Chemosensory transduction in eukaryotic microorganisms: trends for neuroscience?

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It might appear curious to read about yeast, slime molds and protozoa in a journal dedicated to neuroscience. However, despite their distinct lack of synapses, eukaryotic microorganisms hold a wealth of information relevant to the signal-transduction pathways that underly activity in neuronal receptor cells, particularly those subserving the chemical senses. Microorganisms are sensitive to chemical stimuli from their environment and thus have similarities to receptor neurons of the olfactory system and the taste bud. Here, we introduce receptors, second messengers and effectors responsible for chemosensory signal transduction in yeast mating, sea-urchin spermatozoan chemotaxis, slime-mold aggregation and development, and ciliate chemoresponses.

Why turn to eukaryotic microorganisms for information pertinent to neuronal function? First, microorganisms are generally easy to manipulate. Most have well-developed systems for molecular genetic analysis, allowing gene cloning and expression, cell transformation, and the replacing or 'knock-out' of genes. In addition, large quantities of clonal wild-type or mutant cell lines can be produced for biochemical studies. Second, there are first principles to be derived from studying signal transduction across phyla and some of our most detailed information about receptors, G proteins, and kinases – including our first glimpses of eukaryotic chemoreceptors – have come from these microorganisms. The chemoreception systems of yeast mating, chemotaxis of sea-urchin spermatozoa, slime-mold aggregation and development, and ciliate chemoresponses are highlighted here for their parallels and apparent eccentricities as well as their contributions to our understanding of chemoreception in general.

Yeast

The focus of our attention in the budding yeast, *Saccharomyces cerevisiae*, is the mating system (see Refs 1, 5, 10 and 55 for primary citations). The neurobiologist will find here both familiar components of neurotransmitter signal-transduction cascades and some curious twists that make yeast of more interest than just for their use in producing bread and beer. The yeast chemosensory system enables haploid cells of complementary mating type (**a** or α) to recognize each other, set in motion the physiological and morphological changes that are the prelude to mating, and finally fuse to produce a diploid cell. The diploid cells do not respond to mating pheromone and during nutritionally hard times form four spores that, when conditions improve, will germinate into four haploid cells, two of each mating type.

In both **a**- and α-cells, binding of the complementary mating pheromones (the peptides **a**- and α-factor) to their receptors initiates a cascade of events that lead to cell-cycle arrest, gene-transcription changes, morphological changes, agglutination and eventually fusion of the cells and nuclei. Haploid **a**-cells produce and secrete **a**-factor and express membrane receptors for α-factor to respond to α-cells. Similarly, α-cells produce and secrete α-factor and respond through **a**-receptors to the **a**-cells. While not all the details of this cascade are known, Box 1 summarizes one way in which many of the puzzle pieces can be arranged to be consistent with the genetic and biochemical studies.

The yeast signal-transduction pathway begins with the pheromone binding to a receptor that has seven transmembrane regions. Remarkably, the receptors for **a**- and α-factor share no sequence similarity and yet in their secondary and tertiary structure they have conserved all the features of the receptors of the rhodopsin and β-adrenergic family[1,5], which have seven transmembrane regions. (An interesting aside is that in the process of 'courtship', cells distinguish and pair with the complementary cells secreting the most amount of mating factor[6]. This discrimination occurs at the receptor level without the need for the G proteins in the signal-transduction pathway.) The ligand-activated receptor in turn activates a trimeric G protein, in a manner similar to that of activated muscarinic or adrenergic receptors[7]. Here, the pathway makes an interesting deviation from that characterized in other seven transmembrane region receptor systems: the β and γ subunits appear to carry on the flow of information to the downstream effectors. Their positive effects and the α subunit's negative effects on the pathway are illustrated in genetic experiments in which loss of β and γ leads to loss of mating, while loss of G_α function allows conjugation to occur in cells lacking the mating-type receptors (for review see Refs 2 and 8). The downstream effector of the βγ complex remains mysterious, but it sets into motion a kinase cascade that is known to arrest cells in the G1 phase of the cell cycle and induce gene expression. The pheromone-induced arrest of haploid cells in G1 occurs at the restriction point called START when no nuclei are involved in DNA synthesis. This is achieved by inhibiting the action of G1 cyclins (CLN1–3)[9–11], proteins that activate the mitotic kinase CDC28 required for transit through START. The cells must recover from the effects of pheromone in order to carry on their growth and mitotic cycles as diploids, or possibly as haploids if they fail to mate. Recovery is accomplished through several mechanisms, some of which are in common with neuronal systems (see Box 1).

The identities of the effector target of the βγ subunits in the yeast mating system and the second messenger are the major open questions[3]. If the target(s) is a kinase, downstream-information flow could occur by a phosphorylation cascade[11] (as in the bacterial 'chemotaxis' system where information flows by handing on of phosphate from protein to protein), and a diffusible second messenger need not be involved.

The budding yeast has received most of the attention of late, but the fission yeast, *Schizosaccharo-*

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myces pombe, has a similarly interesting mating-pheromone response. The two systems are compared in recent reviews[1,13] and, as expected from studies of the cell-cycle control systems of the two distantly related yeasts, there are many homologous genes shared between the two pheromone systems. Perhaps as interesting as the similarities are the differences; for example, starvation conditions, Ras and G_α are required for mating in *S. pombe*, but *S. cerevisiae* mate while fed, do not require Ras, and utilize $\beta\gamma$ as a positive effector[13].

Sea-urchin spermatozoa

As unlikely as it might seem, research on peptides from sea-urchin eggs, atrial natriuretic peptides and *Escherichia coli* enterotoxins has converged with the identification of the receptors involved in these apparently disparate lines of inquiry. In each of these transduction systems, a cell-surface guanylate cyclase (GC) appears to serve as both receptor and effector to generate the second messenger of the pathway, cGMP. This signaling molecule has caught the attention of neuroscientists because it regulates interesting proteins like kinases and phosphodiesterases as well as ion channels in photoreceptor and olfactory receptor systems[14].

The characteristics of several genes encoding GCs have shown that the receptors are part of a family that includes the sea-urchin receptors, the natriuretic receptors, the enterotoxin receptors, and a retina-specific GC[15,16]. These have a single membrane-spanning region, a C terminus catalytic domain, and an intracellular protein-kinase-like domain. They resemble tyrosine-kinase-containing growth-factor receptors (see Refs 17 and 18 for comparisons). However, the significance of this structural similarity of the two different families of peptide receptors is not yet evident.

Now that the membrane GCs are established as a family of receptors, attention seems to be shifting from the sea-urchin spermatozoa to mammalian cells in order to unravel the signal-transduction pathways. Perhaps the negative regulation of the atrial natriuretic peptide (ANP)-receptor/GC pathway in vascular smooth muscle[19], and the ANP modulation of K^+-channel activity by cGMP-dependent dephosphorylation[20] will be found to be general aspects of signal transduction coupled to GC receptors. We will follow the chronological order of the development of this field, starting with the sea-urchin system.

Sea-urchin eggs release species-specific peptides (resact from *Arbacia punctulata* and speract from *Stronglyocentrotus purpuratus*) that cause changes in spermatozoan motility that might facilitate the spermatozoan coming into the vicinity of the egg, as well as multiple changes in second messenger (cAMP, cGMP, Ca^{2+} and H^+) and respiration levels[21,22]. The roles of these second messengers in the signal-transduction pathway are not yet established.

The sea-urchin peptide receptor was identified as a membrane GC by approaches such as crosslinking and immunological studies. Genes encoding the sea-urchin peptide receptors have been cloned but ligand binding and function have not yet been demonstrated for the recombinant proteins, presumably due to technical problems. For example, dimerization of a related GC and the crosslinking of speract to an associated 77 kDa protein have been demonstrated[23,24] and perhaps a failure of the expressed proteins to dimerize or to interact with an associated factor prevents demonstration of the cloned genes' function. Alternatively, this might be due to problems of expressing a protein at 37°C that is functional at ≤ 20°C. The apparent molecular weight of the receptor changes upon stimulation due to extensive dephosphorylation, which causes the desensitization of the receptor[21,25]. This stands in contrast to the yeast pheromone receptors, which are phosphorylated during desensitization, much like other receptors[26] that have seven transmembrane regions.

The search for receptors of the atrial, brain and type-C natriuretic peptides (ANP, BNP and CNP) and *E. coli* enterotoxin has led to the cloning of the genes for three mammalian GCs. Atrial natriuretic peptide and CNP appear to be ligands for the cloned GC-A and GC-B receptors, respectively, while the BNP could also be a ligand for GC-A or an as yet undiscovered receptor. The enterotoxin peptides that cause diarrhea bind to the GC-C receptor and recently an endogenous ligand for this receptor, guanylin, has been purified and its gene cloned[27].

Recombinant GC-A, GC-B and GC-C bind ligand and exhibit cyclase activity[17,25,28]. Like the sea-urchin receptor, GC-A is dephosphorylated during desensitization[29]. The enterotoxin receptor (GC-C) is now described as an N-linked glycoprotein of molecular weight 120 000, which shows stimulated GC activity with heat-stable enterotoxins and even higher activity with enterotoxins combined with ATP (Ref. 15). Regulation by ATP is a common feature of the receptor GCs and might be mediated through the kinase-like domain[25].

An interesting contrast can be drawn between adenylate cyclase (AC) and GC (Ref. 25). In the AC transduction pathway, the receptor couples to the effector enzyme (AC) through a three-subunit GTP-binding protein. The membrane-associated GCs are receptor, nucleotide (ATP)-binding protein, and enzyme rolled into one – this might reflect the way in which these enzymes with similar substrates and products could have evolved.

A new twist to the GC story is the activation of the soluble form of the enzyme by nitric oxide (NO) produced through stimulated cell activity. Through its actions on GCs, NO might account for vasodilation in long-term potentiation[30]. However, there is no direct evidence for the activation of the membrane-associated GCs by this diffusible transmitter and messenger.

The most important lesson from the sea-urchin spermatozoan system is that GC can serve as both cell-surface receptor (or subunit of one) and effector to produce second messenger. One focus of further research into the GC signal-transduction pathway is the identification of regulatory components. For example, kinases and phosphatases seem likely to modulate GCs' enzyme activity. At present, the candidates for such a role are protein kinases A and C (Ref. 17) and phosphatase 2A (Ref. 29). In addition, the downstream effects of GC, the number of members of the GC-receptor family, their tissue distribution, their function, the role of the protein-kinase-like domain, and the significance of ATP binding have still to be determined for receptor GCs (Ref. 25).

Box 1. Mating pheromone pathway in budding yeast

The *Saccharomyces cerevisiae* pheromones are the α- and **a**-factors, peptides of 12 and 13 amino acids. The α-factor is translated, glycosylated and secreted via the Golgi complex, but the **a**-factor is translated in the cytoplasm, farnesylated and secreted by an unusual transport mechanism that appears to be related to the multidrug-resistance transporter. (See Refs a and b for reviews of the factors and their processing.) Pheromone expressed by a haploid cell binds to its receptor on a complementary haploid cell. An **a**-cell secretes **a**-factor and expresses the α-receptor (product of the *STE2* gene) and an α-cell secretes α-factor and expresses the **a**-receptor (product of the *STE3* gene). The binding of pheromone is conveyed to the heterotrimeric G protein complex (α, β and γ subunits coded for by the *GPA1*, *STE4* and *STE18* genes respectively; reviewed in Refs a, c and d). Through the interaction with ligand-bound receptor, the G_α subunit is activated by exchange of GDP for GTP and the subunit is freed from the β and γ subunits, which appear to carry on the flow of information to the downstream effectors[e,f]. Myristylation of the G_α subunit is required for its activity. The γ subunit is isoprenylated and this modification might serve to target the βγ pair to the membrane and effector[a,b]. While the downstream effector of the βγ complex remains mysterious, the products of the *STE20* (preliminary data reviewed in Ref. b) and *STE5* genes are possible candidates. The *STE20* gene product has homology with serine/threonine kinases and might be responsible for the pheromone-stimulated phosphorylation of the β subunit or might serve as an effector in the pathway. Likewise, STE5 protein might be rapidly phosphorylated upon pheromone treatment. While the βγ interactions with STE20 and STE5 proteins remain uncertain, both STE20 and STE5 are thought to enter into the pathway upstream from a set of kinases (the *KSS1*, *FUS3*, *STE7* and *STE11* gene products) that are essential for the pheromone response and mating. STE11 appears to control phosphorylation and possibly the activities of the STE7 and FUS3 kinases. The *FUS3* gene product is particularly interesting in that it appears to act at two steps in the signal-transduction pathway[a,b]. One or more of its functions can be carried out by the KSS1 protein, which like FUS3 is a member of the MAP/ERK kinase family. Both the *FUS3* and *KSS1* genes must be disrupted to prevent mating, gene induction, and pheromone response. However, *fus3* mutant alleles produce cell-cycle arrest independent of KSS1 activity. (See Ref. b and discussions in Refs g–i for ordering of these five kinases and putative transcription factor in the pathway.) One of the upstream kinases appears to phosphorylate STE12, a transcription factor that is responsible at least in part for induction of genes such as *FAR1*.

Pheromone-stimulated haploid cells arrrest their mitotic cycle in G1 at the restriction point called START when no nuclei will be involved in DNA synthesis. The function of the *CDC28* gene product, the kinase that allows transit through START, is blocked by the effects of the pheromone pathway on all three of the CDC28-activating proteins, the G1 cyclins (CLN1–3)[b,j–m]. FUS3 is responsible for the inactivation of CLN3 and for the phosphorylation of FAR1, allowing FAR1 to asosciate with and inhibit the CDC28–CLN2 kinase[n]. The regulator of CLN1 activity in pheromone-induced cell-cycle arrest has yet to be identified. All three CLN proteins must be inactivated to arrest the cell in G1.

FAR1 is one of several genes whose expression is affected by pheromone stimulation. There is a recognized pheromone response element in the promoter region of genes induced by pheromone[k,l]. This induction is at least in part due to the activation of transcription factor STE12, which changes not its DNA binding but most likely its ability to activate RNA polymerase upon pheromone-stimulated phosphorylation. The kinase that is likely to phosphorylate STE12 is STE11. The kinase FUS3 might play an additional role in STE12 function, a role that is perhaps redundant with that of KSS1, and is involved in inactivation of CLN3.

Cells recover from pheromone stimulation, and are released from the cell-cycle block whether or not they have successfully mated. The recovery involves multiple mechanisms that have parallels in metazoan systems. (1) The pheromone stimuli are degraded – α-factor by protease Bar1 (Ref. o) and **a**-factor by an endopeptidase[p]. (2) As in the β-adrenergic system, the receptor is phosphorylated on the C terminus[q], and mutants with truncated pheromone receptors lacking the multiple serine and threonine phosphorylation sites show increased sensitivity to, and lack of recovery from, pheromone. (3) Unlike the β-adrenergic system, there appears to be no arrestin homolog[q] to bind to the receptor and alter its interaction with G protein. However, modification of the β subunit by phosphorylation might contribute to desensitization[r], possibly with KSS1, among others, serving as the kinase. (*KSS1* was

Dictyostelium discoideum

The literature on chemotaxis in the slime mold *Dictyostelium discoideum* is vast (for reviews see Refs 31–33). While many of the details of this chemosensory signal-transduction system are falling into place, several questions remain to be addressed before the summary pathway (Box 2) can be completed.

Dictyostelium discoideum amebae detect their prey (bacteria) from the folic acid that the bacteria produce. This is a receptor-mediated process[34], but is not as well understood as chemotaxis in response to cAMP (Refs 31, 32). As cells run out of bacteria, they lose their folate receptors and incorporate cAMP receptors into their membranes. These receptors enable cells to respond to pulses of cAMP emanating from a focal cell, toward which the cells in a field migrate and then form a multicellular slug. The slug differentiates into nonviable stalk cells and viable spores, which can weather the bad times and germinate when conditions improve and the amebae can then graze on bacteria again.

A wave of cAMP diffusing past a cell with cAMP receptors initiates not only oriented locomotion of the cell toward the focal cell (chemotaxis pathway through receptor 1 in Box 2) but also the secretion of cAMP to stimulate yet other outlying cells (relay pathway through receptor 2 in Box 2). Both of these responses are transient, the movement lasting 100 s and the cAMP secretion lasting minutes. It is important for the cell to respond to the wave of stimulus and not to its own secreted cAMP. To insure this, the motile response adapts primarily by phosphorylation of the receptor by a specific kinase[35] and by removal of cAMP from the vicinity of the cell by a potent

originally isolated as a suppressor of *sst2*, an allele producing supersensitivity, presumably through lack of desensitization.) (4) There have been suggestions that G_α in the GTP-bound state also promotes desensitization. However, there are alternative interpretations to the data that do not involve desensitization[a]. One mechanism of recovery apparently not at work in yeast is downregulation of receptor numbers[a,b].

Many proteins are phosphorylated in response to pheromone treatment, including receptors (STE2, STE3), kinases (STE7 and FUS3), a transcription factor (STE12) and FAR1[a,b]. Desensitization and recovery could involve specific phosphatases to reverse these modifications. To date, only calcineurin (phosphatase 2B) is implicated in the pheromone response[b]. The Ca^{2+} influx that occurs late in the mating process is postulated to activate the catalytic subunits of phosphatase 2B (products of the *CNA1* and *CNA2* genes).

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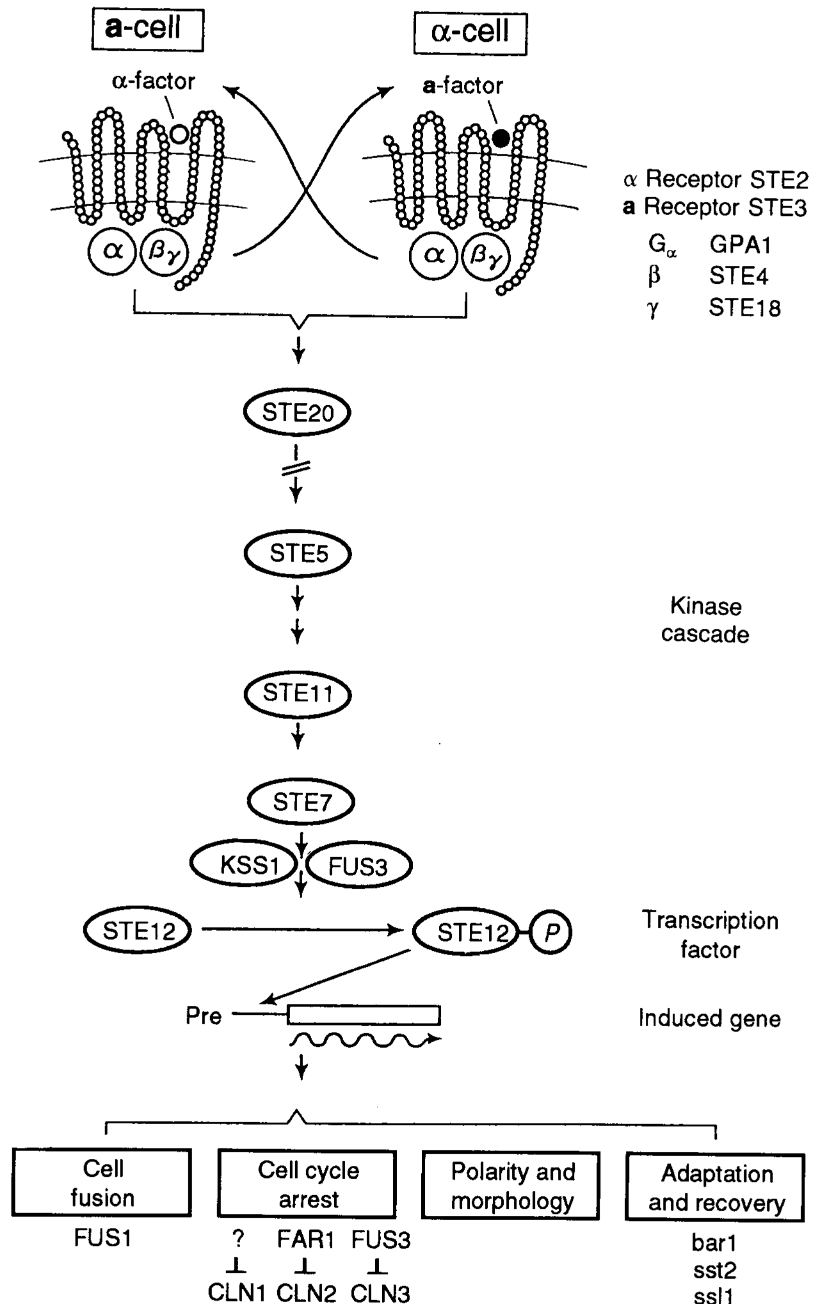


phosphodiesterase and the receptor is subsequently dephosphorylated in order for cell to respond to the next wave of stimulus. Additionally, intracellular signals are degraded to sharpen the timecourse of the motile response[36,37].

Once chemotaxis has brought cells together, cAMP is not abandoned as a signaling molecule. Secreted cAMP is also essential for migration of cells within the aggregation mound and for differentiation of cells into a fruiting body – a structure with stalk cells holding spore cells aloft (for review see Refs 34 and 38). Cells follow spiral trails of cAMP to sort themselves out into prestalk cells at the top and bottom of the aggregation mound, with prespore cells in the middle. The cells at the top produce pulses of cAMP and become the tip of the multicellular slug. Eventually, the prestalk and prespore cells form the fruiting body.

Both the kinetics and concentration of the secreted cAMP are important in the induction of genes for development and morphogenesis. There are three classes of genes: (1) aggregation-related genes that are responsive to nanomolar pulses of cAMP; (2) prestalk-related genes that are responsive to nanomolar pulses and persistent micromolar concentrations of cAMP; and (3) prespore-related genes that respond to persistent micromolar amounts of cAMP. Judicious use of mutants and inhibitors has shown that aggregation, prespore and prestalk genes are induced by different branches of the signal-transduction pathways[34,38]. Divergence of gene-induction pathways might begin at the receptor level: there are at least four receptor genes (*cAR1*, *cAR2*, *cAR3*, *cAR4*) and six G protein genes[34,38]. For example, disruption of *cAR1* interrupts aggregation and

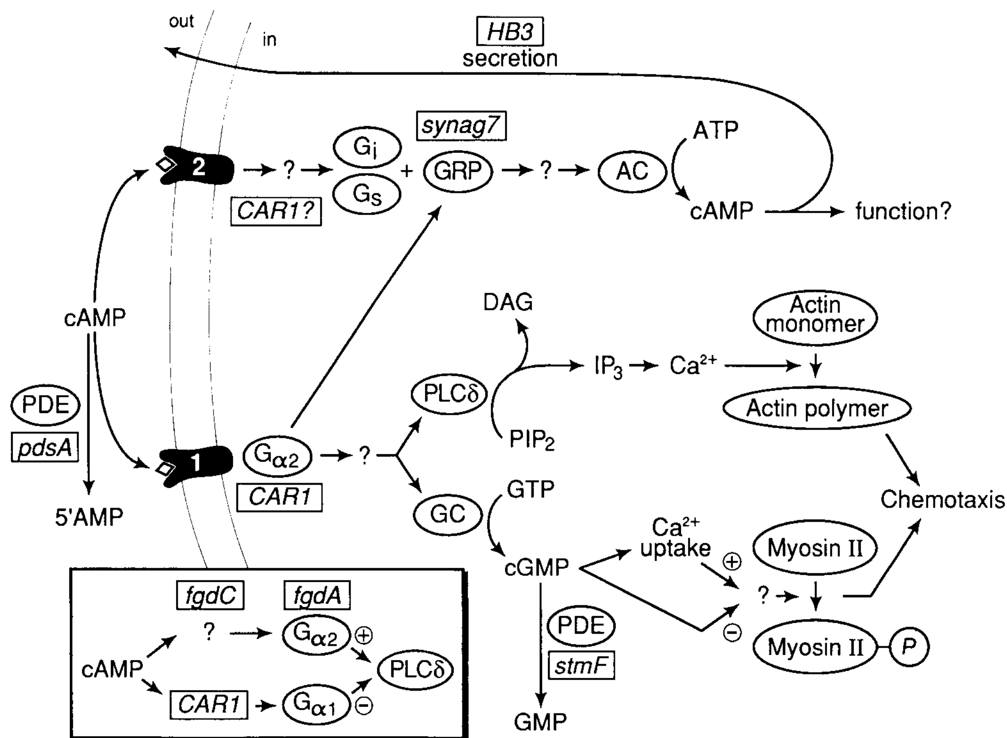
Box 2. *Dictyostelium* chemotaxis and relay signal-transduction pathways

A wave of cAMP approaches the cell and binds to the cell-surface receptors to initiate signal transduction. The genes for a growing family of receptors have been cloned and there are at least four[a–c]. The mRNA for the clone *cAR1* appears at the right time during development to account for the chemotaxis receptors (pathway through receptor 1), and antisense and gene knock-out experiments support this notion[a,d]. The *cAR1* sequence fits the structural motif of the vertrebrate seven transmembrane region receptor[a–c]. In addition to the chemotaxis pathway, *cAR1* might also initiate the relay pathway (pathway through receptor 2) and still other *cAR* clones might be involved in both (Devreotes, P. N., pers. commun.), perhaps with the points of intersection at common G proteins (see below). What once looked like a relatively complex picture of two receptors and subtypes, based on binding kinetic analysis, has to be rethought on the bases of studies of the time of expression, antisense or gene knock-out downregulation, and other molecular manipulation of individual receptor clones[e]. New advances in *Dictyostelium* gene-cloning technology will certainly accelerate progress[f].

Where the chemotaxis pathway bifurcates, the G protein, $G_{\alpha 2}$, is implicated in the coupling of the chemotaxis receptor to both downstream effectors, phospholipase C (PLC) and GC. A deletion mutant of the $G_{\alpha 2}$ gene (HC85 allele of *fgdA*) uncouples the receptor from the rest of the pathway and firmly demonstrates a role for this G protein in the chemotaxis pathway[g]. Additionally, $G_{\alpha 2}$ is expressed at the right time in development to be available for this role in chemotaxis[h]. However, five other G-protein genes have been cloned[e] and some of these code for candidates that could also be involved in the chemotaxis system. For example, $G_{\alpha 1}$ (as well as $G_{\alpha 2}$) is likely to regulate PLC (see inset). The *fgdC* gene product acts as a switch specific to this PLC branch of the pathway[h]. Another interesting aspect of the G proteins is that they can be phosphorylated, but the effect of this is unknown[i].

In the chemotaxis pathway, activated $G_{\alpha 2}$ and $G_{\alpha 1}$ regulate PLC, with the interaction of *fgdC* gene product, and $G_{\alpha 2}$ activates GC (Ref. h). Second messengers diacyl glycerol, inositol 1,4,5-trisphosphate (IP_3), Ca^{2+}, and cGMP are generated, with Ca^{2+} coming from intra- and extracellular sources[j]. At least some of these second messengers have been implicated in the restructuring of the cytoskeleton that underlies the characteristic behavior of the cells to a pulse of cAMP; pause in movement, 'cringing' or rounding up of the cells and then resumed directed movement up the gradient of cAMP (Refs c and k). During chemostimulation, actin and myosin become associated with the Triton-insoluble cytoskeleton, perhaps for reshaping, elongation and other preparation for directed movement of the cell[k].

The Ca^{2+} released by IP_3 action on intracellular Ca^{2+} stores might affect the polymerization of actin for either orientation of the cell or its motility, but this has not been shown *in vivo*. The myosin branch of the pathway is associated with the second messenger cGMP, which is transient and corresponds to a pause in motility of the cells[l]. An *stmF* mutant has a defective cGMP phosphodiesterase and as its cGMP levels remain high longer, it pauses for prolonged periods of time[l]. Myosin accumulation in the cytoskeleton is aberrant in the *stmF* mutants, correlating with prolonged association of myosin with the cytoskeleton and delayed phosphorylation of the myosin. If the myosin association with the cytoskeleton is responsible for the elongation and orientation of the cell, it could explain the improved orientation of the *stmF* mutant, which does show chemotaxis although on a slower timescale. cGMP could affect myosin phosphorylation through activation of a kinase or through a nucleotide-gated Ca^{2+} channel or other mechanism that would explain a prolonged Ca^{2+} influx in *stmF* mutants. An interesting twist is that the myosin heavy chain gene can be disrupted, and cells still move chemotactically toward cAMP[m]. Therefore,



myosin might play a role in efficient elongation and orientation, but is not essential for motility.

Moving along the relay pathway (through receptor 2), stimulatory and possibly inhibitory G_α proteins couple with the cAR1 and perhaps other receptors, resulting in activated adenylate cyclase (AC). The *synag7* gene product is not a GTP-binding protein, but instead acts as a regulator of the G protein (GRP) coupled to the receptor[e,n]. $G_{\alpha 2}$ is required for activation of the relay AC, but it is not the G protein directly coupled to the AC. Therefore, $G_{\alpha 2}$ might be shared between the two pathways and there can be crosstalk between the chemotaxis and relay pathways at the level of the $G_{\alpha 2}$ protein. The genes for the AC have been cloned, and some very interesting information has come from this exercise[o]. Two clones were isolated, one for a protein that resembled other ACs in its inferred structure and one that was significantly smaller. The *ACA* gene product has the expected two sets of six membrane-spanning regions and is the AC involved in aggregation. The *ACG* gene product, in contrast, has one membrane-spanning region, reminiscent of the receptor GCs (above), but has the activity of an AC. *ACG* is expressed during germination and plays a yet undefined role in the pathways. The activated relay pathway results in cAMP production and secretion out of the cell by a process involving the *HB3* gene[e,n]. The function of the cAMP that might remain in the cell is not known.

As in metazoan receptor systems, the *Dictyostelium* chemotaxis and relay systems desensitize and down-regulate. Upon exposure to cAMP, receptor pathways are activated in less than five seconds, following which the receptors become multiply phosphorylated and desensitized[p]. Between one and five minutes of exposure, loss of ligand binding occurs, but receptors are not patched and lost from the surface until 10–20 minutes of exposure to cAMP. After five minutes, receptor mRNA levels decline. Clever use of mutants and inhibitors has defined very different roles for the signal-transduction pathways in these desensitization processes[p]. In the normal time course of chemotaxis, the cells will be desensitized primarily by phosphorylation and recovered for a new wave of cAMP every six minutes[e,p].

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expression of genes that are cell-type specific[39]. Disruption of *cAR3* gives no apparent phenotype, but cAR3 appears in development after cAR1 and before cAR2 (Ref. 40), suggesting that its loss could be compensated by these other receptors. Each of the three receptor proteins, expressed from clones, binds cAMP, but with different affinities[41]. The differences in these receptors with respect to their time of appearance, cAMP affinity and desensitization properties might explain how *Dictyostelium* could make use of the same signal for chemotaxis and morphogenesis.

Perhaps the most interesting lesson from the *D. discoideum* system for metazoan chemoreception is that apparently separate transduction pathways emanating from separate receptors, when examined using detailed molecular genetic and biochemical studies, are seen to cross over and interact. Additionally, G-protein function seems to be associated with 'switch' functions of proteins like the *synag7* and *fgdC* gene products. Lastly, redundancy, which is helpful to the organism, can confound the interpretation of experimental results, particularly when a gene is deleted and no consequence of this is found, as has been the case with genes for myosin II or cAR3 (Refs 41 and 42).

Ciliates

Behavioral responses of ciliates to chemicals in their environment, such as food cues and mating pheromones, are well documented, but the underlying signal-transduction pathways need exploration. Only two examples will be described here: the mating systems of *Euplotes* and the food-sensing systems of *Paramecium* (Fig. 1). Unfortunately, this approach ignores the interesting literature on ciliates such as *Tetrahymena*, *Blepharisma* and *Didinium*, and completely misses *Chlamydomonas* and diatoms[40,43–46].

Euplotes are large cells (>100 μm) with cilia fused into cirri for creeping along the substratum. When its meandering brings the *Euplotes* into the range of a cell of a different mating type, each cell will fall under the influence of the other's pheromone and become mating reactive. Such reactivity is assayed by the pairs that form (even between cells of the same mating type) when stimulated with pheromone[47]. Each cell constitutively produces a pheromone that is determined by the multiple alleles at the *mat* locus and, being diploid, each cell can carry up to two different alleles. In *E. raikovi* and *E. octocarinatus*, the alleles are co-dominant, thus making possible more-complicated mating interactions between heterozygotes.

E. raikovi is a marine ciliate with as many as 12 possible alleles at the *mat* locus. The pheromones, homodimers of 38–40 amino acid peptides (processed from larger prepropeptides[48]), bind specifically and saturably to cells at receptor sites with a K_d of $\approx 10^{-9}$ M (Ref. 49). The interesting twist that distinguishes *Euplotes* from yeast, *Dictyostelium* and sea urchin is that the pheromone appears to bind to a larger, membrane-bound form of the pheromone itself[49,50]. Thus, a cell secretes a pheromone that then binds to the membrane-bound version on its own cell surface, perhaps serving a growth stimulatory or other function in an autocrine fashion. It is only when *E. raikovi* of a different mating type appears that

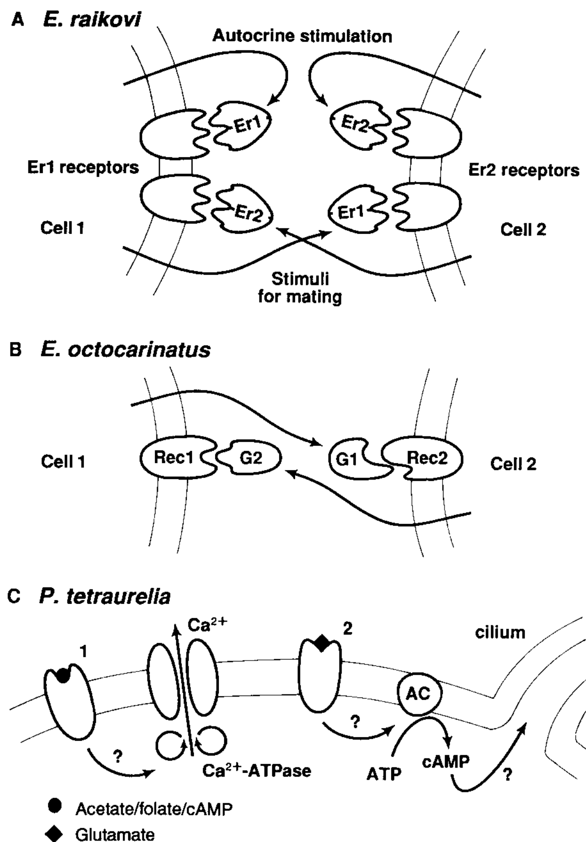

Fig. 1. *Chemoreception in* Euplotes *and* Paramecium. **(A)** Euplotes raikovi *mating pheromone (Er1 or Er2) is secreted by a cell that also synthesizes the receptor for this pheromone. In autocrine fashion, the pheromone binds to its receptor and either stimulates the cell for vegetative growth or does not initiate the signal-transduction pathway. When a secreted pheromone from a different mating-type cell comes into contact with the first cell, the second pheromone also binds to the receptor, but this binding initiates the mating reactivity pathway.* **(B)** *For* Euplotes octocarinatus, *like the situation in yeast, a cell synthesizes a receptor not for its own secreted pheromone but for a complementary one. Binding sets the signal-transduction pathway for mating reactivity into motion.* **(C)** *Two signal-transduction pathways for* Paramecium *are shown. (1) Stimuli such as acetate, folate and cAMP hyperpolarize the cell, probably by stimulating a Ca^{2+}-pump current. The nature of the coupling between the receptor and pump is not known. (2) Glutamate binds to a receptor defined only by its kinetics and pharmacological properties. This binding results in attraction of cells and rapidly increases the concentration of intracellular cAMP. The nature of receptor coupling is not known, nor is the role of intracellular cAMP in stimulation or adaptation.*

there will be a different pheromone to bind to the surface receptors with the same affinity, but with a very different outcome: mating reactivity. The signal-transduction pathways are not well characterized and it is not understood how binding of the autocrine pheromone does not initiate the mating reaction.

The amino acid sequences have been determined for five pheromones, three of which are unique. Genes for at least three of the pheromones and one putative receptor have been cloned and characterized. It appears that the pheromone Er-1 and its membrane-bound receptor originate from the same gene[51]. The receptor shares 75 amino acids with the prepro-pheromone form but has an additional 55 amino acids at the N terminus.

There are interesting developmental aspects of the *E. raikovi* pheromone response. For example, freshly mated cells are 'immature', that is, unresponsive to pheromone. This is probably because they have only about half the number of receptors of an older cell, which has $\sim 10^7$ receptors[52].

Euplotes octocarinatus is a fresh-water ciliate with a different approach to mating. There are ten mating types determined by four co-dominant alleles. Thus, there are only four pheromone molecules to contend with in this system. There are postulated to be four receptors that, as in yeast, are designed to bind pheromones other than the ones secreted by the cell expressing them[53]. The genomic sequences of three, and cDNAs for all four, pheromones are characterized[54,55]. Unlike *E. raikovi, E. octocarinatus* displays the peculiarity of some ciliates to use stop codons to code for amino acids. The resolution of the different approaches of the two *Euplotes* species to the mating response awaits the cloning of the receptor genes from *E. octocarinatus.*

Paramecium mating does not depend on diffusible mating perfumes. However, paramecia home in on a food source by using soluble food odors as stimuli for receptor-mediated signal-transduction pathways[4,56]. Folic acid is used by *Paramecium tetraurelia* as well as *Dictyostelium* for the location of bacteria, their food stuff for vegetative growth. Upon starvation, *Dictyostelium* become responsive to cAMP, and paramecia become mating reactive, but the ciliates remain responsive to folic acid as well as cAMP throughout their growth cycle and might use cAMP as a food cue rather than a social cue. Paramecia accumulate in solutions of folic acid or cAMP. These cells do not orient themselves and crawl directly up a gradient of attractant; rather, paramecia accumulate by biasing their meandering (that is, their straight paths broken by random turns) toward slightly faster swimming with fewer turns when they are moving up a gradient of attractant or down a gradient of repellant[56]. The cells' movement is caused by cilia beating under the control of the membrane potential: ciliary beat frequency increases and decreases with small hyperpolarizations and depolarizations respectively and the spontaneous turns are triggered by Ca^{2+} action potentials that reverse the power stroke of the cilia transiently[57]. Thus, the swimming behavior of paramecia is controlled by modifying membrane currents and permeabilities that change the membrane potential. This makes paramecia appealing to neurobiologists, who can penetrate a *Paramecium* cell with several electrodes. [There is a large body of work on the biophysics of *Paramecium* upon which to build (reviewed in Refs 57–59).]

There are multiple signal-transduction pathways in paramecia and there are large gaps in our current state of understanding in all of them. The first pathway is exemplified by folate and cAMP, which bind specifically and saturably to the cell body, and not to the cilia, of *P. tetraurelia.* The receptor for cAMP has been purified and is a protein of ~48 kDa. The protein appears as a doublet on gels and the bands of the doublet are antigenically similar and related by their peptide composition, differing apparently only at the N terminus. Antibodies against this receptor

render the cells unable to respond to cAMP, but do not affect the cells' ability to respond to the other stimuli tested[56]. Attractants such as cAMP and folate hyperpolarize the cells, which would account for the relatively smooth fast swimming in these attractants. The hyperpolarization is not due to channel activity or closure[60]. Instead, there is indirect evidence for the involvement of a Ca^{2+}-pump current in producing the hyperpolarization[60–62]. The gene for the plasma membrane Ca^{2+} pump is cloned[63] and knowing the sequence might enable the mechanism of coupling the receptors to the pump to be deciphered.

There are other stimuli that fall into a different class that does not affect Ca^{2+} homeostasis and the Ca^{2+} pump[64]. Ammonium chloride (NH_4Cl) is among these stimuli and does not appear to require a surface receptor for its effects. Preliminary measurements of intracellular pH with ion-sensitive dyes confirm that NH_4Cl can enter the cell directly as NH_3 and there alkalize the cell. On leaving the relative attractant NH_4Cl, the cell experiences an acid load[65]. We postulate that these changes in intracellular pH are responsible for the changes in membrane potential and the attraction to NH_4Cl relative to NaCl. Yet another pathway that has attracted our attention is that for glutamate, an attractant, and inosine monophosphate (IMP), a repellent[61,66]. Specific cell-surface receptors for these attractants have been characterized only for kinetic and pharmacological properties at this stage. Binding to receptor results in a rapid three-fold increase in intracellular cAMP in the case of glutamate and a drop by half in the case of IMP (Ref. 66). (Such changes in the levels of cAMP are not seen with stimuli such as acetate or NH_4Cl from other putative pathways.) Again, neither the nature of the coupling between the receptor and the effector enzyme AC nor the role of the cAMP is known at this time. However, cAMP could function directly as a second messenger or play a role in adaptation. Cyclic nucleotides are already known to be associated with shifts in membrane potential and thus with ciliary motility[56,67,68] and could be a direct message to the cilia, resulting in the characteristic behavior to these stimuli. Furthermore, extracellular GTP appears to repel paramecia by an unusual cyclical depolarization[69], suggesting that still other ciliate signal-transduction pathways exist.

News for neuroscience?

Perhaps this review has made apparent the many aspects of sensory-signal transduction in unicellular eukaryotes that cut across phylogenetic lines. The most striking examples are the receptors that have seven transmembrane regions, which couple to G proteins to influence an effector enzyme or channel. Many neurotransmitters signal through such systems, and the nature of the coupling and protein–protein interactions is sufficiently translatable from mammals to yeast that the human β_2-adrenergic receptor can be introduced into yeast to produce a β-agonist-activated pheromone signal-transduction pathway, complete with G1 arrest, morphological changes and transcriptional activation[70]. This is particularly informative given the distinct sequence characteristics of the yeast seven transmembrane region receptor and the unique role of the β and γ subunits of the G protein in carrying on the transduction pathway in yeast (see Ref. 70 for discussion).

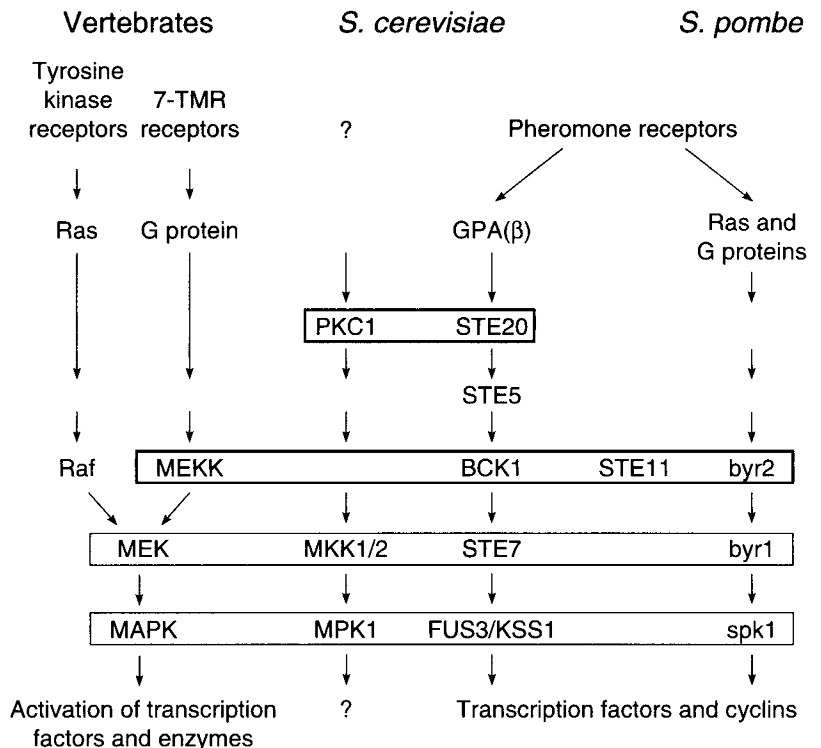

Fig. 2. *Parallels between signal transduction in vertebrates and yeast. The boxes indicate structurally conserved, related proteins. In vertebrates, MAPK (also known as ERK or extracellular-signal-regulated kinase) is the mitogen-activated protein kinase that must be phosphorylated on tyrosine and threonine residues to be activated. MEK (MAPK or ERK kinase) is the kinase that activates MAPK; MEKK (MEK kinase) phosphorylates and activates MEK. Raf is a structurally unrelated kinase that phosphorylates and activates MEK. PKC1 is the yeast homolog of protein kinase C; MKK1 and 2 (MAPK kinase), BCK1 (bypass of C kinase) and MPK1 (MAP kinase) are all kinases homologous to others indicated and positioned in the pathway by suppression and epistasis studies. (See Refs 78–82.)*

A 'hallmark' of neurotransmitter receptors is short-term desensitization through phosphorylation[26]. Multiple kinases phosphorylate serines and threonines of the receptor C terminus over different time scales or concentrations of ligand to achieve homologous or heterologous desensitization. Receptor subtypes differ in their C termini and thus impart useful differences in desensitization kinetics. Likewise, the unicellular eukaryotes' seven transmembrane region receptors are desensitized through phosphorylation. *Dictyostelium* receptor subtypes also differ in their C termini and thus hold similar potential for subtype differences in desensitization kinetics[41].

Only for the moment do the βγ subunits of yeast G protein remain somewhat distinctive in their positive roles in the pheromone signal-transduction pathway. While previously there were a few examples of direct effects of mammalian βγ subunits on phospholipase A_2 and ion channels[71], emerging roles for these subunits now include direct activation of AC as well as ion channels[72], in addition to a role in desensitization and pathway inhibition. For example, brain phosducin bound to βγ acts as an inhibitor of the GTPase in G_s-mediated activation of AC (Ref. 73). β-Adrenergic receptor kinase binds to βγ subunits, which enhances phosphorylation of the β-adrenergic receptors, probably by facilitating the localization of the kinase–βγ complex at the membrane, in close proximity to the receptor[74].

The growing number of members of the neuronal family of receptors having seven transmembrane regions provide enough variety for intercellular communication to accommodate development, tissue specificity, kinetic constraints, or ligand specificity. For example, a very large family of receptors with different odorant specificity is being borne out by the growing number of the olfactory receptor clones[71,75]. Similarly, multiple G proteins can be found within one tissue type, presumably to provide multiple signal-transduction pathways or crosstalk between receptors. In the receptor neurons of the olfactory epithelium and the cells of the taste bud, for example, there are G proteins almost unique to these tissues (G_{olf} and $G_{gustducin}$) amid other G proteins that are found elsewhere in the nervous system[76,77]. An appropriate example is the taste bud in which a variety of G_α's are found, including gustducin, various subunits αq and αi, as well as αs, and even transducin[76].

Despite their apparent homogeneity as a population, unicellular organisms are not to be outdone in displaying an array of receptors or G proteins. *Dictyostelium* has multiple cAMP receptors and multiple G proteins to subserve different requirements of developmental timing or cell-type specificity[33].

Our understanding of the mitogen-activated protein kinase (MAPK) signaling cascade has been revolutionized recently (Fig. 2, reviewed in Refs 11 and 78). The MAP kinase that requires phosphorylation of both threonine and tyrosine for its activation, is the key to the effects of disparate stimuli: growth factors that bind to receptors with built in tyrosine kinase activity and neurotransmitters that bind to the receptors having seven transmembrane regions. The yeast kinase homologs from *S. cerevisiae* and *S. pombe* were identified concurrently as the pheromone signal-transduction pathways unraveled and the yeast sequences *STE11* and *byr2* have even served as probes for the isolation of mammalian upstream-kinase counterparts[80]. MAP kinase is also activated in yeast through a pathway involving a protein kinase C (PKC) analog[81,82]. Protein kinase C1 is required for growth and differentiation and could fit into the vertebrate pathway downstream from activation of tyrosine kinase receptor or the seven transmembrane region pathway. Not all the pieces have fallen into place, but Fig. 2 shows the current state of understanding of the parallel pathways from vertebrates to yeast. Of note are the homologous proteins that serve similar functions and the potential for crosstalk between stimulus pathways. It will be interesting to sort out and compare the roles of Ras and PKC in *S. pombe*, *Dictyostelium* and neuronal cells.

Implicit in Fig. 2 is the potential for neuronal receptors having seven transmembrane regions to activate MAP kinase pathways and function as growth-factor receptors[26]. This stands in contrast to yeast and *Dictyostelium* seven transmembrane region receptors that serve to arrest growth or to activate motility and development. On closer examination of apparent differences, a common aspect of ligand-induced gene expression emerges. Perhaps some of the *Dictyostelium*, yeast, spermatozoa, *Euplotes* and *Paramecium* signal-transduction proteins that are currently without homologs in vertebrates really are for esoteric aspects of life as a microorganism, or perhaps the proteins that, at present, seem to be unique, will be found to be new components for vertebrate signal transduction.

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