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CHEMOSENSORY TRANSDUCTION IN EUKARYOTIC MICROORGANISMS

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INTRODUCTION

Signal transduction is a topic that pervades the current literature, and well it should, since it encompasses studies from mitogenesis to synaptic transmission (1, 19, 124). Recent years have seen pieces of many cell and physiological puzzles fall into place, and the pieces repeatedly are cell surface receptors (30, 130), G proteins (12, 13, 49, 63, 82), second messengers (3, 6, 7, 78b, 90), and protein kinases and phosphatases (33, 43, 58, 65, 72, 97). The excitement of the plot unraveling is being felt in the studies of the chemical senses, in which the primary signals, i.e. ligands for receptors, are chemicals from the outside environment, and the end response can be the sensations of taste or smell in metazoa or the motile or mating responses of unicellular organisms. Here too, at the receptor cell level, the puzzle parts of receptors and signal transduction are being teased out of cells' proteins and genes (16, 24, 67, 78, 105, 141, 147).

One chapter in this volume (J. Armitage) describes the intricate detail into which the bacterial chemoresponse system can be dissected (see also 14). Other chapters concentrate on vertebrate taste and insect olfaction. This chapter focuses on chemosensory transduction in eukaryotic microorganisms. These organisms have proven to be useful in making steady progress in the field of chemical sensing, while technical advances are only now allowing the

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studies of metazoan receptor cells to catch up. It is from these eukaryotic microorganisms that we have caught our first glimpses of chemoreceptors and found them to be similar to internal vertebrate receptors, and yet not predictable in their structure and coupling. Therefore, these microorganisms continue to offer genetically, biochemically, and physiologically tractable cells for the dissection of the complex and fascinating chemosensory transduction pathways (20, 66, 139, 141, 144, 145).

The four examples that are discussed in this chapter are *Dictyostelium,* sea urchin spermatozoan, yeast, and *Paramecium*. These systems are perhaps the most extensively studied (66) and, while there are other extremely interesting chemosensory systems among unicellular eukaryotes (see 141, 145), these four present both common themes and important contrasts. After a description of each system, the receptors, coupling mechanisms, and second messengers of their signal transduction pathways will be contrasted with each other and put into context of metazoan olfaction and taste systems. Whenever possible, review articles will be used for citations.

FOUR EXAMPLES

Dictyostelium

At best one can give a snapshot of the *Dictyostelium* system because discoveries are coming rapidly, particularly with the development of molecular genetic techniques that circumvent the need to produce and analyze Mendelian mutants. While bacteria are plentiful, *Dictyostelium* amoebae graze on this food source and home in on the bacteria by detecting and migrating toward the folic acid that the bacteria produce. This is a receptor-mediated process (136, 145), but one that is not nearly as well studied and understood as the cyclic AMP (cAMP) chemotaxis response that supplants the folate response when the cells begin to starve (25, 26, 135, 66, 121, 96, 47). When the bacteria run out, the folate receptors decline and cAMP receptors are elaborated into the surface membrane. A focal cell will begin to pulse out cAMP and surrounding cells migrate toward this source, oriented by differential occupancy of the cAMP receptors on the cells. The wave of cAMP diffusing past the cell sets into motion not only the oriented locomotion, but also a secretion of cAMP to stimulate yet other outlying cells. As a consequence, the cells move at approximately 20 μm/min toward the focal cell in waves lasting for several min, but the cells soon stop responding and move randomly while the relay system adapts and stops secreting cAMP. The external phosphodiesterase clears the extracellular cAMP, and the receptor loses phosphate groups and becomes responsive to ligand again. The significance of the phosphodiesterase (PDE) is born out in the aggregation mutant Streamer *(stmF),* which has greatly reduced PDE activity (96), and the disruption of chemotaxis by increased activity of PDE (39). Thus the cells are not confounded by their

own relay secretion of cAMP and once again are ready to respond to the next wave of cAMP that lasts for approximately 3 min. At times spiral patterns of migrating cells are set up as they converge on the focal cell.

Once aggregated, approximately 10^5 cells form a multicellular slug that goes on to differentiate into nonviable stalk cells and viable spores. cAMP also plays an important role in gene activation for the developmental changes involved in these phenotypic changes (47, 99, 25, 26). The spores can then develop into new haploid amoebae when environmental conditions improve.

The *Dictyostelium* amoebae communicate using chemical cues, cAMP in this case, to stimulate and direct motility and to produce changes in gene expression that underly its development. A long history of careful studies of this phenomenon, beginning with the identification of the "acrasin" attractants (73), laid the groundwork for the relatively recent identification of the proteins and genes for the major transduction pathways' components that make it possible for external cAMP to exert all of these effects (121, 27, 135). A recent understanding of the relationship of these components in the sensory transduction pathway for production of cAMP and chemotaxis appears in Figure 1 and is discussed below.

The ligand cAMP binds to two different receptors (R_A and R_B) that can be

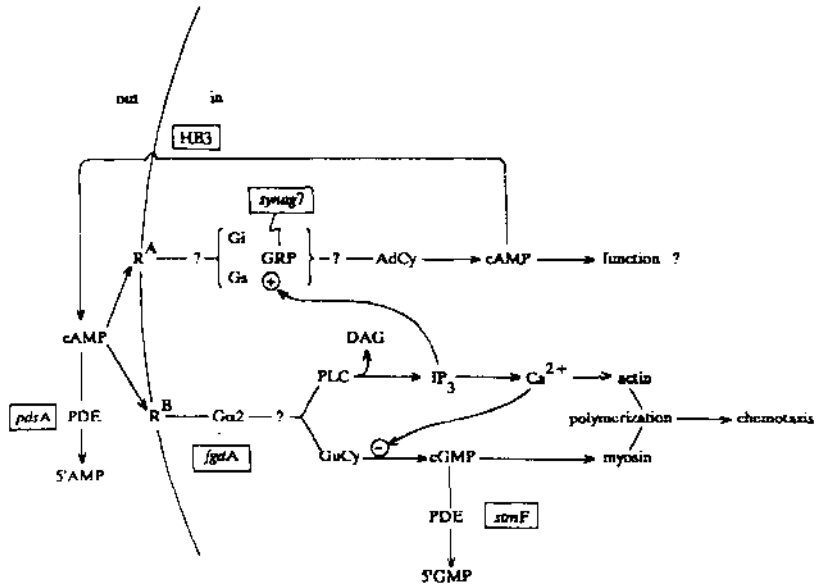

Figure 1 Model for sensory transduction in *Dictyostelium*. Boxes denote mutant with defects in the nearby step of the pathway. All abbreviations are as in the text except GRP, GTP reconstituting protein; GuCy, guanylyl cyclase; AdCy, adenylyl cyclase; DAG, diaclyglycerol. From 135 with permission.

distinguished by their binding kinetics (60) and now by their cloned genes (69, 114, 115, 135). Binding of cAMP to R_A sets into motion the activation of the adenylyl cyclase. The cAMP that is produced is secreted (by an undefined pathway) as the relay of stimulus from one cell to another. A G protein mediates the coupling of receptor to adenylyl cyclase, and a mutant, *synag7*, has a defect in a protein associated with, but probably not identical to, the G protein (see 135 for an overview). There appear to be both activating and inhibitory effects of G proteins on the adenylyl cyclase and differential sensitivity to pertussis toxin. The other receptors (R_B) couple by a different G protein to the enzymes of the chemotaxis pathway. There is a mutant, *fgd A*, that has a defect most likely in the structural gene for Gα2, and hence its receptor is uncoupled from the rest of the pathway (75). Yet another G protein must serve to couple the elusive folate receptor to its chemotaxis pathway (135).

The first G-activated enzyme downstream from the receptor in the chemotaxis pathway is phospholipase C (PLC), which initiates the production of I-1,4,5-P_3 and diacylglycerol from phosphoinositol lipids. IP_3, in turn, liberates Ca^{2+} from internal, non-mitochondrial stores (reviewed in 96). The concommitant production of diacylglycerol should activate protein kinase C, but its role, if any, is not clear. In *Dictyostelium*, diacylglycerol has no arachidonic acid and, therefore, this important second messenger does not appear to play a role in *Dictyostelium* chemotaxis (135).

The activation of the IP_3/Ca^{2+} cascade somehow acts to increase guanylyl cyclase activity. There is no consensus on whether the guanylyl cyclase is activated by increasing levels of Ca_i [as in *Paramecium* (71)] by IP_3, or by yet additional actions of G proteins (96, 98, 135). Nevertheless, cGMP levels do increase rapidly enough to carry on the rapid sensory transduction pathway. The cGMP and Ca^{2+} second messengers mediate the changes in myosin and actin that are necessary to cause the cytoskeletal changes underlying the oriented movement up the gradient of attractant (44, 52, 53, 79).

Recent advances in molecular genetics in general (polymerase chain reaction and antisense technology, for example) and in targeted gene replacement in *Dictyostelium* in particular (34, 122), have made it possible to study *Dictyostelium* sensory transduction pathways at the molecular level. Three representatives of a family of cAMP receptors have been cloned (reviewed in 135). The first was found in a screen of cDNAs in an expression vector (69, 115), and antisense oligonucleotides were used to verify the authenticity of the clone in transformed *Dictyostelium*. The cloned receptors have all the hallmarks of the rhodopsin family of receptors: putative seven transmembrane-spanning regions, small N terminus for a receptor binding a small ligand, and long C terminus for G protein interaction. The clone *car1* has 13 serines in the C terminus and is the candidate for the R_B chemotaxis

receptor that is desensitized by phosphorylation of up to 7 serine residues. Indeed, the phosphorylation of this receptor can be followed by shifts in mobility on SDS polyacrylamide gels, and these shifts exactly track with the cytoskeletal oscillations of cells in response to cAMP and the desensitization of the receptors (25, 26). The receptor kinase has recently been identified (131).

Genes for several G proteins, likewise, have been cloned (62, 75) and appear to have long stretches of sequences identical to mammalian α subunits of the heterotrimeric G proteins. Cloned Gα2 is a candidate for interaction with the R_B receptor. The most compelling evidence comes from the mutant *fgdA* (FrigidA) that has both defective chemotaxis and a mutation in the sequence for Gα2 (62).

Genes for smaller G proteins also have been identified in *Dictyostelium*. There are two *ras* genes, one expressed in vegetative growth, the other during development (110). The overexpression of mutant *ras* with a $gly^{12} \rightarrow thr$ alteration, comparable to activated mammalian oncogene *ras*, disrupts chemotaxis probably by activation of PLC or phosphoinositol kinase and inappropriate increases in IP_3 (36, 136). However, *ras* is not likely to have a normal role in chemotaxis or relay.

Much still remains unexplained in these complex *Dictyostelium* pathways. The second messenger activity can activate protein kinases whose substrates should include transcription factors as well as cytoskeletal elements (88, 99). The sorting out of these enzymes and substrates still leaves work to be done, as does the elucidation of the folate chemoreception pathway.

Sea Urchin Spermatozoa

Several seemingly unrelated lines of research on sea urchin egg peptides, artrial natriuretic peptides, and *E. coli* enterotoxins have recently converged with the identification of their receptors upon a new and apparently widespread transduction mechanism. In each of these cases, a cell surface guanylyl cyclase appears to serve as both receptor and effector to produce an intracellular second messenger, cGMP. Here the focus will be on the spermatozoan sensory transduction pathway (see 21, 46, 134 for reviews).

Species-specific peptides, resact from *Arbacia punculata* and speract from *Stronglyocentrotus purpuratus*, are released from eggs. They activate and cause motility changes in the spermatozoa (46). Whether the motility change is a true chemotactic or -kinetic response is still a matter of debate, but the important point is that intracellular levels of cGMP, cAMP, Ca^{2+}, and H^+ change, as does respiration (46, 134). The receptor that initiates these changes was cross-linked to its ligand and, using immunological and other techniques, was identified as a membrane guanylyl cyclase (see 21, 46 for overviews). The guanylyl cyclase had previously been noted to dramatically change in

apparent molecular weight upon peptide stimulation. The change subsequently was demonstrated to be the result of extensive dephosphorylation of the enzyme as part of the desensitization of the receptor (46, 134).

The genes for eight membrane guanylyl cyclases have since been cloned, two of which are for the sea urchin peptides resact and speract (126, 133). Others are for the atrial natriuretic peptide and enterotoxin (21, 119, 120). Characteristically there is one transmembrane-spanning region, an intracellular protein kinase-like domain that it missing from soluble guanylyl cyclases, and a carboxyl terminal cyclase catalytic region (Figure 2).

The demonstration that these cloned guanylyl cyclases code for the actual peptide receptors (as opposed to a physically, closely associated protein, for example) is most complete for the enterotoxin receptor gene from rat intestine. The expressed protein binds the peptide ligand and ligand stimulates the cyclase activity (119). The sea urchin cloned gene products fail to bind ligand and guanylyl cyclase activity has not yet been observed, but a protein of the appropriate size is expressed from the speract clone (21). The failure to observe enzyme activity and binding may be due to the technical problems of expecting function at 37°C in a heterologous system from a protein that normally functions at ≤ 20°C, or because the receptor possibly has an effector activity other than guanylyl cyclase in vivo (see below).

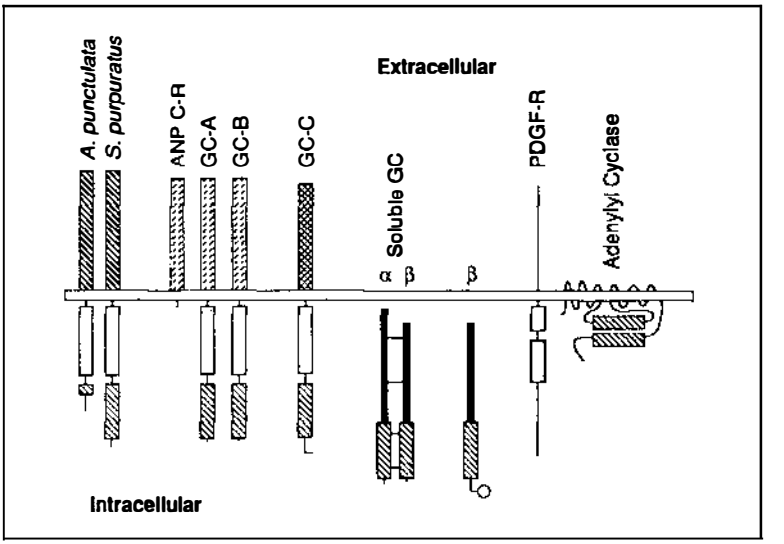

Figure 2 Schematic of structures of receptor guanylyl cyclases compared with soluble guanylyl cyclase, PDGF receptor, and adenylyl cyclase. Boxes with the same pattern denote homologous domains. The gray boxes correspond to protein kinase-like domains; crosshatched boxes in the cytoplasmic side denote cyclase domains. From 120 with permission.

Knowledge of the full sensory transduction pathway of sea urchin is far from complete. It is not certain that cGMP is an obligatory second messenger even though 8-bromo-cGMP can mimic many of the events of peptide stimulation, and an increase in internal cGMP is one of the first biochemically identified responses of spermatozoan to peptide. The doubt arises because of reports of motility changes at peptide levels that are too low to stimulate cGMP production (21, 123). Therefore, the cGMP may be low but compartmentalized, or the peptide receptors may signal by multiple second messenger pathways, perhaps involving the protein kinase domain.

The most important message evolving from the sea urchin and related systems is that guanylyl cyclase can serve as a cell surface receptor (21, 120). There may be many more examples of this growing family of receptors. The presence of a protein kinase-like domain that distinguishs them from other guanylyl cyclases hints at some interesting parallels with peptide growth hormone receptors (21, 72, 120), yet the dephosphorylation with desensitization seems to be a distinguishing feature of receptor guanylyl cyclases. Also intriguing is the modulation of atrial natriuretic peptide activity by nonhydrolyzable ATP analogues (21, 120), which suggests that the guanylyl cyclase receptor holds still more interesting aspects to be elucidated.

Yeast

The story of yeast mating pheromone becomes more exciting and more complicated with the telling. The mating pheromones arrest haploid cells in G1 of the cell cycle in preparation for fusion and production of a diploid cell. Therefore, some components of this sensory transduction process are necessarily integral in the cell cycle control story that is rapidly unfolding in yeast, and is branching out in logical connections to mitogenesis and oncogene activities in mammalian systems (87, 107) (See 9, 41, 63, 77, 141 for general reviews).

Alpha haploid cells produce α-pheromone, a 13-amino acid peptide that binds to α-receptors on **a** cells. **a** cells, in turn, secrete their 12-amino acid pheromone by a unusual transport mechanism (40) and the peptide binds to **a** receptors on α cells. The binding of ligand to receptor initiates a cascade of events leading to cell cycle arrest, gene transcription changes, cell wall alterations, morphological changes, and eventually fusion of the cells. Once a diploid is formed, the cell cycle arrest must be relieved in order for the organism to continue its vegetative growth. The mating signal transduction pathway is shown in Figure 3.

The receptors for both **a** and α factors were found to be coded for by *STE2* and *STE3* genes, and subsequently they have been cloned (18, 51, 94, 95). Judging from the amino acid hydropathy analyses, they are members of the rhodopsin superfamily with seven transmembrane-spanning segments (7-TMS) (55). The hydropathy plots of the two receptors are virtually

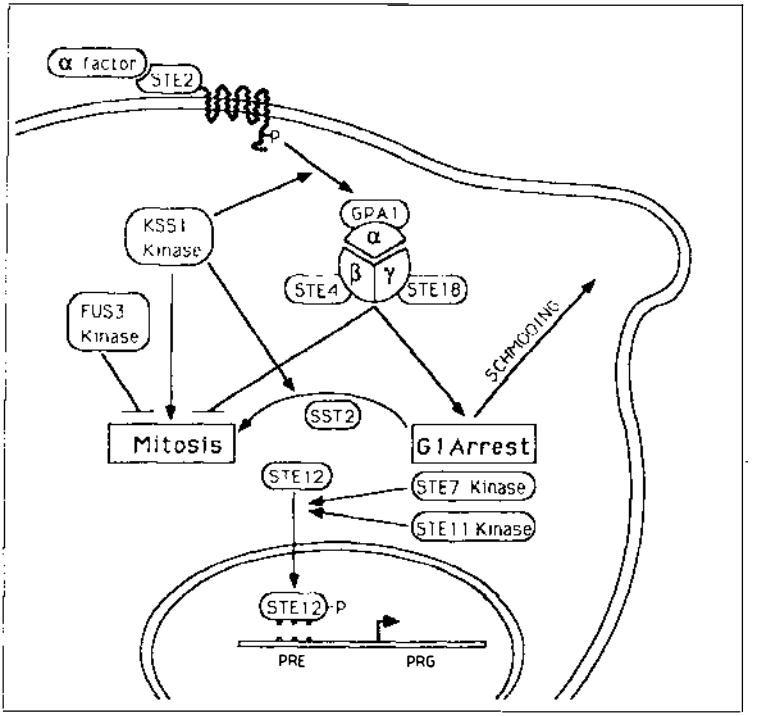

Figure 3 Model of the yeast α-factor signal transduction pathway. Most boxes denote protein components and the genes coding for them. From 57 with permission.

superimposable, yet the actual amino acid sequences bear no resemblance to one another. These genes are testimony to the need to press on with receptor identification because searches for receptors having strong homology with the cannonical sequences may fail to reveal the real breadth and variety in their superfamily.

The transmembrane-spanning segment V, VI, and cytoplasmic loop are involved in G protein binding, as in other 7-TMS receptors (30). TMS VII functions in species-specific ligand recognition as demonstrated through chimeric receptors constructed from receptors of *S. cerevisiae* and *S. kluyveri* (85). The cysteines considered important in the 7-TMS receptors can be genetically eliminated with no loss of function in yeast (30).

The pheromone-arrested yeast cell must eventually recover from the cell cycle block and continue vegetative growth, especially if mating has not been successful. A protease, the product of the *SST1* gene, degrades α-factor in one aspect of recovery (22, 84). As in mammalian long-term desensitization, α-factor receptor is internalized, but new receptors are elaborated into the

surface membrane (61); however, they do not function in pheromone signal transduction. The temporarily non-functional receptors, like their mammalian counterparts, are likely to be phosphorylated on serine and threonine residues within the C terminal domain and, thus uncoupled from G proteins. The C terminus is required not for signalling but for desensitization (74, 108). Another gene, *SST2*, codes for a product that must function in recovery, but it does not seem to be homologous to β-ARK, or require the C terminus of the receptor with its phosphorylation sites (63).

The receptors signal to the cell interior by interaction with G proteins. The G proteins are the large, tripartite proteins as in *Dictyostelium* with α, β, and γ subunits (63, 77). Genes for Gα proteins (*GPA1* and *GPA2*) were identified by screening with rat Gα cDNA (92, 93). *GPA1* is expressed in haploid cells and mediates mating pheromone response (in an unusual way, as described below). The *GPA1* gene was subsequently found to have several mutant alleles: *SCG1*, a suppressor of *sst2*, which confers pheromone supersensitivity; *cdc70*, which bypasses the receptor in activating the mating sensory transduction pathway; and *dac1*, which renders the cell resistant to pheromone (reviewed in 63). *GPA2* is expressed in diploid as well as haploid cells and, along with a separate set of β and γ subunits, appears to regulate cAMP production in concert with the two *RAS* genes of yeast. [The involvement of *RAS* in adenylyl cyclase regulation is another unusual aspect of G protein function in yeast (15, 63).]

As with yeast pheromone receptor genes, the genes for G protein subunits β and γ were first found as mutations that rendered the yeast unable to mate, i.e. sterile. *STE4* and *STE18* were found to encode the genes for β and γ subunits, respectively (150). An attractive aspect of using yeast is its amenability to genetic analysis, and it is through genetic studies that the departure from the now classic interaction of G protein with 7-TMS receptors came to light. These β and γ subunits play a positive effector role and carry on the mating sensory transduction pathway instead of the α subunit doing so (8, 95, 150). Loss of these β and γ genes leads to loss of mating, while loss of α function (e.g. *cdc70* allele) allows conjugation in cells missing the mating type receptor (59). An allele of *GPA1* (*SCG1*) was isolated as a multicopy suppressor of a mutation in *sst2*, which confers supersensitivity to pheromone (28). These observations are consistent with Gα acting in a negative role to inhibit sensory transduction through the pheromone pathway, and with β and γ subunits acting in a positive manner following ligand-receptor binding to carry on the pathway. However, Gα does seem to have a positive role in the mating process downstream from the actions of β and γ. *GPA1*, when constitutively activated, leads to supersensitivity to pheromone (i.e. growth arrest and gene induction at 100-fold lower concentrations of pheromone than necessary for wild type), but also enhanced recovery and long-term resistance to pher-

omone. Thus a second pathway, perhaps with Gα playing a positive role, needs to be characterized (see 77 for a discussion of alternative models of G protein involvement in pheromone sensory transduction).

Paramecium

Paramecium tetraurelia is a diploid eukaryotic unicell that presents technical advantages for the study of excitable cells and has been likened to swimming neurons (56). Although the electrophysiological studies of paramecia began in the 1960s (see 32, 76, 83 for reviews), progress continues to be made because of improving technology (54, 100, 111, 112, 122). Now there are at least seven currents identified and a Mendelian mutant available for almost every current.

Paramecia are chemosensory cells that alter swimming behavior when confronted with a chemical stimulus. Attractants are compounds that indicate that bacteria, their food, is nearby (137). Acetate, lactate, folate, NH_4^+, and IMP are examples of attractants that, at pH 7, cause accumulation of cells relative to Cl^-. cAMP also is an attractant, thus *Paramecium tetraurelia* and *Dictyostelium discoideum* share attraction to both folate and cAMP. *Paramecium,* however, differs by responding to both stimuli at all stages of growth. (The possible functions of cAMP and IMP as stimuli for paramecia are discussed in 140.) The accumulation of cells in response to stimulus can be assayed in a very simple T-maze (147) in which cells distribute between buffers with attractant and buffers with control salt, with cations and pH kept constant between the two. The behavioral responses are specific and saturable, consistent with receptor-mediated processes (127, 137, 142).

The cells accumulate or disperse by biasing their normally random walk, i.e. by changing frequency of turning and speed of swimming to make mean free paths longer or shorter (137, 146). There must be adaptation if there is to be accumulation or dispersal by these kinetic mechanisms (116), and the cells' behaviors do adapt (137, 143). Unlike *Dictyostelium,* there is no orientation, no tactic component (see 145, 146 for an historical perspective on the studies of chemoresponse in *Paramecium*).

Since their ciliary beat frequency, angle, and direction of power stroke are all under membrane electrical control (83, 137, 138), the cells' swimming behavior is a clear and accessible indicator of their membrane properties. In general, small hyperpolarizations increase swimming speed and small depolarizations slow down the cells. A sufficient depolarization will trigger a calcium action potential that, in turn, transiently reverses the ciliary beat and causes an abrupt turn. Thus underlying any swimming behavior changes, including chemoresponse, are membrane potential changes. A broad generalization would be that attractant stimuli hyperpolarize the cells and cause accumulation primarily by increasing mean free path lengths, i.e. cause

a biased random walk. Repellents generally depolarize the cells, increasing the frequency of action potentials and turns, and hence repulsion by short mean free paths in a biased random walk. There are exceptions to this generalization, such as repellents that hyperpolarize the cells much more than attractants and effect dispersal by extremely fast and smooth swimming (137, 147). However, membrane potential is the key to *Paramecium* behavior, and among all these cases of chemoresponse behavior, there is no exception to its basic physiological rules.

Surface receptors for folate and cAMP specifically and saturably bind radioligand stimuli (118, 127), complex with photo-affinity or affinity-labeled stimuli (113, 142), and produce specific, saturable, and additive membrane potential changes (103, 127, 138). Moreover, mutants with single-site mutations are defective in responses to specific stimuli and binding of ligand, thus implying that receptor function could be disrupted by gene mutation (127, 118). The binding sites for radioligand are almost exclusively on the cell body, not cilia, which is covered with about 50% of the cell surface membrane (31). The cilia can be removed along with the voltage-gated Ca^{2+} channels (31) with no impairment of the attractant-induced hyperpolarization. [An exception to this may be the ciliary binding sites that correlate with a response to glutamate (101).] Therefore, the chemoreceptors studied to date reside on the cell body, where the search for receptor proteins began.

The cAMP receptor has proven to be more yielding to purification efforts than the folate receptor. The receptor can be purified from cell body membranes as a doublet of proteins of 48 kd (140, 148). The two proteins are inseparable by reverse phase HPLC, but do have different pIs (6.6 and 9.1 respectively), and different N terminal amino acid sequences (J. Van Houten & B. Cote, unpublished results). Monoclonal antibodies recognize epitopes in common to both (S. Ahmed & J. Van Houten, unpublished results). It is most likely that the smaller protein is produced by a degradative cleavage of the larger. While it is possible that, as in *Dictyostelium* and sea urchins, covalent modification could account for different mobility between the two bands, there is no evidence for this to date.

The most compelling evidence for the receptor function of the 48-kd proteins is the specific and total blocking effect that antiserum produced against the doublet has on whole cells' chemoresponse to cAMP and no other stimuli (148). An open question about the receptors regards their distribution. Folate and acetate responsiveness is not evenly distributed across the cell surface, but falls into a gradient from anterior to posterior (104). More avid antibodies to the cAMP receptor will help to determine whether cAMP-binding sites are in a similar distribution.

Following the binding of stimulus to receptor, there is a change in membrane potential that is rapid and sustained (138) (see 102 for an exception).

Electrophysiological studies of this hyperpolarization have detected no reversal potential and no effect of altering external K and Na alone or in combination (103). Resistance measurements show no consistent pattern across attractants. Therefore, the 0.2 namp current that can be measured in response to acetate, for example, must be generated by a voltage-independent mechanism. The best candidate, for several reasons, is a calcium pump: LiCl deranges chemoresponse and inhibits normal Ca^{2+} efflux by 50% (140, 148, 151); a mutant defective in Ca^{2+} homeostasis (37) is not attracted to most stimuli, with the exception of NH_4 Cl (140); internal Ca^{2+} is slightly reduced upon stimulation as indicated by calcium-sensitive fluorescent dyes (M. Frantz & J. Van Houten, unpublished results).

There is in the cell body surface membrane a Ca-ATPase activity with the biochemical hallmarks of a pump and a corresponding protein that forms a phospho-enzyme intermediate and binds calmodulin in overlays, also characteristics of a surface Ca^{2+} pump (140, 151). Because surface membranes are complex (64, 131) and the association of pumping activity with the Ca-ATPase is only correlative, it remains to be proven that the activity and pump are one in the same. However, the activity and protein are clearly not from potentially contaminating intracellular membranes (140, 152). It is not possible to demonstrate increases in Ca-ATPase activity with chemical stimuli because the activity needs to increase by only a few percent in order to generate the current necessary to hyperpolarize the cells. Therefore, the involvement of the pump in chemoresponse awaits the cloning of the Ca^{2+}-ATPase gene that will make possible alternate methods by which the function in chemoresponse can be studied.

Ca^{2+} then appears to be the second messenger for *Paramecium* chemoresponse. If a surface Ca^{2+} pump is confirmed as part of the transduction mechanism, it will be a novel addition to the transduction repertoire. It may also help to account for other observations of hyperpolarization and ciliary beat control that are consistent with reduced Ca_i (91).

The use of LiCl as a pharmacological tool and a Ca^{2+}-homeostasis mutant, K-shy, in studying chemoresponse has not only led to suspecting Ca^{2+} as a second messenger, it has also suggested another transduction mechanism. The organic compounds that are indicators of bacterial activity (acetate, folate, lactate, cAMP) induce behavioral responses that are greatly affected by incubation of cells in LiCl, while NH_4 Cl elicits an attraction response that is resistant to LiCl (148, 151). It is possible that the effects of LiCl on Ca^{2+} homeostasis interfere with the attractant stimuli that couple to the Ca^{2+} pump and that NH_4Cl, by affecting internal pH, causes the membrane potential and motility changes needed for attraction without the involvement of the pump. Perhaps *Paramecium* will share pH_i changes with the cascade of signal transduction events in the chemoresponse of sea urchin spermatozoa and

neutrophils and in mitogenesis in mammalian cells (4, 11, see 141 for review of neutrophils).

The mechanism of coupling receptor to the pump or any intracellular component of the transduction pathway in order to generate a hyperpolarizing current is not yet clear. There is no evidence for G protein interactions based on GTPase activation or GTP binding upon stimulation (J. Zhang & J. Van Houten, unpublished). However, these are characteristics of the heterotrimeric G protein interactions and, to date, only members of the small *ras*-like family have been identified (R. Hinrichsen, personal communication). Other second messengers, cAMP and IP_3, apparently play no essential role in this transduction pathway (140, 152). Therefore numerous cloning and biochemical studies remain to be done to sort out the transduction components.

TRANSDUCTION MECHANISMS

Receptors

The structures of three sets of receptors from these unicellular systems have been deduced from the DNA sequences of clones and, in the case of *Dictyostelium* and yeast, have been found to be members of the 7-TMS receptor superfamily that includes rhodopsin and β-adrenergic receptors (30). The desensitization of the sensory transduction pathway is at least in part due to phosphorylation of these receptors on serines in the C terminal domain, again a characteristic of the 7-TMS receptor superfamily.

The first *Dictyostelium* receptor clone was found by screening a cDNA expression library with antibodies against the protein (69, 114). This clone appears to code for the receptor of the chemotaxis pathway, but the additional clones that were found using sequences from the first do not have assigned function. They differ from the original primarily in the N and C terminal domains, particularly in the potential serine phosphorylation sites (135). Curiously, the two yeast receptors are not conserved in their primary sequence, and on this level do not resemble each other. On the secondary and tertiary level, however, they appear to have conserved all the features of a 7-TMS receptor. Together these three receptors support the arguments that unicellular organisms are relevant because they are similar to and divergent from higher systems. Unicellular organisms make it possible to identify components of a complex transduction pathway without having to use searches based solely on homology. Hence, the true breadth and variety of a superfamily can possibly be sampled.

The 7-TMS receptors generally show long- and short-term desensitization (30, 125). Long term is mediated by the loss of receptors from the cell surface. Short term is rapid, reversible and the result of covalent modification, specifically phosphorylation. The phosphorylation can be catalyzed by a

protein kinase, such as PKA, that is activated by any ligand and transduction pathway that increases adenylyl cyclase activity. Hence, this is referred to as heterologous desensitization. Homologous desensitization requires a substrate-activated kinase, such as β-ARK, that phosphorylates the β-adrenergic receptor when it is activated by its ligand. The phosphorylation by either mechanism works to uncouple G protein function from the receptor and thus temporarily stops the signaling pathway. In the case of the β-adrenergic receptor, β-ARK is necessary but not sufficient for desensitization. An additional factor, β-arrestin, is needed possibly to interact with the receptor at sites where G protein normally would.

In the *Dictyostelium* and yeast chemoresponse there are mechanisms of desensitization comparable to those of vertebrates. Both *Dictyostelium* and yeast utilize extracellular enzymes to clear the stimulus, i.e. removal of receptors from the cell surface for long-term desensitization, and reversible phosphorylation of the C terminal domain for short-term desensitization. Only in *Dictyostelium* has a kinase specific to the receptor been identified (131), thus much information is yet to be gathered about the kinases and phosphatases that figure into these transduction pathways (43, 57). There is no lack of kinases in the yeast sensory transduction pathway, and at least one modifies receptor-G protein signaling (*KSS1*, although the receptor is apparently not its substrate), and two are responsible for transcription factor activation for gene expression specific to G1 (57). The cyclin/cdc2/CDC28 kinase complex that is instrumental in G1/S growth control is likely to be under the influence of some aspect of the mating transduction system since mating factors arrest cells in G1, and a G1 cyclin (*CLN3*) may be a substrate of kinase *KSS1* gene product (57, 107).

The other class of receptor arising from the study of unicellular eukaryotes is the ligand-binding guanylyl cyclase. Interestingly, dephosphorylation and not phosphorylation is associated with its desensitization (21). Guanylyl cyclases have since been found to be the receptors for atrial natriuretic factor and enterotoxin and, therefore, this different kind of transduction mechanism is not esoteric to the sea urchin (21, 120). This example also reinforces the usefulness of unicellular eukaryotes in providing insight into the mechanisms of higher organisms.

G Proteins

G proteins are named for their ability to bind GTP. Generally, when bound with GTP they are in an appropriate conformation for interacting with and affecting enzyme activity such as adenylyl cyclase. The effect can be excitatory or inhibitory. G proteins also have endogenous GTPase activity, and eventually the bound GTP is hydrolyzed to GDP and the G protein no longer functions as an effector. There are several classes of cellular GTP-binding

proteins, but those that are involved in signal transduction fall roughly into two classes: the small G proteins and the larger ones that physically associate with surface receptors to couple them to the transduction pathway. The latter G proteins, such as Gα, which couples the β-adrenergic receptor to adenylyl cyclase, also associate with β and γ subunits and when so associated are inactive and bound with GDP. When receptor that couples to the heterotrimeric G protein complex is bound with ligand, GDP bound to the Gα subunit is exchanged for GTP. The α subunit dissociates from β and γ and is now free to act on target enzymes or other proteins. There is evidence that the β and γ subunits have activity of their own, activating ion channels, for example. However, generally, receptor-associated G proteins are thought to continue the sensory transduction pathway through activities of the GTP bound form of Gα. (See 11, 12, 63 for reviews.)

The small G proteins that do not directly couple surface receptors to enzymes or other targets range from 17–25 kd in mass. The small G proteins, $p21^{ras}$ in particular, are regulated by a GTPase-activating protein (GAP) and a GDP/GTP exchange factor. The function of GAP may not be simply to terminate the activity of *ras,* but it may play some effector function as well (see 63, 82, 86 for reviews).

The G proteins that thus far figure into the unicellular chemosensory transduction pathways are heterotrimeric proteins that typically can couple to receptors of the 7-TMS superfamily. In *Dictyostelium,* the genes for several α subunits have been examined (75, 135). One clone, Gα2, seems to code for the Gα that activates PLC of the chemotaxis pathway, while others, still unidentified, must couple the receptors of the relay pathway to the adenylyl cyclase: the receptors for development, to differential gene expression; and folate receptors, to the folate chemotaxis pathway PLC. The G protein of the relay system may be regulated by cross-talk from the chemotaxis pathway, by IP_3 in particular (135). Multiple G proteins coupling differentially to receptors or to effector systems are a common theme of vertebrate G proteins as well (70).

Yeast cells, likewise, utilize heterotrimeric G proteins to couple mating pheromone receptors to the sensory transduction pathway, and these receptors, like those of *Dictyostelium,* are members of the 7-TMS superfamily. Yeast genetics has brought to light an important distinction: the β and γ subunits are the downstream effectors in the signal transduction pathway, while α plays either no role or a negative one (see 77 for models). However, α may play a positive effector role in a parallel pathway for desensitization (63). While the yeast *RAS* genes are involved in the regulation of adenylyl cyclase (15), they are not directly involved in mating signal transduction. We may find that the trimeric mating pathway G proteins as well as the *RAS* gene products all figure into growth control in yeast because both nutrient limita-

tion (the adenylyl cyclase pathway) and mating factor arrest cells in G1 of the cell cycle (15, 107).

There is no evidence for a role for G proteins in the activation of the sea urchin guanylyl cyclase/receptor or in the coupling of the *Paramecium* cAMP chemoreceptor pathway (J. Zhang & J. Van Houten, unpublished observations). However, a recent report (5) describes the effects of exogenous GTPγS on ciliary beat reversal and suggests that a G protein may be part of the regulation of axonemal movement that underlies ciliary motility, or that a G protein affects ion channel function and, hence, motility in *Paramecium*. There is yet no compelling evidence for receptor G interactions in this ciliate's chemoresponse.

Second Messengers

The message of ligand binding to receptors, with or without G proteins, must be transduced into second messengers in the cell in order to carry on the signal transduction pathway. These second messages include cyclic nucleotides, diacylglycerol, inositol phosphates, pH alterations, and calcium. Only arachidonic acid seems to be missing from the repertoire, but still may figure into these pathways in ways that are not yet evident. In short, these organisms utilize the small cadre of second messengers that are common to signal transduction pathways from mitogenesis to synaptic transmission (3, 6, 7, 11, 45, 78b, 90). Therefore the enzymes and channels that generate these second messengers must also be common to these unicellular organisms. In the case of *Dictyostelium,* cAMP receptors R_A and R_B couple to PLC or phosphotidylinositol kinase and adenylyl cyclase, respectively, (Figure 1) in order to generate the IP_3 and cAMP of the chemotaxis and relay systems. Calcium released from non-mitochondrial stores by IP_3 (96) and cGMP are among the next second messengers to be generated. Ultimately, the myosin- and actin-based motility system must be affected by these second messengers in order to orient the cell up the gradient of attractant and to increase motility (52, 53, 96). While the major details are similar to mammalian signal transduction, there are important enzyme activity differences coming to light, for example, in the metabolism of phosphoinositol lipids (135).

In yeast, the pathway clearly involves the activation of G proteins, but the second messenger systems that are subsequently activated are not obvious (Figure 2). Ultimately, as in the signal transduction of mitogenesis (1, 19, 50, 99), the activity of transcription factors comes into play, and this implies the activation of protein kinases by second messengers (57). The yeast system is a particularly good one in which to study protein kinases; more than 30 have been identified to date by a variety of techniques (57). Since the mating pheromone arrests cells in G1 at the start site, the potential components of the yeast mating system expand to include the cell division cycle-(*CDC*-) gene products that control cell cycling in G1. While this appears to complicate the

picture, it also could serve to simplify the search for transduction pathway components since these may already be known in the CDC system. This also seems to be typical of the way in which the understanding of the CDC system seems to be progressing: ever widening circles of connections to other systems of embryonic mitotic control, fertilization, oncogene activity, and more (57, 87, 107).

Multiple second messengers are produced upon activation of sea urchin spermatozoa with egg peptide. The causal relationships of these changes are not yet established, and it is not clear whether cGMP is an obligatory second messenger generated from the activation of guanylyl cyclase/receptor by ligand binding. The roles of changing cAMP, Ca^{2+}, and H^+ in the activation process as well as the control of their synthesis remain to be determined (46, 134).

The second messengers of the *Paramecium* system, likewise, remain elusive. While the receptor and downstream membrane electrical changes of the *Paramecium* chemoresponse pathway are being directly characterized, the second messenger steps in between have been established by correlative evidence and process of elimination (140, 141, 144, 151). While cyclic nucleotides can affect ciliary beating (10, 122), they do not appear to mediate chemosensory signal transduction. Moreover, phosphoinositol lipid turn over does not seem to respond to chemical stimuli (151) (see 140 for discussion). The characteristic membrane potential changes in response to folate and acetate stimuli do not appear to be mediated by a channel with a reversal potential (103), but correlative evidence does point to a cation pump as the mechanism of hyperpolarization in these attractant stimuli. If the Ca^{2+}-ATPase pump that is currently under study is the source of the hyperpolarizing current, this will be yet another novel transduction mechanism established through the studies of unicellular eukaryotes. If the attraction to NH_4Cl is mediated by changes in internal pH, it may prove to be a transduction process in common with signal transduction pathways involved in mitogenesis or other complex surface receptor-mediated processes (4, 11).

CONTEXT OF OLFACTION AND TASTE

Chemosensory transduction crosses phylogenetic boundaries from bacteria to vertebrates, and the chemosensory transduction mechanisms are numerous and varied. The clearest parallels between the unicellular eukaryotes of this chapter are with olfactory and taste receptor cells and are briefly outlined below. The basic elements of the insect olfactory system and vertebrate taste systems are thoroughly discussed in other chapters in this volume and can be generalized to other taste and olfactory systems. Therefore, the reader is referred to these chapters and reviews (24, 67, 105, 128, 132, 149).

Olfaction

Olfactory receptor cells are thought to detect stimuli dissolved in mucous, sensillar lymph, or water by cell surface receptors. However, the lipophilic nature and large variety of the stimuli called into question whether receptors were involved (78). Now there seems to be some potential resolution to the question of receptors through molecular genetics (17, 48). Genes for several putative receptors have been cloned by polymerase chain reaction using primers fashioned after conserved regions of the 7-TMS receptors (17) (see 35 for polymerase chain reaction technology). The size of the gene family and their function as odorant receptors still need to be demonstrated. The *Dictyostelium* and yeast receptors, likewise, are from the 7-TMS superfamily, but the yeast example (55) cautions that some receptors of a broad and varied family might be missed by screening with conserved primary sequences. Instead, secondary or tertiary structure may be the conserved aspect among the family members.

Olfactory receptors appear to couple to G proteins, and an olfactory-specific G protein has been identified (see 16 for review). These are members of the heterotrimeric G proteins and to date only the α subunit has been implicated in sensory transduction similar to *Dictyostelium* and differing from yeast. The G proteins couple to adenylyl cyclase or phospholipase C to stimulate generation of cAMP or IP_3 (2, 38, 109, 132), which in turn increases $Ca^{2+}{}_i$. This is reminiscent of *Dictyostelium*, which combines these receptor-activated pathways in one cell. At present, the variety of receptors and pathways within one olfactory receptor cell is not known.

A distinct difference from the unicellular systems comes in the action of cAMP and IP_3, not upon kinases, but upon cation channels in olfactory cells (23, 81, 109, 132). The opening of channels depolarizes the cells and generates the action potentials whose frequency, latency, and pattern across the entire olfactory epithelium encode the quality and quantity of the odor. In the unicellular examples and in mitogenesis signal transduction in mammalian cells, IP_3 liberates Ca^{2+} from internal stores (19, 90, 96), but in olfaction IP_3 appears to open surface channels directly to allow an influx of extracellular Ca^{2+} (109). Channel activation in general does not figure into *Dictyostelium* and yeast sensory transduction (135), but may be involved in the Ca^{2+} fluxes in spermatozoa and in the hyperpolarization of *Paramecium* in attractants such as NH_4Cl. Olfactory receptor cells can also hyperpolarize to modulate the receptor cell activity and may account for the dichotomy of cAMP and IP_3 (excitatory and inhibitory) functions in the same receptor cell (38, 89). Electrophysiological studies of receptor cells, particularly new patch techniques, are shedding light on the ion fluxes initiated by second messengers (38, 42, 68, 80). Among the four unicellular examples, only in *Paramecium* is electrophysiology regularly used to assay channel function, and the currents

in this cell in response to some chemical stimuli may be generated by a cell surface pump.

Additional considerations in olfaction are perireceptor events (89), that is the actions on the stimulus at the cell surface other than direct interaction with the receptor. In olfactory cells, an odorant carrier (olfactory-binding protein) in the mucous or sensillar lymph combines with odorant to concentrate, protect, and/or remove the stimulus from the area of the receptor (128, 149). This has no apparent counterpart in the unicellular systems. *Dictyostelium* and yeast degrade stimuli to remove them from the area of the receptor, and these stimuli are not generally taken up by the cells or readily cross the membranes. It has been documented, especially in insects and crustacea (20, 149), that, as in *Dictyostelium* and yeast, olfactory dendrites rapidly degrade stimuli. Additionally, olfactory-specific cytochrome P450 and UDP-glucuronosyltransferase are thought to prevent hydrophilic stimuli from interfering with cell metabolism or diffusing back into the extracellular fluid where they can interact with receptor and be confused with the next wave of stimuli (29, 78a). These metabolic activities do not seem to play a part in unicellular perireceptor events.

In *Dictyostelium* and yeast, an important consequence of chemoreception is the activation of transcription, probably through the activities of protein kinases activated by second messengers. Considering the potential number of second messengers in olfaction and taste (see below) and the kinases that they regulate, activation of specific gene transcription most likely is a consequence of stimulating the olfactory or gustatory signal transduction pathways.

Taste

While there are at least two signal transduction pathways in olfaction, there is a larger variety of mechanisms in taste (24, 67). In taste receptor cells in general, there must be a depolarization or enzymatic change to produce the increase in $Ca^{2+}{}_i$ that is required for secretion of neurotransmitter, and there is evidence for receptors mediating sweet and some bitter taste responses (67). Other tastants can act directly to block K channels or pass through Na channels, for example (24, 67). Likewise, *Paramecium* chemoresponse to organic stimuli is receptor-mediated and elicits a change in membrane potential. *Paramecium* can also respond as a neuron to changing external inorganic salt concentrations and thus has some additional parallels with taste cells, even down to the effect of amiloride on its behavior (144).

The tastants that may be receptor-mediated activate increasing levels of second messengers that may act directly on channels, as opposed to indirectly through kinases. In contrast, the receptor-coupled enzymes of the unicellular eukaryotes generate second messengers that seem to act primarily through kinases or on cytoskeletal elements as well as kinases. As in olfaction,

electrophysiological techniques will provide some important insights into the cause and effect relationships of the signal transduction pathway (24, 67).

This chapter has emphasized that, while the general themes of sensory transduction are held in common, there are a great many variations on these themes. While it is important to look to common themes to identify basic mechanisms, variations are important to investigate as well. Some variations will be peculiar to the organism, e.g. *Paramecium* or hamster taste bud cell, but some will provide new insights into mechanisms of transduction that had not previously been considered. The unicellular systems that are relatively easily manipulated and dissected (66) have already brought to light some variations that were subsequently found elsewhere, and thus have led to a new understanding of transduction mechanisms. Therefore, the chemosensory signal transduction of these organisms should continue to be of interest in the field of chemical senses.

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