry and Physiology of Protozoa, Vol 4. New York: Academic Press, pp 67–124.

Van Houten J, Preston RR (1987) Chemoreception in single-celled organisms. In Finger TE, Silver WL (eds): Neurobiology of Taste and Smell. New York: John Wiley & Sons, pp 11–38.

Van Houten J, Preston RR (1988) Chemokinesis. In Görtz H-D (ed): Paramecium. New York: Springer-Verlag, pp 282–300.

Van Houten J, Van Houten JC (1982) Computer analysis of *Paramecium* chemokinesis. J Theor Biol 98:453–468.

Van Ments-Cohen M, Van Haastert PJM (1989) The cyclic nucleotide specificity of eight cAMP-binding proteins in *Dictyostelium discoideum* is correlated into three groups. J Biol Chem 264:8717–8722.

Verghese MW, Smith CD, Snyderman R (1985) Potential role for a guanine nucleotide regulatory protein in chemoattractant receptor mediated polyphosphomosetide metabolism, Ca^{2+} mobilization and cellular response by leukocytes. Biochem Biophys Res Commun 127:450–457.

Vogt RG (1987) The molecular basis of pheromone reception: Its influence on behavior. In Prestwich GD, Blomquist GS (eds): Pheromone Biochemistry. New York: Academic Press, pp 385–431.

Vogt RG, Kohne AC, Dubnau JT, Prestwich GD (1989) Expression of pheromone binding proteins during antennal development in the gypsy moth *Lymantria dispar*. J Neurosci 9:3322–3346.

Vogt RG, Prestwich GD, Lerner MR (1990a) Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. J Neurobiol 22:74–84.

Vogt RG, Prestwich GD, Riddiford LM (1988) Sex pheromone receptor proteins. J Biol Chem 263:3952–3959.

Vogt RG, Riddiford LM, Prestwich GD (1985) Kinetic properties of a pheromone degrading enzyme: The sensillar esterase of *Antheracea polyphemus*. Proc Natl Acad Sci USA 82:8827–8831.

Vogt RG, Rybczynski R, Lerner MR (1990b) The biochemistry of odorant reception and transduction. In Schild D (ed): Chemosensory Information Processing. Berlin: Springer-Verlag, pp 33–76.

Volpi M, Molski TFP, Naccache PH, Feinstein MB, Sha'afi RI (1985) Phorbol 12-myristate, 13 acetate potentiates the action of the calcium ionophore in stimulating arachidonic acid release and production of phosphatidic acid in rabbit neutrophils. Biochem Biophys Res Commun 128:594–600.

Webster RO, Wysolmerski RB, Lagunoff D (1986) Enhancement of human polymorphonuclear leukocyte adherence to plastic and endothelium by phorbol myristate acetate. Am J Pathol 125:369–378.

Weinstock RS, Wright HN, Spiegel AM, Levine MA, Moses AM (1986) Olfactory dysfunction in humans with deficient guanine nucleotide–binding protein. Nature 322:635–636.

Weiss RM, Chasalow S, Koshland DE (1990) The role of methylation in chemotaxis. J Biol Chem 265:6817–6826.

Wessels D, Soll DR, Knecht D, Loomis WF, DeLozanne A, Spudich J (1988) Cell motility and chemotaxis in *Dictyostelium* amoebae lacking myosin heavy chain. Dev Biol 128:164–177.

White JR, Huang C-K, Hill JM, Naccache PH, Becker EL, Sha'afi RI (1984) Effect of phorbol 12-myristate 13-acetate and its analogue 4α-phorbol 12,13-didecanoate on protein phosphorylation and lysosomal enzyme release in rabbit neutrophils. J Biol Chem 259:8605–8611.

Wright TM, Hoffman RD, Nishijima J, Jakoi L, Snyderman R, Shin HS (1988) Leukocyte chemoattraction by 1,2-diacylglycerol. Proc Natl Acad Sci USA 85:1869–1873.

Wright MV, Van Houten J (1988) Effects of lithium on chemoresponse and ion regulation in *Paramecium*. Chem Senses 13:748.

Wright MV, Van Houten J (1990) Characterization of putative transporting Ca^{2+}-ATPase from pellicles of *Paramecium tetraurelia*. Biochim Biophys Acta 1029:241–251.

Wurster B, Schubigerk, Wick U, Gerisch G (1977) Cyclic GMP in *Dictyostelium discoideum*: Oscillations and pulses in response to folic acid and cyclic AMP signals. FEBS Lett 76:141–144.

Wymann MP, VonTscharner V, Demleau DA, Baggiolini M (1987) The onset of the respiratory burst in human neutrophils. J Biol Chem 262:12048–12053.

Yin HL (1987) Gelsolin: Calcium- and polyphosphoinositide-regulated actin-modulating protein. BioEssays 7:176–179.

Yuli I, Oplatka A (1987) Cytosolic acidification as an early transductory signal of human neutrophil chemotaxis. Science 235:340–342.

Yuli I, Tomonaga A, Snyderman R (1982) Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. Proc Natl Acad Sci USA 79:5906–5910.

Zigmond SH (1989) Chemotactic response of neutrophils. Am J Respir Cell Mol Biol 1:451–453.

Zigmond SH, Sullivan SJ (1979) Sensory adaptation of leukocytes to chemotactic peptides. J Cell Biol 82:517–527.

Zuberi AR, Bischoff DS, Ordal GW (1991) Nucleotide sequence and characterization of a *Bacillus subtilis* gene encoding a flagellar switch protein. J Bact 173:710–719.

Zufall F, Stengl M, Franke C, Hildebrand JG, Hatt H (1991) Ionic currents of cultured olfactory receptor neurons from antennae of male *Manduca sexta*. J Neurosci 11:956–965.