

G PROTEINS AND OLFACTORY SIGNAL TRANSDUCTION

Gabriele V. Ronnett¹ and Cheil Moon

Departments of Neuroscience and Neurology¹, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; e-mail: gronnett@jhmi.edu; cmoon@jhmi.edu

Key Words olfaction, olfactory receptor neuron, adenylyl cyclase, sensory transduction, second messengers, cross-talk, desensitization

■ **Abstract** The olfactory system sits at the interface of the environment and the nervous system and is responsible for correctly coding sensory information from thousands of odorous stimuli. Many theories existed regarding the signal transduction mechanism that mediates this difficult task. The discovery that odorant transduction utilizes a unique variation (a novel family of G protein–coupled receptors) based upon a very common theme (the G protein–coupled adenylyl cyclase cascade) to accomplish its vital task emphasized the power and versatility of this motif. We now must understand the downstream consequences of this cascade that regulates multiple second messengers and perhaps even gene transcription in response to the initial interaction of ligand with G protein–coupled receptor.

INTRODUCTION

Cell signaling systems have developed to serve the diverse intracellular and intercellular communications needs of complex organisms. Study of these signaling pathways has revealed that cells employ variations of common transduction motifs to generate their responses. This is true even for sensory signaling, in which environmental stimuli must be interpreted as the first step in sensory perception. The correct analysis of sensory input is vital to an organism's survival. The olfactory system has challenged many researchers seeking to understand the molecular aspects of sensory signal transduction and coding mechanisms (1–5).

The olfactory system must discriminate among thousands of odors comprised of chemically divergent structures (odorants). As for other sensory modalities, a combination of molecular, electrophysiological, and cell biological approaches was required to delineate odorant transduction. What has emerged is that odorant transduction combines unique receptive molecules with common G protein–mediated transduction cascades to detect odorants. Many of these features are conserved across phyla, as recently reviewed by Hildebrand & Shepherd (5). Although G protein cascades are involved in the initial events of odor perception,

what has emerged is that a variety of signal cascades are activated in addition to a G protein cascade. The roles of these other signals are only beginning to be understood, and many controversial issues remain (6).

CELLULAR COMPOSITION OF THE OLFACTORY EPITHELIUM

The initial events of odor detection occur at the peripheral olfactory system, which is well adapted structurally to perform its function. The primary olfactory sensory receptor neurons are located in the olfactory epithelium, where they are in direct contact with inhaled odorants. There are three principal cell types in the olfactory epithelium: olfactory receptor neurons (ORNs), supporting sustentacular cells, and several types of basal cells (7, 8).

ORNs are bipolar, extending apical dendrites to the surface of the neuroepithelium and sending unmyelinated axons through the basal lamina and cribriform plate (of the ethmoid bone) to terminate in the brain on dendrites of mitral and tufted neurons in the glomeruli of the olfactory bulb. The apical dendrites form dendritic knobs from which arise specialized, nonmotile cilia, where the initial events of olfactory transduction occur (2, 9, 10). Electrophysiological studies indicate that odorant sensitivity and the odorant-induced current are uniformly distributed along the cilia, suggesting that all the components of the immediate responses to odorants are localized to the cilia. Immunoelectron microscopic studies have confirmed the cilial localization of many of these components (11, 12). ORNs comprise 75–80% of the cells in the epithelium (13) and are functionally homogeneous: They all detect odorants. ORNs senesce and die throughout life at a regular rate. They are replenished by the differentiation of globose basal cells (14–16). As they mature, ORNs move apically in the epithelium, permitting determination of neuronal age by position (17), with mature ORNs expressing olfactory marker protein (18, 19). Interestingly, this neurogenesis can be hyperinduced by ablation of the olfactory bulb (termed bullectomy) (20). Thus, the understanding of the functions of signaling components in signal transduction can be facilitated by studies of the spatial organization and development of ORNs.

Sustentacular cells are in general considered to be supportive cells and share features in common with glia. They stretch from the epithelial surface to the basal lamina, where they maintain foot processes (2, 3). Sustentacular cells electrically isolate ORNs, secrete components into the mucus, and contain detoxifying enzymes (21). The sustentacular cells contain high concentrations of cytochrome P450-like enzymes (22). Regarding odorant transduction, it is thought that these enzymes may modify odorants to make them less membrane permeable or inactivate them. Recent studies indicate that sustentacular cells may produce growth factors important to ORN development (23). Neuropeptide Y (NPY) is an amidated

neuropeptide that performs many functions in mammalian physiology (24, 25). NPY mRNA is upregulated following peripheral axotomy and in pheochromocytoma and ganglioneuroblastoma tissue (26). Whereas NPY is expressed in developing ORNs during embryogenesis, it is expressed in sustentacular cells in the adult olfactory epithelium, functioning as a neuroproliferative factor for olfactory neuronal precursors *in vivo* and *in vitro* (23). Thus, NPY is the first of possibly many growth factors that maintain ORN homeostasis.

The basal cells underlie the ORNs and serve as precursors for the generation of new ORNs throughout adulthood (7, 8, 16). Basal cells have been divided into two general classes. Horizontal cells are morphologically flat and express cytokeratin (7, 27), and globose basal cells are rounded in shape and express several markers, including GBC-1, GBC-3, and GBC-5 (28, 29). Compared with other neurons, ORNs have a shorter average lifetime, in the range of several months. This may in part be due to the fact that ORNs are exposed to a variety of toxic or infectious agents. Given the turnover of ORNs throughout life, the role of the globose basal cells in providing new ORNs is crucial to the maintenance of the sense of smell.

GENERAL FEATURES OF ODORANT TRANSDUCTION

Odorant signal transduction is initiated when odorants interact with specific receptors on the cilia of ORNs (1, 30–32) (Figure 1). Receptors subsequently couple to a G protein to stimulate adenylyl cyclase (33–35). Electrophysiological and biochemical studies confirm that cAMP is the key messenger in the initial phase of odorant detection (33, 34, 36–41). The concentration of cAMP in the cilia rises, gating open a cyclic nucleotide-gated channel, resulting in an influx of Na⁺ and calcium (42, 43). The immediate response is the generation of a graded receptor potential (44, 45).

In addition, several other second messenger cascades that are activated upon odorant detection may regulate secondary events or odorant adaptation. These include pathways activated downstream of the cyclic nucleotide-gated channel and include the consequence of the channels' substantial calcium permeability (46, 47). Odorants also increase phosphoinositide hydrolysis and the production of inositol-1,4,5-trisphosphate (IP₃) (35, 48–50). Cyclic GMP production is also increased with odorant exposure (51, 52). Interestingly, the ORN's response to odorant-induced cGMP production is much slower than the cAMP or IP₃ responses, which normally peak within 500 ms. Thus, the cGMP response does not appear to function in the immediate detection phase of olfaction, such as modulating cyclic nucleotide-gated cation channels or IP₃ receptors, but rather in desensitization or the modulation of the cellular response during longer exposures to odorants (53–56). The relationship of these messengers to the G protein-coupled cascade is discussed in subsequent sections.

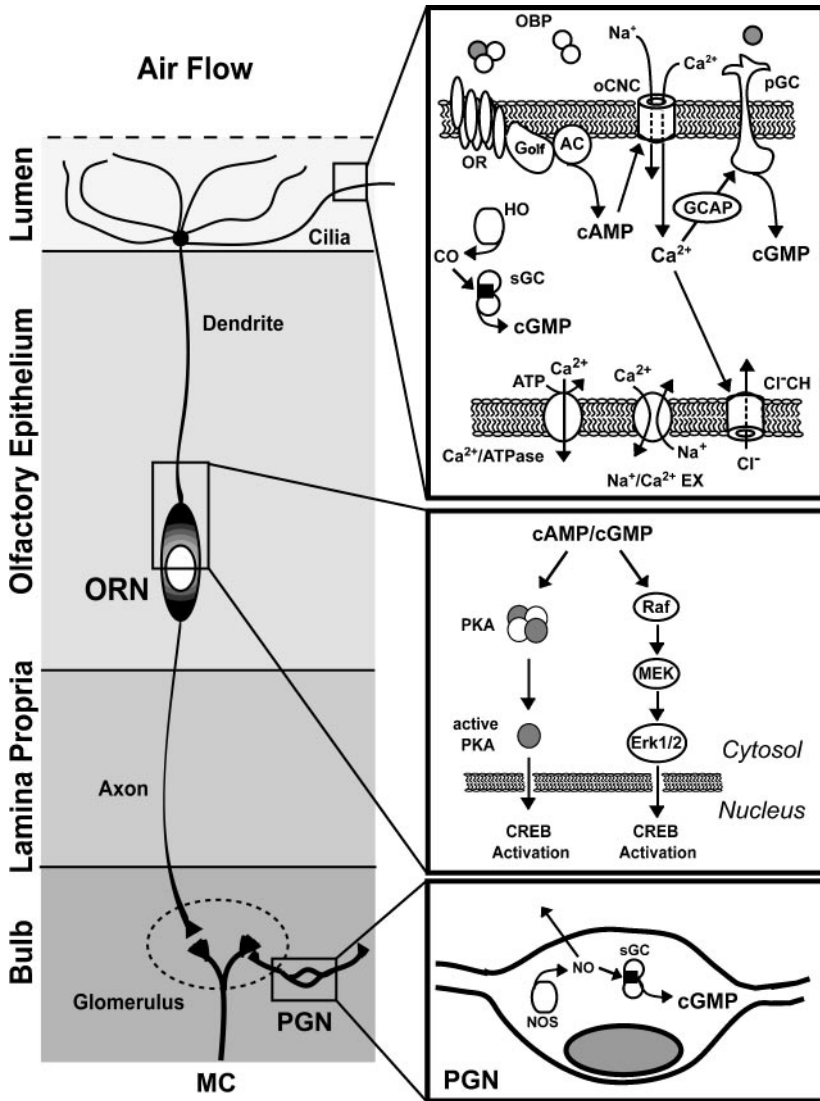


Figure 1 Model of odorant signal transduction. See text for details. There are signaling cascades that mediate the initial phase of odorant detection and that mediate potential long-term responses to odorant detection. Abbreviations: AC, adenylyl cyclase; CO, carbon monoxide; CREB, cAMP-responsive element binding protein; GCAP, guanylyl cyclase activating protein; G_{olf} , olfactory G protein; HO, heme oxygenase; MEK, MAP or ERK kinase; OBP, odorant binding protein; oCNC, olfactory cyclic nucleotide-gated channel; OR, odorant receptor; pGC, particulate guanylyl cyclase; PGN, periglomerular neuron; PKA, cAMP-dependent protein kinase; Raf, MEK kinase; sGC, soluble guanylyl cyclase.

COMPONENTS OF THE ODORANT TRANSDUCTION CASCADE

Odorant-Binding Proteins

The existence of carrier proteins for odorants resident in the nasal mucus was predicted based upon the fact that hydrophobic odorants must travel through the aqueous mucus barrier toward the cilia of ORNs. In fact, odorant-binding proteins (OBP) were discovered by several laboratories in early attempts to identify odorant receptors using radioactive odorants such as 3-isobutyl-2-methoxy-pyrazine (57–59). Native OBP purified from olfactory mucus is a homodimer of two 19-kDa subunits with an affinity for odorants in the micromolar range (60).

The molecular cloning of OBP helped clarify its function. OBP is a member of the lipophilic molecule carrier protein family; a well-characterized member of this family is a retinol-binding protein of the retina. This protein conveys retinol from retinal pigment epithelium to rods and cones where it is incorporated into rhodopsin (61). *In situ* hybridization studies using probes to visualize OBP mRNA revealed its selective concentration in the lateral nasal gland, the largest of 20 discrete nasal glands in mammals (62). OBP thus appears to be secreted from this gland down a long duct to the tip of the nose, where watery secretions are atomized to humidify inspired air. OBP thus localized might trap odorants and carry them with inhalation to ORNs. Alternatively, OBP may function to remove odorants from the sensory epithelium and cilia.

Further studies have revealed that more than one form of OBP is expressed in the nasal epithelium. Rabbitts and colleagues (63) identified a second form of OBP, OBPII. OBPII encodes a secretory protein with significant homology to OBPI, and it is also expressed in the lateral nasal gland, which is the site of OBP expression. Interestingly, the OBPII sequence shows significant homology to the VEG protein, which is thought to be involved in taste transduction (64). Breer and colleagues demonstrated that although OBP proteins appear to share many structural features, recombinant rat OBPI and OBPII each interact with distinct sets of odorants and therefore contain distinct ligand specificities (65). OBPI binds specifically to a pyrazine derivative, 2-isobutyl-3-methoxy-pyrazine, whereas OBPII binds to the chromophore, 1-anilinonaphthalene 8-sulfonic acid (1,8-ANS), specifically. In other vertebrates multiple forms of OBP have been identified. There are four OBPs in mice (66), three OBPs in rabbit (67), and two OBPs in cow (68, 69). OBP has also been cloned from insects (70, 71).

Odorant Receptors

Mammals perceive a huge variety of environmental odors. The initial step in odor recognition involves the interaction of odorous ligands with specific receptors in the ciliary membrane of ORNs (1, 30–32). Based upon the assumption derived from biochemical evidence that odorant signal transduction involves G proteins and G protein-coupled receptors, a very large gene family of closely related olfactory-specific seven-transmembrane spanning domain receptors was identified

by polymerase chain reaction (1, 72, 73). In vertebrates the family of odorant receptors (ORs) encodes as many as 1000 genes, suggesting that some of the steps following odorant recognition occur within the primary sensory neurons themselves. To date, OR genes have been isolated from 12 vertebrate species: rat, mouse, human, catfish, zebrafish, dog, frog, chicken, pig, opossum, mudpuppy, and lamprey (74). In humans estimates for the size of the receptor family range from 500 to 1000 genes. Interestingly, compared with the other species, human OR clones display a high frequency of pseudogenes (74).

The genes encoding ORs may be initially classified as Class I (fish-like) and Class II (tetrapod-specific) ORs. Class I ORs are specific for recognizing water-soluble odorants, whereas Class II ORs bind airborne odorants. All human Class I ORs are localized in a single large cluster, and half of those ORs are apparently functional (75). Expression of Class I ORs has already been reported in rats (76) and in human (77). Class II families are all present in more than one chromosome each, except for a small family of 12 (75). The genes encoding ORs are devoid of introns within their coding regions (72). Mammalian OR genes are typically organized in clusters of 10 or more members and located on many chromosomes. There is a strong correlation between the localization of an OR in a particular chromosomal cluster and its position in a phylogenetic dendrogram derived from comparison of full-length OR protein sequences (78). The repertoire of human OR genes contains a large fraction of pseudogenes, suggesting that olfaction became less important in the course of primate evolution.

Our knowledge of human ORs has been facilitated through the Human Genome Project. The availability of this sequence information indicated that the overall number of human ORs may be in excess of 1000, as previously predicted (79), and that only one third of the human ORs appear to be functional, consistent with previous reports showing a large proportion of pseudogenes (80). One group predicts that 906 human OR genes are present in the human genome, of which approximately 60% appear to be pseudogenes (81), compared with fewer than 5% pseudogenes in rodents or lower primates.

The locations of human ORs have been elucidated in the past several years. Human ORs contain large genomic segments that have been duplicated to many locations in the genome (82), particularly near telomeres (83). The overall localization of human ORs on all chromosomes except 20 and Y is in agreement with previous work based on fluorescence *in situ* hybridization (80). Human ORs are predominantly localized to the middle of the q arms with several additional genes located near the p telomere. Human chromosome 11 appears to be the origin of the human OR repertoire. This chromosome contains 42% of all human ORs and is the only one containing Class I receptors. Moreover, this chromosome contains the most diverse collection of OR families, 9 out of the 13 Class II families. It also has the two largest clusters in the genome, each with more than 100 ORs. Interestingly, this chromosome shows contiguous conserved synteny in species from humans to the earliest mammals (84).

The expression pattern of ORs in ORNs shows an unusual spatial distribution (85, 86). *In situ* hybridization studies showed that OR mRNAs are expressed within

one of several broad, nonoverlapping zones. Within a zone ORs are expressed in a random manner. Each zone occupies about a quarter of the olfactory epithelium (85) and is represented on the turbinates and on the septum (87). However, the physiological relevance of zonal expression remains unclear.

Although a number of studies have been done on the expression and distribution of ORs at the message level, relatively little is known about the expression of OR proteins. Polyclonal antibodies have been raised against some ORs, permitting visualization of OR proteins. In rats, an OR is expressed as early as E14 in a zonally restricted pattern (88). The expression of ORs appears restricted to the cilia and dendritic knobs of ORNs. The cilia-specific expression of ORs supported a role for ORs in olfactory transduction (12, 89, 90). A concern with studies utilizing antibodies to identify discrete members of the OR family is the specificity of the antibodies, given the large numbers of receptors. Thus, despite the general utility of antisera for immunohistochemical and biochemical studies, the enormous size of the OR repertoire limits the feasibility of proving the specificity of an antibody for a specific receptor.

Significant difficulties with the heterologous expression of ORs have severely limited studies designed to provide functional confirmation of the role of ORs. The most convincing data concerning function have been provided by four approaches. One approach utilized genetic studies in *Caenorhabditis elegans*, which demonstrated that the ODR-10 mutant lacked a seven-transmembrane receptor and was deficient in its ability to detect acetyl (91). Krautwurst et al. (92) achieved similar functional heterologous expression of rodent ORs also using HEK-293 cells. This group generated an expression library of mouse ORs and identified three ORs responding to carvone, (–) citronellal, and limonene using micromolar concentrations of these odorants. Firestein and colleagues also demonstrated functional expression of a cloned OR in rat nasal epithelium by using a recombinant adenovirus containing a putative OR to infect rat nasal epithelium in vivo (93). They demonstrated that this specific OR was overexpressed in the rat olfactory epithelium and, by electro-olfactogram recording, that the expressed OR conferred a response to a small subset of odorants. Malnic et al. (31) performed single-cell polymerase chain reaction on ORNs whose odorant responses had been determined as isolated cells in culture, to demonstrate that a combinatorial code of receptor gene expression exists for odorant perception. These approaches of developing functional expression systems to study ORs may prove to be extremely useful for the screening of ORs on a large scale, as well as understanding the molecular mechanism of odorant recognition.

Besides functioning in odorant transduction at the dendritic end of the bipolar ORN, ORs may be involved in determining or guiding ORN axonal projections to the olfactory bulb and possibly to specific glomeruli (94, 95). In rodents the axons of ORNs that express the same OR message converge to defined glomeruli in the olfactory bulb, suggesting that the rodent olfactory bulb is topographically organized and that an ORN expressing a specific OR projects to and synapses with the representing glomeruli in the olfactory bulb. This type of organizational “wiring” prompts an interesting hypothesis. It suggests that the signals from ORNs

expressing a single OR gene (out of the approximately 2000 alleles of the rat OR repertoire) are represented in glomeruli as a topographical map in the olfactory bulb; as a consequence an environmental odor is encoded by activation of a specific set of glomeruli.

Some studies indicate that receptors closely related to OR genes may be expressed in tissues other than the olfactory epithelium. This finding suggests that there may be alternative biological roles for this family of chemosensory receptors. Expression of various ORs was reported in human and murine erythroid cells (77), developing rat heart (96), avian notochord (97), and lingual epithelium (98). The best case for the existence of ORs is the finding that genes related to mammalian ORs are transcribed in testes and expressed on the surface of mature spermatozoa, suggesting a possible role for ORs in sperm chemotaxis (99, 100).

G Proteins

The first evidence for the involvement of G proteins in odorant transduction was obtained through biochemical experiments in which investigators demonstrated that the odorant-induced stimulation of olfactory sensory cilia was dependent upon the presence of GTP (101). Subsequently, a G protein was cloned from an olfactory cDNA library. This clone, termed G_{olf} , was highly and almost exclusively expressed in ORNs (102). G_{olf} was able to stimulate adenylyl cyclase in heterologous systems. Aside from its expression in ORNs of the olfactory epithelium, G_{olf} was expressed in basal ganglia (103). As mentioned, odorants also increased IP_3 production, causing many to postulate that cilia might contain olfactory-specific G_q proteins. To date, an olfactory-specific class of G_q proteins has not been reported.

Mice with targeted disruption of the gene for G_{olf} displayed a striking reduction in the electrophysiological response of ORNs to a wide variety of odors, supporting the hypothesis that G_{olf} , and thus this G protein-mediated cascade, is required for odorant signal transduction (104). Despite this intense attenuation in response to odors, the topographic map of ORN projections to the olfactory bulb was unaltered in G_{olf} -deficient mice. Thus, odorant stimulation may or may not be an essential process in determining the targets of ORN axonal projections to the olfactory bulb. However, for a conclusive answer, these studies may need to be done at higher resolution.

SIGNALING CASCADES AND THEIR COMPONENTS IN ODORANT DETECTION

cAMP

The first direct biochemical studies reported an odorant-induced cAMP response in olfactory sensory cilia isolated from both frog and rat (33, 34). The olfactory sensory cilia were prepared by subcellular fractionation after calcium shock of the olfactory epithelium (101). The odorant-stimulated production of cAMP was tissue

specific and occurred only in the presence of GTP, suggesting the involvement of receptors coupled to G proteins.

Electrophysiological studies provided further evidence for the central role of cAMP in odorant detection. Recordings from excised membrane patches of cilia demonstrated a cAMP-gated conductance (42). Investigators proposed that an odorant would increase intracellular cyclic nucleotide concentration to gate a cationic conductance, initiating a depolarizing response. Kinetic studies of odorant-induced currents recorded in the whole-cell configuration (43, 105) suggested that the latency of the odorant response (several hundred milliseconds) indeed supported a role for a second messenger such as cAMP. Further biochemical characterization using isolated rat olfactory sensory cilia showed that cAMP was best produced by fruity, floral, and herbaceous odors (34, 42). Screening many odorants at a single concentration revealed only minimal cAMP production by some, generating the hypothesis that those odorants with small or no cAMP responses employed another cascade, perhaps inositol phosphates (34, 42). These initial measurements were made 15 minutes after the exposure of isolated cilia to odorants.

To demonstrate that the production of cAMP occurs on a relevant time scale, sub-second kinetics of odorant-induced changes were analyzed using a rapid quench-flow device (39, 106). In this device cilia membranes and odorant solutions were reacted together using computer-controlled mixing, with subsequent quenching of samples at intervals from 8–500 ms. cAMP was produced rapidly and transiently in response to odorants, with increases evident as early as 25 ms. Certain odorants, such as fruity odors, were able to stimulate cAMP production at concentrations as low as 10 nM, whereas others, such as putrid odors, had no effect, even at higher concentrations. Those odorants that did not stimulate cAMP production were hypothesized to act through the phosphoinositide cycle. High (millimolar) levels of calcium inhibited the response; intermediate concentration ranges, however, were not tested.

The odorant-induced cAMP response was investigated further using isolated rat olfactory cilia determining the generality of the odorant-induced cAMP response and the calcium dependence of this response (38). Odorants indeed cause rapid and transient elevations of cAMP, as well as the more sustained signal, as seen by Pace et al. (33) and Sklar et al. (34). Different from the observation from Breer's group (39, 106), all odorants stimulated cAMP production. Interestingly, responses were nonlinear. Thus, there was an initial dose-dependent cAMP response with increasing odorant concentration that decreased at higher odorant concentrations, and for some odorants increased at even greater concentrations. Basal and odorant-induced cAMP levels in cilia demonstrated a biphasic calcium dependence, with peak cAMP stimulation in the range of 1–10 μ M free calcium. Dose-response curves done at two calcium levels showed that the influence of calcium on odor responses was complex, suggesting the possible involvement of calcium in both signal generation and termination.

To evaluate odorant signal transduction in intact cells, a primary culture system of olfactory epithelium enriched in ORNs was developed (35, 36, 107). Using this

primary culture system, cAMP responses to odorant stimulation were monitored in intact ORNs. Odorants were quite potent at producing cAMP, with as little as 0.1 nM isobutylmethoxypyrazine (IBMP) generating a response (35, 36). Responses were multiphasic; cAMP production increased with increasing odorant concentration, decreased at even higher odorant concentrations, and sometimes reappeared at still higher (1–10 μ M) concentrations. Signals were calcium dependent, with maximal adenylyl cyclase activity at 10 μ M free calcium and inhibition at higher calcium concentrations. Odorant induction of cAMP production was rapid, with peak effects observed at 10–15 sec, but signals continued well above baseline for minutes, confirming results from Sklar et al. (34) and Pace & Lancet (108). The duration of the cAMP response observed in whole cells was significantly longer than that measured using isolated cilia. This may be because of differences between the preparations, or because cAMP functions in more than the initial rapid phase of odor detection. This latter possibility is considered in later sections.

Adenylyl Cyclases

Cyclic AMP is generated by adenylyl cyclases. There are at least nine known isoforms of adenylyl cyclases (109). Bakalyar & Reed (110) cloned a novel adenylyl cyclase, AC3. Northern blot analysis revealed that AC3 mRNA was enriched in the olfactory epithelium and that AC3 message disappeared after bulbectomy. When expressed in HEK293 cells, AC3 had almost no basal activity. In contrast, two other isoforms of adenylyl cyclase, AC1 and AC2, have high basal activities. The low basal activity of AC3 may be relevant to its role in sensory transduction. G_{olf} and AC3 have been ultrastructurally localized to olfactory cilia, indicating that G_{olf} may mediate the activation of AC3 (111).

To evaluate the role of AC3 in the olfactory transduction, the AC3 gene has been disrupted in mice (41). Odorant-induced responses measured by electro-olfactogram were completely eliminated in AC3-null mice. Moreover, odor-dependent learning was impaired in these mice. Interestingly, both fruity odors (transduced by cAMP) and putrid odors (transduced by IP_3) failed to evoke any response in these animals. This observation was mimicked by a pharmacological study that showed that adenylyl cyclase antagonists reversibly inhibit electro-olfactogram responses, even to putrid odors (formerly thought to act through IP_3) (112). Taken together, these results confirmed earlier biochemical studies that implicated cAMP as essential for the initial phases of odorant transduction. IP_3 was therefore postulated to play more of a modulatory role in the odorant transduction in mammals.

Certain adenylyl cyclases are rather broadly expressed, whereas others are restricted in their distribution (113). Although AC3 is highly enriched in ORNs, especially in cilia, other adenylyl cyclases, such as AC2 or AC4, have also been associated with olfactory neuroepithelium, raising the issue that other adenylyl cyclases may be important in different aspects of olfactory signal transduction.

Adenylyl cyclases are regulated by different mechanisms. Studies by Storm and colleagues (114–116) indicated that the mechanisms of regulation of adenylyl cyclases may not only be dependent upon the specific kind of adenylyl cyclase expressed in a tissue, but by local influences and the expression of regulatory molecules in that specific cell. Thus, whereas ectopically expressed AC3 may be stimulated by calcium, *in vivo* studies in certain tissues argue for the inhibition of AC3 by calcium. Equally diverse are the effects of protein kinases on adenylyl cyclases. Phorbol esters are used to mimic the effects of protein kinase C (PKC) activation and elicit a stimulatory effect on AC2 but barely stimulate AC1 or AC8. These latter adenylyl cyclases are stimulated up to eightfold by calcium (117). Frings (118) has reported that activation of PKC by phorbol esters increased cAMP in frog olfactory tissue. Calmodulin mediates the stimulation by calcium of AC1, AC3, and AC8 (119); it is unclear how the calcium sensitivity of the calcium inhibition of AC5 and AC6 is achieved. There is also evidence that PKA may affect adenylyl cyclase activity.

Olfactory Phosphodiesterases

The ambient level of cAMP in a cell is dependent upon both the synthesis and degradation of cAMP. Odorants clearly activate adenylyl cyclase, but is there any effect of odorants on phosphodiesterases (PDEs)? There are at least seven different gene families of PDEs whose activities are regulated by calcium, cyclic nucleotides, and phosphorylation (120–123). Thus, odorants could have an indirect effect on the degradation of cAMP, potentially providing a second site of regulation for the odorant-induced cAMP response. Several forms of cAMP-PDE are expressed in rat olfactory cilia (124–125). A novel calcium/calmodulin PDE (CaM-PDE) is selectively found in ORNs, with prominent ciliary expression. This novel CaM-PDE has a high affinity (K_m of 1.4 μM) for cAMP and could be activated by odorants in response to intraciliary calcium increases. Cloning of the high-affinity PDE revealed it to have a higher affinity for cAMP than any known brain isoform (126). This PDE, designated PDE1C2, is well suited for restoring the submicromolar levels of cAMP after odorant stimulation. In an ectopic expression system, maximum activation by calcium was reached at 10 μM calcium concentration.

A subset of olfactory neurons expresses cGMP-stimulated phosphodiesterase (PDE2) (127). In these specific ORNs, guanylyl cyclase type-D (GC-D) is also expressed, suggesting that it may play an important role in odorant transduction for a specific subset of responses. PDE2 and GC-D are both expressed in olfactory cilia of these neurons; however, only PDE2 is expressed in axons (127). In contrast to most other ORNs, these neurons appear to project to a distinct group of glomeruli in the olfactory bulb similar to the subset that have been termed necklace glomeruli. Furthermore, this subset of neurons are unique in that they do not contain several of the previously identified components of olfactory signal transduction cascades involving cAMP and calcium, including a calcium/calmodulin-dependent PDE (PDE1C2), AC3, and cAMP-specific PDE (PDE4A) (127, 128).

Interestingly, these latter three proteins are expressed in the same neurons; however, their subcellular distributions are distinct. PDE1C2 and AC3 are expressed almost exclusively in the olfactory cilia, whereas PDE4A is present only in the cell bodies and axons. Taken together, these data strongly suggest that selective compartmentalization of different PDEs and cyclases is an important feature for the regulation of signal transduction in ORNs.

A recent study identified some ORNs devoid of G_{olf} , ACIII, PDE1C2, and the cyclic nucleotide-gated channel subunits $\alpha 3$ and $\beta 1b$ that are expressed in prototypical ORNs (127). These particular ORNs express GC-D, PDE2, and cGMP-selective $\alpha 2$ channels (127, 128). GC-D is related to the Ca^{2+} -regulated retinal GC forms GC-E and GC-F, as opposed to the receptor GCs, GC-A, GC-B, and GC-C, which are activated by peptide ligands (129). In particular, GC-D and GC-E/F share characteristic sequence similarity in a regulatory domain that is involved in binding of GCAPs (130). This similarity raises the intriguing possibility that GC-D activity is under the dual control of an unknown extracellular ligand and Ca^{2+} (54). These ORNs project their axons in glomeruli different from ACIII-expressing ORNs and form necklace-shaped synapses in the glomeruli. The necklace glomeruli in the olfactory bulb are spared from the morphological alterations observed in the typical glomeruli of the G_{olf} null-mice, suggesting that a typical glomeruli receives innervation from a subset of receptor neurons that use a pathway independent of cAMP signaling (131).

Channels

In ORNs, ion channels are expressed in ciliary processes of dendritic endings, where they amplify the odor-induced receptor current. Ca^{2+} signals generated by cyclic nucleotide-gated channels (CNCs) are at the heart of sensory transduction in vision and olfaction. The ability of CNCs to conduct Ca^{2+} determines both the rise time and the amplitude of the olfactory receptor current, as well as its termination after the stimulus. Ca^{2+} -gated Cl^{-} channels are triggered by odor-induced Ca^{2+} influx through CNCs and cause a depolarizing Cl^{-} efflux that amplifies the receptor current (132). The extrusion of Ca^{2+} ions is mediated by Na^{+}/Ca^{2+} exchange mechanisms in cilia and probably involves Ca^{2+} -ATPases in knobs, dendrites, and cilia (133–137).

OLFACTORY CYCLIC NUCLEOTIDE-GATED CHANNELS

General

The gating of CNCs accounts for the initial component of the odor-induced electrical response, and this event is crucial to recognizing the environmental signal in the central nervous system. In fact, mice deficient in $CNC\alpha 3$, which is essential for forming functional CNCs, suffer from general anosmia (40, 138). Retinal and olfactory CNCs share a high degree of sequence similarity (over 80% amino

acids identity) in the cyclic nucleotide binding sites, but they show very different characteristics. First, cyclic nucleotide selectivities of these CNCs are very different (139). The retinal CNCs show much higher apparent affinity for cGMP (140, 141), whereas cAMP and cGMP have very similar effects on the olfactory CNCs (42, 140). cAMP and cGMP are varied only in their purine ring structure. Next, the olfactory CNC (oCNC) has a larger single-channel conductance (55 versus 20 ps), a lower degree of selectivity among monovalent cations, and a larger apparent pore diameter (6.3 versus 5.8 Å, determined from organic cation permeability) than the retinal CNC (142). Notably, in the absence of divalent cations, unit conductance of the oCNC is 25–40 ps (143, 144).

oCNCs have a higher affinity for cyclic nucleotides than visual CNCs and display higher affinity for cGMP than for cAMP (145). However, cGMP may not gate oCNCs in the earlier olfactory signal transduction because the cGMP response is not large and fast enough to gate oCNC (37, 54).

Composition

The oCNC was identified based on the visual CNC. Initially, the α -subunit was cloned, but the expressed channel showed different characteristics from the wild type. Later the β -subunit was cloned and conferred the affinity for cAMP (146). To date, three subunits that form the oCNCs of ORNs have been identified, and the rod photoreceptor channels have at least two subunits (147–150). In addition, a second type of modulatory subunit is part of the olfactory channels (146, 151, 152). Native oCNCs appear to contain not only α and β subunits but also a splice variant of the β subunit of rod photoreceptor CNC (153). Recent research revealed that native oCNCs comprise a heteromeric channel complex consisting of $\alpha 3$, $\alpha 4$, and $\beta 1b$, which is highly permeable to Ca^{2+} (150, 154).

Exogenous expression of α subunits alone generates functional CNCs in oocytes. In contrast, the β subunit does not yield functional channels when expressed on its own. However, coexpression of the β subunit with the α subunit shifts the $K_{1/2}$ values for cAMP to lower values and yields channels whose properties resemble more closely the native rat olfactory channel (146, 153). However, the $K_{1/2}$ values for cAMP are still threefold higher than those in the native channels (146, 153).

Regulation

The activity of oCNCs is regulated in various ways. The most rapid is the negative feedback inhibition of oCNCs by Ca^{2+} /calmodulin (155, 156). Ca^{2+} /calmodulin causes a decrease in the apparent cyclic nucleotide affinity of both rod (157, 158) and olfactory (155, 159) CNCs. Elevation of Ca^{2+} owing to influx through oCNCs reduces its affinity for cAMP, resulting in a lowering of its open probability (156). This modulation occurs by the direct binding of Ca^{2+} /calmodulin to the amino-terminal region of the CNCs and does not involve the action of a kinase (155). The decrease in apparent cyclic nucleotide affinity arises from a decrease in the stability of the allosteric opening transition, consistent with the effect of mutations

in the amino-terminal region (155). Ca^{2+} -mediated desensitization appears to act through an allosteric mechanism with the effect of stabilizing a closed state of the channel.

Divalent cations like Ca^{2+} and Mg^{2+} suppress the conductance of the oCNCs (42). First, Ca^{2+} regulates the activity of oCNCs. In the presence of Ca^{2+} , the open probability of single CNCs was reduced from 0.6 to 0.09 in the presence of 100 μM cAMP, whereas the single channel conductance remained unchanged (160). Kramer & Sieglebaum (161) demonstrated a similar action by intracellular Ca^{2+} in catfish olfactory neurons. The effect of Ca^{2+} is to reduce the open probability by shifting the affinity of the channel for cAMP to higher concentrations; this effect was overcome by application of high concentrations of cAMP (162). Extracellular Ca^{2+} also lowers the affinity of the oCNC for cAMP by a different mechanism (163). Ni^{2+} also produces an inhibition in the oCNCs and may primarily bind to the channel when it is closed (158). This effect is the opposite of the observation in the rCNC. The binding site for the inhibitory effect of Ni^{2+} on the oCNC is localized to a single histidine residue (H396) at a position just three amino acids downstream from the homologous potentiation site in the rod channel.

Phosphorylation is the most common posttranslational regulatory mechanism. The affinity of the oCNCs for cAMP is affected by phosphorylation of oCNC subunits (164). When Ser93 of the oCNC α subunit is phosphorylated, the affinity for cAMP increases. The protein kinases involved in this effect are PKCs (γ , δ , and τ). When the ORNs are activated by phorbol ester, a PKC activator, the ORNs showed larger responses. Interestingly, the oCNC β subunit is a subject of phosphorylation by the protein tyrosine kinase (PTK), whereas the α subunit is not affected by PTK inhibitor (165, 166). Homomeric channel complexes with α subunits are unaffected by geniestin, whereas heteromeric channel complex $\alpha + \beta$ channels are inhibited, indicating that the β subunit may interact with the PTK. This is different from the rCNC, in which the α subunit is crucial for allowing interaction with PTK (166). The two subunits that are unable to interact with the PTK (α subunit of oCNC and β subunit of rCNC) share a homologous domain in the NH2 terminus that enables these subunits to interact with Ca^{2+} /calmodulin (155, 159). Expression of oCNC subunits is especially widespread in the nervous system and elsewhere, but channels in different locations may differ in their subunit compositions (167, 168), potentially providing for differential modulation by protein kinases.

Expression of Cyclic Nucleotide-Gated Channels in Other Systems

CNCs have been found elsewhere in the nervous system (153, 169) and have been implicated in processes as diverse as synaptic modulation and axon outgrowth in animals ranging from the nematode to mammals (170, 171). CNCs have also been found in a variety of other cell types including kidney, testis, and heart (47, 172), where they may fulfill various physiological functions.

Transcripts for subunit α of both the rod photoreceptor (rCNC α) and the olfactory receptor cell (oCNC α) subtype of CNC were detected in adult rat hippocampus in most principal neurons, including pyramidal granule cells (169). Two genes are colocalized in individual neurons. Comparison of the patterns of expression of type 1 cGMP-dependent protein kinase and the CNCs suggests that hippocampal neurons can respond to changes in cGMP levels with both rapid changes in CNC activity and slower changes induced by phosphorylation. Recent studies have shown that increased cGMP levels in hippocampal neurons can lead to increased transmitter release and that Ca^{2+} entry into presynaptic terminals plays a critical role. Although some of these effects may be mediated by protein kinases, the CNCs may also contribute to some of the actions of cAMP and cGMP in hippocampus and other regions of the central nervous system. It is possible that they exert their effects at both postsynaptic and presynaptic sites. This will be answered when the subcellular localization of CNCs in hippocampus is determined.

Ca^{2+} -DEPENDENT Cl^- CONDUCTANCE Many odorants elicit the activation of a Ca^{2+} -permeable nonselective cation conductance (46, 173, 174), followed by a nonlinearly Ca^{2+} -dependent Cl^- conductance (175, 176). Owing to the specific ion concentrations in the mucus, these result in an influx of extracellular Ca^{2+} followed by outflux of intracellular Cl^- , which cooperatively depolarizes the cilia membrane (177). The chloride conductance significantly amplifies the odor-induced depolarization of the cilia, and the nonlinear Ca^{2+} sensitivity of this current is thought to introduce an excitation threshold to improve the signal-to-noise ratio of the transduction process (154, 178). If the stimulus strength is sufficient, the depolarization of the cilia propagates by passive electrotonic spread and finally triggers the generation of action potentials at the initial segment of the axon (179). A Cl^- channel blocker reduces the receptor current by 85% in rat olfactory cells. Cl^- efflux amplifies the cationic current in terrestrial animals. The chloride channel mediating this current remains to be identified.

K^+ CONDUCTANCE Repolarization of the cells seems to involve Ca^{2+} -dependent and fast inactivation potassium conductances. Repolarization of the action potential is achieved by the activation of various K^+ channels (180–186). A transient, 4-aminopyridine (4-AP)-sensitive K^+ conductance and Ca^{2+} -activated K^+ conductances measured in various species seem to contribute to the repolarization and an increase of the impedance of ORNs (180–189). However, in cultured ORNs of rat, the fast 4-AP-sensitive current and a Ca^{2+} -dependent K^+ current are absent, whereas a large delayed-rectifier K^+ conductance is observed (190).

Na^+ CONDUCTANCE Excitation of an olfactory neuron generates a receptor potential, and when the membrane potential reaches the firing threshold, Na^+ channels activate and initiate spike generation. The Na^+ currents are increased via cGMP-dependent phosphorylation, whereas the delayed rectifier K^+ currents are not affected by PKG-mediated phosphorylation (191). Cyclic GMP may lower the

threshold in olfactory perception by decreasing the current threshold to generate spikes, and also prevent the saturation of odor signals by increasing the maximum spike frequency (191). Variability in this observation could be because these channels are primarily located in the axonal membrane, which is partially lost during preparation (189, 192).

Ca²⁺ CONDUCTANCE High-voltage-activated Ca²⁺ currents in ORNs have been described in various species (180, 181, 185–187, 189, 190). Quantitative ratiometric Ca²⁺ imaging with fluo3 and FuraRed revealed that the high-voltage-activated Ca²⁺ channels in *Xenopus* ORNs are primarily situated on the soma and the proximal dendrite (193).

A low-voltage-activated Ca²⁺ current may also be involved in action potential formation. The low-voltage-activated Ca²⁺ currents play a particular role in relatively large ORNs that have high capacitances and ion membrane time constants (194, 195).

OTHER SIGNALING CASCADES IN OLFACTION

Although targeted deletion studies support the central role of cAMP in odorant detection, many investigators have provided evidence demonstrating that other signaling pathways are activated in response to odor stimulation.

Inositol-1,4,5-trisphosphate (IP₃)

In the brain and peripheral tissues receptor-mediated stimulation of phospholipase C generates IP₃, which releases calcium from endoplasmic reticulum stores by binding to specific IP₃ receptors (196, 197). Plasma membrane IP₃ receptors have been identified in lymphocytes (198) and neurons (199, 200) that permit calcium entry from extracellular sources. Thus, calcium may be made available to affect a variety of targets in response to odor stimulation. There are now five families of IP₃ receptors (201–203).

Studies in several species implicate IP₃ in olfaction. However, electrophysiological experiments have in many cases failed to demonstrate a role for IP₃ in the initial phases of the response to odorants. Huque & Bruch (204) showed phospholipase C activity in isolated catfish olfactory cilia. Restrepo and collaborators (205) showed that amino acids enhanced calcium flux in isolated catfish ORNs. Utilizing the rapid mixing technique, Breer and colleagues (39) demonstrated increases in IP₃ levels in response to some odorants.

Studies in primary cultures of ORNs confirmed that odorants stimulate the production of IP₃. Exposure of cells to low nanomolar concentrations of odorants resulted in IP₃ formation (35, 206). All odorants stimulate cAMP and IP₃ production in primary culture, although with different potencies, suggesting interactions with different receptors. The enhancement by single odors of both cAMP and IP₃ production affords a mechanism for increased specificity of odor detection. However,

these studies were only performed at longer (1 s and beyond) times after odor encounter. Ache and coworkers confirmed that odors differentially stimulate dual pathways in isolated lobster antennules (207). Odors elevated cAMP and IP₃ in the outer dendritic membranes of lobster *in vitro*. IP₃ carried the stimulatory current, while cAMP was inhibitory, providing a mechanism for fine-tuning of the responses. The relevance of IP₃ to mammalian olfaction has been questioned by several groups, whose knock-outs affecting the cAMP signaling cascade resulted in loss of the electro-olfactogram responses, suggesting that cAMP is the sole odorant-generated second messenger (40, 41, 104). These discrepancies may be reconciled if cAMP is indeed the primary second messenger required for the initial events of odor detection and cellular depolarization, whereas IP₃ is involved in other secondary responses, such as adaptation or activity-driven cellular responses, not electro-olfactogram generation.

IP₃ receptors have been localized immunohistochemically to the ciliary surface membrane (208), positioning IP₃ to trigger the influx of extracellular calcium. There is also evidence for plasma membrane IP₃-sensitive channels in lobster ORNs (209, 210). Kalinoski and colleagues have also demonstrated an IP₃-like receptor in isolated catfish cilia, although its micromolar K_d for IP₃ suggests a different type of IP₃ receptor (211). Several phospholipase C isoforms have been demonstrated in olfactory epithelium (209, 212, 213).

Reconciliation of the data thus far obtained for IP₃ will require further work. For some time debate existed as to whether cGMP or calcium was the visual second messenger (214). We now know that, whereas cGMP is central, calcium is the major modulator of cGMP levels (215–218). Additionally, there are striking interspecies differences: Whereas IP₃ is important in amphibian phototransduction, no role has thus far been found in mammals. Olfaction may have similar complexities.

cGMP

Cyclic GMP is well established to be the primary second messenger in visual signal transduction. A number of studies indicate that cGMP may play an important role in olfactory transduction. Odorants increase cGMP levels in olfactory tissues (56) and ORNs (52). When compared with the odorant-induced increase in cAMP and IP₃ levels, the rise in cGMP levels occurred with a slower, sustained time course. This delayed response suggests that cGMP may not be involved in initial signaling events, but rather in long-term cellular events such as desensitization (219) or in the activation of neuronal activity-dependent transcription (55). cGMP levels are regulated by two distinct classes of guanylyl cyclases, soluble guanylyl cyclase and receptor guanylyl cyclase. Soluble guanylyl cyclase is activated by gaseous messengers such as NO or CO, whereas receptor guanylyl cyclase is activated by specific extracellular ligands or calcium. Both guanylyl cyclases are expressed in ORNs, implying a complex regulation of cGMP levels in olfaction (52, 220).

Diffusible gaseous messenger molecules such as NO or CO can stimulate soluble guanylyl cyclase by binding to the heme group in soluble guanylyl cyclases

(221). NO and CO are produced by NO synthase and heme oxygenase (HO), respectively. In ORNs NO synthase is expressed at embryonic stages and is markedly reduced at early postnatal stages, whereas HO is highly expressed after birth (222, 223). These data suggest that NO plays an important role during development, whereas HO functions in mature ORNs. Two forms of HO have been identified: HO-1 and HO-2. HO-1 is a heat shock protein (hsp-32) induced by heme, heavy metals, stress, and hormones (224–229) and is highly expressed in the spleen and liver, where it is responsible for the destruction of heme from red blood cells.

HO-2 is not inducible and is highly expressed in the brain, especially in neurons of the olfactory epithelium and in the neuronal and granule cell layer of the olfactory bulb. In situ hybridization analysis showed that guanylyl cyclase and HO-2 were found in ORNs (52). Incubation of ORNs with the HO inhibitor, zinc protoporphyrin-9 (Zn PP-9), lowered cGMP levels in ORNs (222). In addition, odorants augment cGMP levels in ORNs (52, 222). This odorant-induced cGMP increase could be inhibited by Zn PP-9, but not by an NO synthase inhibitor. Interestingly, the inhibition of HO could not entirely deplete cGMP levels in ORNs, suggesting that particulate guanylyl cyclases may also contribute to cGMP production in ORNs (222). Exposure of isolated cilia derived from OR neurons to various odorants increased cGMP levels (220). Thus, there is a strong suggestion that both soluble and receptor guanylyl cyclases have roles in olfactory signal transduction.

The observation that the inhibition of HO in ORNs could not totally block the cGMP response suggested the involvement of receptor guanylyl cyclases in odorant transduction. The fact that an NO donor and soluble guanylyl cyclase activator, sodium nitroprusside, could not alter the cGMP levels in isolated cilia supported the idea that a receptor guanylyl cyclase might play a role in olfactory cilia. An olfactory-specific receptor guanylyl cyclase, guanylyl cyclase-D (GC-D), has been identified in olfactory epithelium (129). GC-D has been suggested to function as the receptor of sensory neurons to specific odors. The role and regulation of these guanylyl cyclases in the olfactory system are unclear.

Recent studies have identified an odorant-responsive receptor guanylyl cyclase in rat olfactory sensory cilia (220). At least two receptor guanylyl cyclases exist in cilia, a low K_m and a high K_m isoform (220). Odorants were shown to elevate cGMP levels in cultured ORNs (222) and in isolated olfactory cilia (220) in a calcium-dependent manner. A number of experiments suggested that calcium plays a role in odorant transduction and can fluctuate upon odorant exposure (172, 230, 231). Hence, it was hypothesized that an OR guanylyl cyclase could be regulated by a calcium-binding protein, such as guanylyl cyclase-activating protein (GCAP), similar to that found in the visual transduction pathway. Immunohistochemical studies using anti-GCAP1 antibodies revealed that GCAP1 was highly localized to the olfactory cilia (220). Moreover, GCAP1 regulated the odorant-induced cGMP response in isolated rat olfactory cilia in a calcium-dependent manner (220). Thus, ORNs contain multiple cGMP pathways that mediate delayed and sustained cGMP responses to odorants.

Calcium

Calcium regulates diverse cellular functions, and in general these functions are mediated by a variety of calcium-binding proteins (232). Odorant stimulation of ORNs results in a calcium influx, which in turn can modulate a number of transduction pathways. Calmodulin and other calcium-binding proteins may participate in the processing of olfactory information. Therefore, study of the calcium-binding proteins may provide important background information about the complex signal transduction pathway involved in olfaction. Olfactory tissue contains various calcium-binding proteins: calmodulin, calretinin, calbindin-D28k, neurocalcin, and recoverin (233). Another calcium-binding protein, S-100, is restricted to glial cells, primarily around the cribriform plate.

Calmodulin is found in olfactory cilia at a concentration of about 1 mM (234). The odorant-induced intracellular elevation of calcium is thought to promote adaptation because calcium/calmodulin can reduce the affinity of the oCNC for cAMP by 20-fold (155, 235). Extracellular calcium is absolutely required for the decay phase of the odorant-induced whole cell current, which in the absence of extracellular calcium remains at a steady state (236). Calcium/calmodulin can also affect CNC activity (237).

Neurocalcin is a calcium-binding protein with three EF hand motifs and is also expressed in the rat olfactory epithelium (238). Neurocalcin is expressed in ORNs, where it is associated with outer mitochondrial membrane, endoplasmic reticulum, and axon fibers. The intracellular distribution of neurocalcin in ORNs suggested that this protein may participate in cytoskeletal arrangement in ORNs. The expression of neurocalcin in postnatal development was also studied (233, 239). Neurocalcin showed a gradient of expression pattern descending from the central to the lateral areas in the nasal cavity during childhood; this expression pattern became identical to the adult profile after 20 days.

Additional calcium-binding proteins have been localized to the olfactory system. A 26-kDa calcium-binding protein named p26olf was identified in frog olfactory epithelium (240). p26olf contains two S-100-like regions and is localized to the cilia layer of the olfactory epithelium, suggesting that it is a dimeric form of S-100 protein that may be involved in the olfactory transduction or adaptation. Visinin-like protein (VILIP), a member of the neuronal subfamily of EF-hand calcium-sensor proteins, was associated with ORNs of the rat olfactory epithelium (241). VILIP is localized prominently to cilia and dendritic knobs. *In vitro* recombinant VILIP attenuates odorant-induced cAMP formation in a calcium-dependent manner. The observation that VILIP does not interfere with odorant-induced receptor desensitization and that VILIP inhibits the forskolin-induced cAMP production suggests that it may directly affect adenylyl cyclases and in turn may play a role in adaptation of ORNs.

A GCAP1-like calcium-binding protein is present in rat olfactory cilia (220). GCAP1 was purified and later cloned from bovine retina by Palczewski and colleagues (130). GCAP1 is a 21-kDa cytosolic EF-hand-family protein and has

been proposed to function as a photoreceptor-specific calcium-binding protein to activate particulate guanylyl cyclase, thus restoring the cGMP level in light-activated photoreceptor cells. Immunohistochemical studies revealed the presence of GCAP1 in rat olfactory cilia (220). Interestingly, purified GCAP1 potentiated cGMP production at high calcium concentrations in isolated rat olfactory cilia (220). In photoreceptor cells, GCAP1 activates particulate guanylyl cyclase when the intracellular calcium level is low. The size of the olfactory GCAP (19 kDa) was not identical to the retinal GCAP1. Thus, the olfactory GCAP is considered a GCAP1-like protein. The cloning of the olfactory GCAP will answer the precise function and mechanism of the olfactory guanylyl GCAP in olfaction.

Calcium itself mediates Cl^- conductance in ORNs (132, 176, 242). The odor-induced currents show little rectification. It appears that the depolarizing current has two components, an initial inward cationic conductance followed by an inward anionic Cl^- conductance (132, 237, 242). Calcium, which enters the cilia through the cyclic nucleotide-gated channel, triggers a calcium-activated Cl^- channel in olfactory cilia membrane (176). This conductance may serve as a “fail safe” so that cells can depolarize, irrespective of changes in extracellular milieu.

DESENSITIZATION

Desensitization of receptor-mediated responses can occur through a variety of processes, including phosphorylation, internalization, and receptor-effector uncoupling (243–245). The homologous desensitization of G protein-coupled receptors is well established in β 2-adrenergic receptor (β AR-2) as a model (246, 247). Phosphorylation of receptors by a specific receptor kinase such as β -adrenergic receptor kinases (β -ARKs) or G protein receptor kinases (GRKs) mediate homologous desensitization. Complete quenching of signal transduction requires the binding of a protein called β -arrestin (β ARR) to a phosphorylated receptor (248).

Specific isoforms of GRKs and β ARR, β ARK-2, and β ARR-2 were localized to olfactory neurons, specifically to olfactory cilia and dendritic knobs (249). Other isoforms of β ARK or β ARR were not present in these cells. Functional studies of β ARK-2 and β ARR-2 in the olfactory cilia were performed (249, 250). The odorant-induced cAMP production was monitored in the presence or absence of neutralizing antibodies against specific isoforms of β ARK and β ARR. Preincubation of olfactory cilia with neutralizing antibodies to β ARK-2 and β ARR-2 increased the absolute levels of odorant-induced cAMP as high as fourfold and completely blocked desensitization. Later mice with targeted disruption of β ARK-2 have been available, and cilia preparations derived from the β ARK-2-deficient mice showed lack of the agonist-induced desensitization (251). Taken together, the expression of β ARK-2 and β ARR-2 within the olfactory cilia, the inhibition of desensitization with β ARK-2 and β ARR-2 neutralizing antibodies, and the lack of the agonist-induced desensitization in the β ARK-2 deficient mice suggest that β ARK-2 and β ARR-2 mediate the odorant-dependent desensitization in olfaction.

In addition to this mechanism for homologous desensitization, it has been suggested that PKA or PKC may play a role in odorant-related heterologous desensitization (252). PKA has been implicated in olfactory desensitization following the increase in cAMP by odorant stimulation, whereas PKC may mediate desensitization following phosphoinositide cycle activation by odorant stimulation. However, these results need to be reexamined, in light of more recent data using knockout animals that indicate that cAMP mediates odorant detection.

Cyclic GMP may also play a role in desensitization. Zufall & Leinders-Zufall (53) showed that cGMP mediated a long-lasting form of odor response adaptation in tiger salamander. The long-lasting adaptation lasted for several minutes and was attributable to cyclic nucleotide-gated channel modulation by cGMP. They showed that this form of long-lasting adaptation was abolished selectively by HO inhibitors (which prevent CO release and cGMP formation), whereas odor excitation was unaffected. The results suggest that endogenous CO/cGMP signals contribute to olfactory desensitization.

LONG-TERM RESPONSES TO ODORANT DETECTION

The theory that extracellular signals, such as hormones, growth factors, and neuronal activity, can modulate transcriptional events to produce long-term changes in cellular activity is well established (253). However, the long-lasting effects of odorant stimulation in ORNs has only recently been studied.

A delayed cAMP response upon odorant stimulation was characterized and was mediated by cGMP via activation of a cGMP-dependent protein kinase (PKG) (220). Based on the kinetics of the delayed cAMP response discussed above, it was suggested that cGMP might mediate a delayed cAMP response to regulate long-term cellular responses to odorant detection, including the regulation of gene expression. Recent studies support this idea. Odorant stimulation could therefore potentially result in transcriptional changes via CREB activation (55). Incubation of ORNs with either 8-Br-cGMP or a soluble guanylyl cyclase activator (sodium nitroprusside) increased CREB activation. Thus, cGMP produced upon odorant stimulation may generate a sustained cAMP signal capable of activating CREB.

Involvement of the Ras-MAPK (mitogen-activated protein kinase) signal transduction pathway in olfaction was recently reported in *C. elegans* (254). The Ras-MAPK pathway plays important roles in cellular proliferation and differentiation in response to extracellular signals. Mutational inactivation and hyperactivation of this pathway impaired efficiency of chemotaxis to a set of odorants. The activation of MAPK upon odorant stimulation was dependent on calcium fluxes via the nucleotide-gated channel and the voltage-activated calcium channel. More recently, Watt & Storm demonstrated that odorants activate MAPK in rodent ORNs (255). The odorant-activation of the MAPK pathway led to the activation of cAMP response element (CRE)-mediated transcription. The odorant stimulation of MAPK activation was ablated by inhibition of CaM-dependent protein

kinase II (CaMKII), suggesting that odorant activation of MAPK is mediated through CaMKII. Moreover, discrete populations of ORNs display CRE-mediated gene transcription when stimulated by odorants in mice. These data suggest that ORNs may undergo long-term adaptive changes mediated through CRE-mediated transcription.

CONCLUSIONS

Our understanding of olfactory transduction has advanced rapidly in the past decade with the realization that G proteins and seven-transmembrane spanning domain receptors are involved in odorant detection. It remains to be determined how other transduction cascades interface with these G protein-coupled receptors to either fine-tune the response or mediate other aspects of odor detection. Understanding the olfactory code will allow us to manipulate olfactory perception in both health and disease. Our appreciation of the ability of odor perception to influence long-term neuronal responses, and potentially neuronal survival, may provide clues to understanding this process in other neuronal systems.

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED

- Buck LB. 1996. Information coding in the vertebrate olfactory system. *Annu. Rev. Neurosci.* 19:517–44
- Getchell TV. 1986. Functional properties of vertebrate olfactory receptor neurons. *Physiol. Rev.* 66:772–818
- Getchell TV, Margolis FL, Getchell ML. 1985. Perireceptor and receptor events in vertebrate olfaction. *Prog. Neurobiol.* 23:317–45
- Firestein S. 1996. Olfaction: scents and sensibility. *Curr. Biol.* 6:666–67
- Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu. Rev. Neurosci.* 20:595–631
- Gold GH. 1999. Controversial issues in vertebrate olfactory transduction. *Annu. Rev. Physiol.* 61:857–71
- Graziadei PPC, Monti-Graziadei GA. 1979. Neurogenesis and neuron regeneration in the olfactory system of mammals. *J. Neurocytol.* 8:1–18
- Moulton DG, Beidler LM. 1967. Structure and function in the peripheral olfactory system. *Physiol. Rev.* 47:1–52
- Labarca P, Bacigalupo J. 1988. Ion channels from chemosensory olfactory neurons. *J. Bioenerg. Biomembr.* 20:551–69
- Lowe G, Gold GH. 1993. Contribution of the ciliary cyclic nucleotide-gated conductance to olfactory transduction in the salamander. *J. Physiol.* 462:175–96
- Menco BPM, Brunch RC, Dau B, Danho W. 1992. Ultrastructural localization of olfactory transduction components: the G protein subunit $G_{\text{olf}\alpha}$ and type III adenylyl cyclase. *Neuron* 8:441–53
- Menco BPM. 1997. Ultrastructural aspects of olfactory signaling. *Chem. Senses* 22:295–311
- Farbman AI. 1992. Development and plasticity. In *Cell Biology of Olfaction*, ed. PW Barlow, D Bray, PB Green, JMW Slack, pp. 167–206. Cambridge, UK: Cambridge Univ. Press

14. Graziadei PPC. 1973. Cell dynamics in the olfactory mucosa. *Tissue Cell* 5:113–31
15. Graziadei PPC, Metcalf JF. 1971. Autoradiographic and ultrastructural observation on the frog's olfactory mucosa. *Zellforsch* 116:305–18
16. Caggiano M, Kauer JS, Hunter DD. 1994. Globose basal cells are neuronal progenitors in the olfactory epithelium: a lineage analysis using a replication-incompetent retrovirus. *Neuron* 13:339–52
17. Roskams AJI, Cai X, Ronnett GV. 1998. Expression of neuron-specific beta-III tubulin during olfactory neurogenesis in the embryonic and adult rat. *Neuroscience* 83:191–200
18. Margolis FL. 1980. A marker protein for the olfactory chemoreceptor neuron. In *Proteins of the Nervous System*, ed. RA Bradshaw, DM Schneider, pp. 59–84. New York: Raven
19. Farbman AI, Margolis FL. 1980. Olfactory marker protein during ontogeny: immunohistochemical localization. *Dev. Biol.* 74:205–15
20. Carr VM, Farbman AI. 1993. The dynamics of cell death in the olfactory epithelium. *Exp. Neurol.* 124:308–14
21. Okano TM. 1974. Secretion and electrogenesis of the supporting cell in the olfactory epithelium. *J. Physiol.* 242:353–70
22. Lazard D, Zupko K, Poria Y, Nef P, Lazarovits J, et al. 1991. Odorant signal termination by olfactory UDP-glucuronosyl transferase. *Nature* 349:790–93
23. Hansel DE, Eipper BA, Ronnett GV. 2001. Neuropeptide Y: functions as a neuroproliferative factor. *Nature* 410:940–44
24. Baraban SC, Hollopeter G, Erickson JC, Schwartzkroin PA, Palmiter RD. 1997. Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. *J. Neurosci.* 17:8927–36
25. Danger JM, Tonon MC, Jenks BG, Saint-Pierre S, Martel JC, et al. 1990. Neuropeptide Y: localization in the central nervous system and neuroendocrine functions. *Fundam. Clin. Pharmacol.* 4:307–40
26. Adrian TE, Allen JM, Bloom SR, Ghatei MA, Rossor MN, et al. 1983. Neuropeptide Y distribution in human brain. *Nature* 306:584–86
27. Calof AL, Chikaraishi DM. 1989. Analysis of neurogenesis in a mammalian neuroepithelium: proliferation and differentiation of an olfactory neuron precursor in vitro. *Neuron* 3:115–27
28. Goldstein BJ, Schwob JE. 1996. Analysis of the globose basal cell compartment in rat olfactory epithelium using GBC-1, a new monoclonal antibody against globose basal cells. *J. Neurosci.* 16:4005–16
29. Huard JM, Youngentob SL, Goldstein BJ, Luskin MB, Schwob JE. 1998. Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *J. Comp. Neurol.* 400:469–86
30. Rhein LD, Cagan RH. 1980. Biochemical studies of olfaction: isolation, characterization and odorant binding activity of cilia from rainbow trout olfactory rosettes. *Proc. Natl. Acad. Sci. USA* 77:4412–16
31. Malnic B, Hirono J, Sato T, Buck L. 1999. Combinatorial receptor codes for odors. *Cell* 96:713–23
32. Dwyer ND, Troemel ER, Sengupta P, Bargmann CI. 1998. OR localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell* 93:455–66
33. Pace U, Hanski E, Salomon Y, Lancet D. 1985. Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature* 316:255–58
34. Sklar PB, Anholt RRR, Snyder SH. 1986. The odorant-sensitive adenylate cyclase of olfactory receptor neurons. *J. Biol. Chem.* 261:15538–43
35. Ronnett GV, Cho H, Hester LD, Wood SF, Snyder SH. 1993. Odorants differentially enhance phosphoinositide turnover

- and adenylyl cyclase in olfactory receptor neuronal cultures. *J. Neurosci.* 13:1751–58
36. Ronnett GV, Parfitt DJ, Hester LD, Snyder SH. 1991. Odorant-sensitive adenylyl cyclase: rapid potent activation and desensitization in primary olfactory neuronal cultures. *Proc. Natl. Acad. Sci. USA* 88:2366–69
37. Ronnett GV, Snyder SH. 1992. Molecular messengers of olfaction. *Trends Neurosci.* 15:508–12
38. Jaworsky DE, Matsuzaki O, Borisy FF, Ronnett GV. 1995. Calcium modulates the rapid kinetics of the odorant-induced cyclic AMP signal in rat olfactory cilia. *J. Neurosci.* 15:310–18
39. Breer H, Boekhoff I, Tareilus E. 1990. Rapid kinetics of second messenger formation in olfactory transduction. *Nature* 345:65–68
40. Brunet LL, Gold GH, Ngai J. 1996. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* 17:681–93
41. Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, et al. 2000. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* 27:487–97
42. Nakamura T, Gold GH. 1987. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* 325:442–44
43. Firestein S, Werblin FS. 1989. Odor-induced membrane currents in vertebrate olfactory receptor neurons. *Science* 244:79–82
44. Getchell TV, Shepherd GM. 1978. Adaptive properties of olfactory receptor analysed with odour pulses of varying durations. *J. Physiol.* 282:541–60
45. Ottoson D. 1956. Analysis of the electrical activity of the olfactory epithelium. *Acta Physiol. Scand.* 122:1–83
46. Frings S, Seifert R, Godde M, Kaupp UB. 1995. Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. *Neuron* 15:169–79
47. Kaupp UB. 1991. The cyclic nucleotide-gated channels of vertebrate photoreceptors and olfactory epithelium. *Trends Neurosci.* 14:150–57
48. Breer H, Boekhoff I. 1991. Odorants of the same odor class activate different second messenger pathways. *Chem. Senses* 16:19–29
49. Miyamoto T, Restrepo D, Cragoe EJ, Teeter JH. 1992. IP₃ and cAMP-induced responses in isolated olfactory receptor neurons from the channel catfish. *J. Membr. Biol.* 127:173–83
50. Schandar M, Laugwitz KL, Boekhoff I, Kroner C, Gudermann T, et al. 1998. Odorants selectively activate distinct G protein subtypes in olfactory cilia. *J. Biol. Chem.* 273:16669–77
51. Ingi T, Cheng J, Ronnett GV. 1996. Carbon monoxide: an endogenous modulator of the nitric oxide-cyclic GMP signaling system. *Neuron* 16:835–42
52. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. 1993. Carbon monoxide: a putative neural messenger. *Science* 259:381–84
53. Zufall F, Leinders-Zufall T. 1997. Identification of a long-lasting form of odor adaptation that depends on the carbon monoxide/cGMP second-messenger system. *J. Neurosci.* 17:2703–12
54. Moon C, Jaber P, Otto-Bruc A, Baehr W, Palczewski K, Ronnett GV. 1998. Calcium-sensitive particulate guanylyl cyclase as a modulator of cAMP in olfactory receptor neurons. *J. Neurosci.* 18:3195–205
55. Moon C, Sung YK, Reddy R, Ronnett GV. 1999. Odorants induce the phosphorylation of the cAMP response element binding protein in olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* 96:14605–10
56. Breer H, Klemm T, Boekhoff I. 1992. Nitric oxide mediated formation of cyclic

- GMP in the olfactory system. *NeuroReport* 3:1030–31
57. Pelosi P, Baldaccini NE, Pisanelli AM. 1982. Identification of a specific olfactory receptor for 2-isobutyl-3-methoxy-pyrazine. *Biochem. J.* 201:245–48
58. Pevsner J, Sklar PB, Snyder SH. 1986. Odorant-binding protein: localization to nasal gland and secretions. *Proc. Natl. Acad. Sci. USA* 83:4942–46
59. Pevsner J, Trifiletti RR, Strittmatter SS, Snyder SH. 1985. Isolation and characterization of an olfactory receptor protein for odorant pyrazines. *Proc. Natl. Acad. Sci. USA* 82:3050–54
60. Pevsner J, Hou VX, Snyder SH. 1990. Odorant-binding protein: characterization of ligand binding. *J. Biol. Chem.* 265: 6118–25
61. Bok D. 1990. Processing and transport of retinoids by the retinal pigment epithelium. *Eye* 4:326–32
62. Pevsner J, Hwang PM, Sklar PB, Venable JC, Snyder SH. 1988. Odorant-binding protein and its mRNA are localized to lateral nasal gland implying a carrier function. *Proc. Natl. Acad. Sci. USA* 85:2383–87
63. Dear TN, Boehm T, Keverne EB, Rabbits TH. 1991. Novel genes for potential ligand-binding proteins in subregions of the olfactory mucosa. *EMBO J.* 10:2813–19
64. Burova TV, Rabesona H, Choiset Y, Janowski CK, Sawyer L, Haertle T. 2000. Why has porcine VEG protein unusually high stability and suppressed binding ability? *Biochim. Biophys. Acta* 1478:267–79
65. Lobel D, Marchese S, Krieger J, Pelosi P, Breer H. 1998. Subtypes of odorant-binding proteins—heterologous expression and ligand binding. *Eur. J. Biochem.* 254: 318–24
66. Pes D, Pelosi P. 1995. Odorant-binding proteins of the mouse. *Comp. Biochem. Physiol. B* 112:471–79
67. Garibotti M, Navarrini A, Pisanelli AM, Pelosi P. 1997. Three odorant-binding proteins from rabbit nasal mucosa. *Chem. Senses* 22:383–90
68. Bianchet MA, Bains G, Pelosi P, Pevsner J, Snyder SH, et al. 1996. The three-dimensional structure of bovine odorant binding protein and its mechanism of odor recognition [see comments]. *Nat. Struct. Biol.* 3:934–39
69. Dal Monte M, Andreini I, Revoltella R, Pelosi P. 1991. Purification and characterization of two odorant-binding proteins from nasal tissue of rabbit and pig. *Comp. Biochem. Physiol. B* 99:445–51
70. Vogt RG, Prestwich GD, Lerner MR. 1990. Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *J. Neurobiol.* 22:74–84
71. Vogt RG, Rybczynski R, Lerner MR. 1991. Molecular cloning and sequencing of general odorant-binding proteins GOBP1 and GOBP2 from the tobacco hawk moth *manduca sexta*: comparisons with other insect OBPs and their signal peptides. *J. Neurosci.* 11:2972–84
72. Buck L, Axel R. 1991. A novel multigene family may encode ORs: a molecular basis for odor recognition. *Cell* 65:175–87
73. Buck LB. 1992. The olfactory multigene family. *Curr. Biol.* 2:467–73
74. Mombaerts P. 1999. Seven-transmembrane proteins as odorant and chemosensory receptors. *Science* 286:707–11
75. Glusman G, Yanai I, Rubin I, Lancet D. 2001. The complete human olfactory subgenome. *Genome Res.* 11:685–702
76. Raming K, Konzelmann S, Breer H. 1998. Identification of a novel G-protein coupled receptor expressed in distinct brain regions and a defined olfactory zone. *Recept. Channel* 6:141–51
77. Feingold EA, Penny LA, Nienhuis AW, Forget BG. 1999. An olfactory receptor gene is located in the extended human beta-globin gene cluster and is expressed in erythroid cells. *Genomics* 61:15–23
78. Zozulya S, Echeverri F, Nguyen T. 2001.

- The human olfactory receptor repertoire. *Genome Biol. Res.* 0018.1–0018.12
79. Lancet D, Ben-Arie N, Cohen S, Gat U, Gross-Isseroff R, et al. 1993. Olfactory receptors: transduction, diversity, human psychophysics and genome analysis. *Ciba Found. Symp.* 179:131–41
 80. Rouquier S, Taviaux S, Trask BJ, Brand-Arpon V, van den Engh G, et al. 1998. Distribution of olfactory receptor genes in the human genome. *Nat. Genet.* 18:243–50
 81. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al. 2001. The sequence of the human genome. *Science* 291:1304–51
 82. Trask BJ, Massa H, Brand-Arpon V, Chan K, Friedman C, et al. 1998. Large multi-chromosomal duplications encompass many members of the olfactory receptor gene family in the human genome. *Hum. Mol. Genet.* 7:2007–20
 83. Trask BJ, Friedman C, Martin-Gallardo A, Rowen L, Akinbami C, et al. 1998. Members of the olfactory receptor gene family are contained in large blocks of DNA duplicated polymorphically near the ends of human chromosomes. *Hum. Mol. Genet.* 7:13–26
 84. O'Brien SJ, Eisenberg JF, Miyamoto M, Hedges SB, Kumar S, et al. 1999. Genome maps 10. Comparative genomics. Mammalian radiations. Wall chart. *Science* 286:463–78
 85. Ressler KJ, Sullivan SL, Buck LB. 1993. A zonal organization of OR gene expression in the olfactory epithelium. *Cell* 73:597–609
 86. Vassar R, Ngai J, Axel R. 1993. Spatial segregation of OR expression in the mammalian olfactory epithelium. *Cell* 74:309–18
 87. Mombaerts P. 1999. Molecular biology of ORs in vertebrates. *Annu. Rev. Neurosci.* 22:487–509
 88. Koshimoto H, Katoh K, Yoshihara Y, Nemoto Y, Mori K. 1994. Immunohistochemical demonstration of embryonic expression of an odor receptor protein and its zonal distribution in the rat olfactory epithelium. *Neurosci. Lett.* 169:73–76
 89. Menco BP, Cunningham AM, Qasba P, Levy N, Reed RR. 1997. Putative odour receptors localize in cilia of olfactory receptor cells in rat and mouse: a freeze-substitution ultrastructural study. *J. Neurocytol.* 26:691–706
 90. Menco BP, Jackson JE. 1997. A banded topography in the developing rat's olfactory epithelial surface. *J. Comp. Neurol.* 388:293–306
 91. Senhupta P, Chou JH, Bargmann CI. 1996. odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell* 84:899–909
 92. Krautwurst D, Yau KW, Reed RR. 1998. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* 95:917–26
 93. Zhao H, Ivic L, Otaki JM, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian OR. *Science* 279:237–41
 94. Ressler KJ, Sullivan SL, Buck LB. 1994. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79:1245–55
 95. Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, et al. 1996. Visualizing an olfactory sensory map. *Cell* 87:675–86
 96. Drutel G, Arrang JM, Diaz J, Wisniewsky C, Schwartz K, Schwartz JC. 1995. Cloning of OL1, a putative olfactory receptor and its expression in the developing rat heart. *Recept. Channel* 3:33–40
 97. Nef S, Nef P. 1997. Olfaction: transient expression of a putative OR in the avian notochord. *Proc. Natl. Acad. Sci. USA* 94:4766–71
 98. Abe K, Kusakabe Y, Tanemura K, Emori Y, Arai S. 1993. Multiple genes for G protein-coupled receptors and their expression in lingual epithelia. *FEBS Lett.* 316:253–56

99. Walensky LD, Ruat M, Bakin RE, Blackshaw S, Ronnett GV, Snyder SH. 1998. Two novel OR families expressed in spermatids undergo 5'-splicing. *J. Biol. Chem.* 273:9378–87
100. Walensky LD, Roskams JA, Lefkowitz RJ, Snyder SH, Ronnett GV. 1995. ORs and desensitization proteins colocalize in mammalian sperm. *Mol. Med.* 1:130–41
101. Rhein LD, Cagan RH. 1983. Biochemical studies of olfaction: binding specificity of odorants to cilia preparation from rainbow trout olfactory rosettes. *J. Neurochem.* 41:569–77
102. Jones DT, Reed RR. 1987. Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium. *J. Biol. Chem.* 262:14241–49
103. Drinnan SL, Hope BT, Snutch TP, Vincent SR. 1991. G_{olf} in the basal ganglia. *Mol. Cell. Neurosci.* 2:66–70
104. Belluscio L, Gold GH, Nemes A, Axel R. 1998. Mice deficient in G(olf) are anosmic. *Neuron* 20:69–81
105. Firestein S, Shepherd GM, Werblin FS. 1990. Time course of the membrane current underlying sensory transduction in salamander olfactory receptor neurones. *J. Physiol.* 430:135–58
106. Boekhoff I, Tareilus E, Strotmann J, Breer H. 1990. Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO J.* 9:2453–58
107. Ronnett GV, Hester LD, Snyder SH. 1991. Primary culture of neonatal rat olfactory neurons. *J. Neurosci.* 11:1243–55
108. Pace U, Lancet D. 1986. Olfactory GTP-binding protein: signal transducing polypeptide of vertebrate chemosensory neurons. *Proc. Natl. Acad. Sci. USA* 83:4947–51
109. Hanoune J, Defer N. 2001. Regulation and role of adenylyl cyclase isoforms. *Annu. Rev. Pharmacol. Toxicol.* 41:145–74
110. Bakalyar HA, Reed RR. 1990. Identification of a specialized adenylyl cyclase that may mediate odorant detection. *Science* 250:1403–6
111. Menco BPM, Bruch RC, Dau B, Danho W. 1992. Ultrastructural localization of olfactory transduction components: the G protein subunit G_{olf} and type III adenylyl cyclase. *Neuron* 8:441–53
112. Chen S, Lane AP, Bock R, Leinders-Zufall T, Zufall F. 2000. Blocking adenylyl cyclase inhibits olfactory generator currents induced by “IP(3)-odors.” *J. Neurophysiol.* 84:575–80
113. Mons N, Cooper DMF. 1995. Adenylate cyclases: critical foci in neuronal signaling. *Trends Neurosci.* 18:536–42
114. Choi EJ, Xia Z, Storm DR. 1992. Stimulation of the type III olfactory adenylyl cyclase by calcium and calmodulin. *Biochemistry* 31:6492–98
115. Choi E-J, Wong ST, Dittman AH, Storm DR. 1993. Phorbol ester stimulation of the type I and type III adenylyl cyclases in whole cells. *Biochem. J.* 32:1891–94
116. Wayman GA, Impey S, Storm DR. 1995. Ca²⁺ inhibition of type III adenylyl cyclase *in vivo*. *J. Biol. Chem.* 270:21480–86
117. Cooper DMF, Mons N, Karpen JW. 1995. Adenylyl cyclases and the interaction between calcium and cAMP signaling. *Nature* 374:421–24
118. Frings S. 1993. Protein kinase C sensitizes olfactory adenylate cyclase. *J. Gen. Physiol.* 101:183–205
119. Tang W-J, Gilman AG. 1992. Adenylyl cyclases. *Cell* 70:869–72
120. Beavo JA, Conti M, Heaslip RJ. 1994. Multiple cyclic nucleotide phosphodiesterases. *Mol. Pharmacol.* 46:399–405
121. Beltman J, Sonnenburg WK, Beavo JA. 1993. The role of protein phosphorylation in the regulation of cyclic nucleotide phosphodiesterases. *Mol. Cell Biochem.* 127/128:239–53
122. Beavo JA. 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol. Rev.* 75:725–48

123. Burns F, Zhao AZ, Beavo JA. 1996. Cyclic nucleotide phosphodiesterases: gene complexity, regulation by phosphorylation, and physiological implications. *Adv. Pharmacol.* 36:29–48
124. Borisy FF, Hwang PM, Ronnett GV, Snyder SH. 1993. High affinity cyclic AMP phosphodiesterase and adenosine localized in sensory organs. *Brain Res.* 610:199–207
125. Borisy FF, Ronnett GV, Cunningham AM, Juilfs D, Beavo J, Snyder SH. 1991. Calcium/calmodulin activated phosphodiesterase selectively expressed in olfactory receptor neurons. *J. Neurosci.* 12: 915–23
126. Yan C, Zhao AZ, Bentley JK, Loughney K, Ferguson K, Beavo JA. 1995. Molecular cloning and characterization of a calmodulin-dependent phosphodiesterase enriched in olfactory sensory neurons. *Proc. Natl. Acad. Sci. USA* 92:9677–81
127. Juilfs DM, Fülle HJ, Zhao AZ, Houslay MD, Garbers DL, Beavo JA. 1997. A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc. Natl. Acad. Sci. USA* 94:3388–95
128. Meyer MR, Angele A, Kremmer E, Kaupp UB, Muller F. 2000. A cGMP-signaling pathway in a subset of olfactory sensory neurons. *Proc. Natl. Acad. Sci. USA* 97:10595–600
129. Fülle H-J, Vassar R, Foster DC, Yang R-B, Axel R, Garbers DL. 1995. A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. *Proc. Natl. Acad. Sci. USA* 92:3571–75
130. Palczewski K, Subbaraya I, Gorczyca WA, Helekar BS, Ruiz CC, et al. 1994. Molecular cloning and characterization of retinal photoreceptor guanylyl cyclase-activating protein. *Neuron* 13:395–404
131. Baker H, Cummings DM, Munger SD, Margolis JW, Franzen L, et al. 1999. Targeted deletion of a cyclic nucleotide-gated channel subunit (OCNC1): biochemical and morphological consequences in adult mice. *J. Neurosci.* 19:9313–21
132. Lowe G, Gold GH. 1993. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* 366:283–86
133. Dionne VE. 1998. New kid on the block: a role for the Na/Ca exchanger in odor transduction. *J. Gen. Physiol.* 112:527–28
134. Jung A, Lischka FW, Engel J, Schild D. 1994. Sodium/calcium exchanger in olfactory receptor neurones of *Xenopus laevis*. *NeuroReport* 5:1741–44
135. Menco BP, Birrell GB, Fuller CM, Ezech PI, Keeton DA, Benos DJ. 1998. Ultrastructural localization of amiloride-sensitive sodium channels and Na⁺,K⁺-ATPase in the rat's olfactory epithelial surface. *Chem. Senses* 23:137–49
136. Noe J, Tareilus E, Boekhoff I, Breer H. 1997. Sodium/calcium exchanger in rat olfactory neurons. *Neurochem. Int.* 30: 523–31
137. Reisert J, Matthews HR. 1998. Na⁺-dependent Ca²⁺ extrusion governs response recovery in frog olfactory receptor cells. *J. Gen. Physiol.* 112:529–35
138. Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P. 2000. Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. *Neuron* 26:81–91
139. Varnum MD, Black KD, Zagotta WN. 1995. Molecular mechanism for ligand discrimination of cyclic nucleotide-gated channels. *Neuron* 15:619–25
140. Zufall F, Firestein S, Shepherd GM. 1994. Cyclic nucleotide-gated ion channels and sensory transduction in olfactory receptor neurons. *Annu. Rev. Biophys. Biomol. Struct.* 23:577–607
141. Fesenko EE, Kolesnikov SS, Lyubarsky AL. 1985. Induction by cGMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* 313:310–13

142. Picco C, Menini A. 1993. The permeability of the cGMP-activated channel to organic cations in retinal rods of the tiger salamander. *J. Physiol.* 460:741–58
143. Firestein S, Zufall F, Shepherd GM. 1991. Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. *J. Neurosci.* 11:3565–72
144. Kurahashi T, Kaneko A. 1991. High density cAMP-gated channels at the ciliary membrane in the olfactory receptor cell. *NeuroReport* 2:5–8
145. Nakamura T. 2000. Cellular and molecular constituents of olfactory sensation in vertebrates. *Comp. Biochem. Physiol. A* 126:17–32
146. Liman ER, Buck LB. 1994. A second subunit of the olfactory cyclic nucleotide-gated channel confers high sensitivity to cAMP. *Neuron* 13:611–21
147. Chen T-Y, Peng Y-W, Dhallan RS, Ahmed B, Reed RR, Yau K-W. 1993. A new subunit of the cyclic nucleotide-gated cation channel in retinal rods. *Nature* 362:764–67
148. Korschen HG, Illing M, Seifert R, Sesti F, Williams A, et al. 1995. A 240 kDa protein represents the complete beta subunit of the cyclic nucleotide-gated channel from rod photoreceptor. *Neuron* 15:627–36
149. Sautter A, Zong X, Hofmann F, Biel M. 1998. An isoform of the rod photoreceptor cyclic nucleotide-gated channel beta subunit expressed in olfactory neurons. *Proc. Natl. Acad. Sci. USA* 95:4696–701
150. Bonigk W, Bradley J, Muller F, Sesti F, Boehhoff I, et al. 1999. The native rat olfactory cyclic nucleotide-gated channel is composed of three distinct subunits. *J. Neurosci.* 19:5332–47
151. Bradley J, Li J, Davidson N, Lester HS, Zinn K. 1994. Heteromeric olfactory cyclic nucleotide-gated channels: a subunit that confers increased sensitivity to cAMP. *Proc. Natl. Acad. Sci. USA* 91:8890–94
152. Shapiro MS, Zagotta WN. 1998. Stoichiometry and arrangement of heteromeric olfactory cyclic nucleotide-gated ion channels. *Proc. Natl. Acad. Sci. USA* 95:14546–51
153. Bradley J, Zhang Y, Bakin R, Lester HA, Ronnett GV, Zinn K. 1997. Functional expression of the heteromeric “olfactory” cyclic nucleotide-gated channel in the hippocampus: a potential effector of synaptic plasticity in brain neurons. *J. Neurosci.* 17:1993–2005
154. Dzeja C, Hagen V, Kaupp UB, Frings S. 1999. Ca²⁺ permeation in cyclic nucleotide-gated channels. *EMBO J.* 18:131–44
155. Chen T-Y, Yau K-W. 1994. Direct modulation by Ca²⁺-calmodulin of cyclic nucleotide-activated channel of rat olfactory receptor neurons. *Nature* 368:545–48
156. Kurahashi T, Menini A. 1997. Mechanism of odorant adaptation in the olfactory receptor cell. *Nature* 385:725–29
157. Chen TY, Illing M, Molday LL, Hsu YT, Yau KW, Molday RS. 1994. Subunit 2 (or beta) of retinal rod cGMP-gated cation channel is a component of the 240-kDa channel-associated protein and mediates Ca(2+)-calmodulin modulation. *Proc. Natl. Acad. Sci. USA* 91:11757–61
158. Gordon SE, Zagotta WN. 1995. A histidine residue associated with the gate of the cyclic nucleotide-activated channels in rod photoreceptors. *Neuron* 14:177–83
159. Liu M, Chen TY, Ahamed B, Li J, Yau KW. 1994. Calcium-calmodulin modulation of the olfactory cyclic nucleotide-gated cation channel. *Science* 266:1348–54. Erratum. 1994. *Science* 266:1933
160. Zufall F, Shepherd GM, Firestein S. 1991. Inhibition of the olfactory cyclic nucleotide-gated ion channel by intracellular calcium. *Proc. R. Soc. Biol.* 246:225–30
161. Kramer RH, Siegelbaum SA. 1992. Intracellular Ca²⁺ regulates the sensitivity of cyclic nucleotide-gated channels in olfactory receptor neurons. *Neuron* 9:897–906

162. Firestein S, Zufall F. 1994. The cyclic nucleotide gated channel of olfactory receptor neurons. *Semin. Cell Biol.* 5:39–46
163. Kleene SJ. 1999. Both external and internal calcium reduce the sensitivity of the olfactory cyclic-nucleotide-gated channel to cAMP. *J. Neurophysiol.* 81:2675–82
164. Muller F, Bonigk W, Sesti F, Frings S. 1998. Phosphorylation of mammalian olfactory cyclic nucleotide-gated channels increases ligand sensitivity. *J. Neurosci.* 18:164–73
165. Molokanova E, Maddox F, Luetje CW, Kramer RH. 1999. Activity-dependent modulation of rod photoreceptor cyclic nucleotide-gated channels mediated by phosphorylation of a specific tyrosine residue. *J. Neurosci.* 19:4786–95
166. Molokanova E, Savchenko A, Kramer RH. 2000. Interactions of cyclic nucleotide-gated channel subunits and protein tyrosine kinase probed with genistein. *J. Gen. Physiol.* 115:685–96
167. Berghard A, Buck LB. 1996. Sensory transduction in vomeronasal neurons: evidence for G α o, G α i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. *J. Neurosci.* 16:909–18
168. Wiesner B, Weiner J, Middendorff R, Hagen V, Kaupp UB, Weyand I. 1998. Cyclic nucleotide-gated channels on the flagellum control Ca $^{2+}$ entry into sperm. *J. Cell. Biol.* 142:473–84
169. Kingston PA, Zufall F, Barnstable CJ. 1996. Rat hippocampal neurons express genes for both rod retinal and olfactory cyclic nucleotide-gated channels: novel targets for cAMP/cGMP function. *Proc. Natl. Acad. Sci. USA* 93:10440–45
170. Coburn CM, Bargmann CI. 1996. A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. *Neuron* 17:695–706
171. Zufall F, Shepherd GM, Barnstable CJ. 1997. Cyclic nucleotide gated channels as regulators of CNS development and plasticity. *Curr. Opin. Neurobiol.* 7:404–12
172. Yau K-W. 1994. Cyclic nucleotide-gated channels: an expanding new family of ion channels. *Proc. Natl. Acad. Sci. USA* 91:3481–83
173. Frings S. 1999. Tuning Ca $^{2+}$ permeation in cyclic nucleotide-gated channels. *J. Gen. Physiol.* 113:795–98
174. Frings S, Lindemann B. 1991. Current recording from sensory cilia of olfactory receptor cells in situ. *J. Gen. Physiol.* 97:1–15
175. Frings S, Reuter D, Kleene SJ. 2000. Neuronal Ca $^{2+}$ -activated Cl $^{-}$ channels—homing in on an elusive channel species. *Prog. Neurobiol.* 60:247–89
176. Kleene SJ, Gesteland RC. 1991. Calcium-activated chloride conductance in frog olfactory cilia. *J. Neurosci.* 11:3624–29
177. Reuter D, Zierold K, Schroder WH, Frings S. 1998. A depolarizing chloride current contributes to chemolectrical transduction in olfactory sensory neurons in situ. *J. Neurosci.* 18:6623–30
178. Kleene SJ. 1997. High-gain, low-noise amplification in olfactory transduction. *Biophys. J.* 73:1110–17
179. Zufall F, Leinders-Zufall T, Greer CA. 2000. Amplification of odor-induced Ca $^{2+}$ transients by store-operated Ca $^{2+}$ release and its role in olfactory signal transduction. *J. Neurophysiol.* 83:501–12
180. Corotto FS, Piper DR, Chen N, Michel WC. 1996. Voltage- and Ca $^{2+}$ -gated currents in zebrafish olfactory receptor neurons. *J. Exp. Biol.* 199:1115–26
181. Firestein S, Werblin FS. 1987. Gated currents in isolated olfactory receptor neurons of the larval tiger salamander. *Proc. Natl. Acad. Sci. USA* 84:6292–96
182. Lynch JW, Barry PH. 1991. Properties of transient K $^{+}$ currents and underlying single K $^{+}$ channels in rat olfactory receptor neurons. *J. Gen. Physiol.* 97:1043–72
183. Lucero MT, Chen N. 1997. Characterization of voltage- and Ca $^{2+}$ -activated K $^{+}$ channels in squid olfactory receptor neurons. *J. Exp. Biol.* 200:1571–86

184. Miyamoto T, Restrepo D, Teeter JH. 1992. Voltage-dependent and odorant-regulated currents in isolated olfactory receptor neurons of the channel catfish. *J. Gen. Physiol.* 99:505–29
185. Nevitt GA, Moody WJ. 1992. An electrophysiological characterization of ciliated olfactory receptor cells of the coho salmon *Oncorhynchus kisutch*. *J. Exp. Biol.* 166:1–17
186. Schild D. 1989. Whole-cell currents in olfactory receptor cells of *Xenopus laevis*. *Exp. Brain Res.* 78:223–32
187. Delgado R, Labarca P. 1993. Properties of whole cell currents in isolated olfactory neurons from the Chilean toad *Caudiverbera caudiverbera*. *Am. J. Physiol. Cell Physiol.* 264:C1418–C27
188. Maue RA, Dionne VE. 1987. Patch-clamp studies of isolated mouse olfactory receptor neurons. *J. Gen. Physiol.* 90:95–125
189. Trotier D. 1986. A patch-clamp analysis of membrane currents in salamander olfactory receptor cells. *Pfluegers Arch.* 407:589–95
190. Trombley PQ, Westbrook GL. 1991. Voltage-gated currents in identified rat olfactory receptor neurons. *J. Neurosci.* 11:435–44
191. Kawai F, Miyachi E. 2001. Modulation by cGMP of the voltage-gated currents in newt olfactory receptor cells. *Neurosci. Res.* 39:327–37
192. Restrepo D, Okada Y, Teeter JH, Lowry LD, Cowart B, Brand JG. 1993. Human olfactory neurons respond to odor stimuli with an increase in cytoplasmic Ca^{2+} . *Biophys. J.* 64:1961–66
193. Schild D, Jung A, Schultens HA. 1994. Localization of calcium entry through calcium channels in olfactory receptor neurons using a laser scanning microscope and the calcium indicator dyes Fluo-3 and Fura-Red. *Cell Calcium* 15:341–48
194. Kawai F, Kurahashi T, Kaneko A. 1997. Nonselective suppression of voltage-gated currents by odorants in the newt olfactory receptor cells. *J. Gen. Physiol.* 109:265–72
195. Trotier D, MacLeod P. 1986. Intracellular recordings from salamander olfactory supporting cells. *Brain Res.* 374:205–11
196. Berridge MJ, Irvine RF. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312:315–21
197. Berridge MJ, Irvine RF. 1989. Inositol phosphates and cell signalling. *Nature* 341:197–204
198. Kuno M, Gardner P. 1987. Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* 326:301–4
199. Fijimoto T, Nakade S, Miyawaki A, Mikoshiba K, Ogawa K. 1992. Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. *J. Cell Biol.* 119:1507–13
200. Bush KT, Stuart RO, Li SH, Moura LA, Sharp AH, et al. 1994. Epithelial inositol 1,4,5-trisphosphate receptors. Multiplicity of localization, solubility, and isoforms. *J. Biol. Chem.* 269:23694–99
201. Joseph SK. 1996. The inositol triphosphate receptor family. *Cell. Signal.* 8:1–7
202. Taylor CW, Richardson A. 1991. Structure and function of inositol trisphosphate receptors. *Pharmacol. Ther.* 51:97–137
203. Taylor CW, Traynor D. 1995. Calcium and inositol trisphosphate receptor. *J. Membr. Biol.* 145:109–18
204. Huque T, Bruch RC. 1986. Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia. *Biochem. Biophys. Res. Commun.* 137:36–42
205. Restrepo D, Miyamoto T, Bryant BP. 1990. Odor stimuli trigger influx of Ca^{2+} into olfactory neurons of the channel catfish. *Science* 249:1166–68
206. Ronnett GV, Cho H, Hester LD, Wood SR, Snyder SH. 1993. Odorants differentially enhance phosphoinositide turnover and adenylyl cyclase in olfactory receptor neuronal cultures. *J. Neurosci.* 13:1751–58

207. Boekhoff I, Michel WC, Breer H, Ache BW. 1994. Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J. Neurosci.* 14:3304–9
208. Cunningham AM, Ryugo DK, Sharp AH, Reed RR, Snyder SH, Ronnett GV. 1993. Neuronal inositol 1,4,5-trisphosphate receptor localized to the plasma membrane of olfactory cilia. *Neuroscience* 57:339–52
209. Munger SD, Gleeson RA, Aldrich HC, Rust NC, Ache BW, Greenberg RM. 2000. Characterization of a phosphoinositide-mediated odor transduction pathway reveals plasma membrane localization of an inositol 1,4,5-trisphosphate receptor in lobster olfactory receptor neurons. *J. Biol. Chem.* 275:20450–57
210. Fadool DA, Ache BW. 1992. Plasma membrane inositol 1,4,5-trisphosphate-activated channels mediate signal transduction in lobster olfactory receptor neurons. *Neuron* 9:907–18
211. Kalinoski DL, Aldinger SB, Boyle AG, Huque T, Maracek JF, et al. 1992. Characterization of a novel inositol 1,4,5-trisphosphate receptor in isolated olfactory cilia. *Biochem. J.* 281:449–56
212. Bruch RC, Abogadie FC, Farbman AI. 1995. Identification of three phospholipase C isotypes expressed in rat olfactory epithelium. *NeuroReport* 6:233–37
213. Abogadie FC, Bruch RC, Wurzbarger R, Margolis FL, Farbman AI. 1995. Molecular cloning of a phosphoinositide-specific phospholipase C from catfish olfactory rosettes. *Brain Res.* 31:10–16
214. Zuker CS. 1996. The biology of vision of *Drosophila*. *Proc. Natl. Acad. Sci. USA* 93:571–76
215. Somlyo AV, Walz B. 1995. Ca²⁺ in visual transduction and adaptation in vertebrate and invertebrates. *Cell Calcium* 18:253–55
216. Wang TL, Sterling P, Vardi N. 1999. Localization of type I inositol 1,4,5-trisphosphate receptor in the outer segments of mammalian cones. *J. Neurosci.* 19:4221–28
217. Udovichenko IP, Cunnick J, Gonzalez K, Takemoto DJ. 1994. The visual transduction and the phosphoinositide system: a link. *Cell. Signal.* 6:601–5
218. Coccia VJ, Cote RH. 1994. Regulation of intracellular cyclic GMP concentration by light and calcium in electropermeabilized rod photoreceptors. *J. Gen. Physiol.* 103:67–86
219. Leinders-Zufall T, Shepherd GM, Zufall Z. 1996. Modulation by cyclic GMP of the odour sensitivity of vertebrate olfactory receptor cells. *Proc. R. Soc. Biol.* 263:803–11
220. Moon C, Jaber P, Otto-Bruc A, Baehr W, Palczewski K, Ronnett G. 1998. Calcium-sensitive particulate guanylyl cyclase as a modulator of cAMP in olfactory neurons. *J. Neurosci.* 18:3195–205
221. Snyder SH. 1994. Nitric oxide and carbon monoxide: unprecedented signalling molecules in the brain. In *Encyclopedia Britannica*, ed. F-Y Sat, D Calhoun, pp. 84–101. Chicago: Britannica
222. Ingi T, Ronnett GV. 1995. Direct demonstration of a physiological role for carbon monoxide in olfactory receptor neurons. *J. Neurosci.* 15:8214–22
223. Roskams JA, Bredt DS, Ronnett GV. 1994. Nitric oxide expression during olfactory neuron development and regeneration. *Am. Chem. Soc.* 16:308
224. Bauer I, Wanner GA, Rensing H, Alte C, Miescher EA, et al. 1998. Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver. *Hepatology* 27:829–38
225. Beschorner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, et al. 2000. Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol.* 100:377–84
226. Ewing JF, Raju VS, Maines MD. 1994. Induction of heart heme oxygenase-1 (HSP32) by hyperthermia: possible role

- in stress-mediated elevation of cyclic 3':5'-guanosine monophosphate. *J. Pharmacol. Exp. Ther.* 271:408–14
227. Hirata K, He JW, Kuraoka A, Omata Y, Hirata M, et al. 2000. Heme oxygenase 1 (HSP-32) is induced in myelin-phagocytosing Schwann cells of injured sciatic nerves in the rat. *Eur. J. Neurosci.* 12: 4147–52
228. Koistinaho J, Miettinen S, Keinanen R, Vartiainen N, Roivainen R, Laitinen JT. 1996. Long-term induction of haeme oxygenase-1 (HSP-32) in astrocytes and microglia following transient focal brain ischaemia in the rat. *Eur. J. Neurosci.* 8: 2265–72
229. Kutty RK, Maines MD. 1989. Selective induction of heme oxygenase-1 isozyme in rat testis by human chorionic gonadotropin. *Arch. Biochem. Biophys.* 268:100–7
230. Hatt H, Ache BW. 1994. Cyclic nucleotide and inositol phosphate-gated ion channels in lobster olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* 91:6264–68
231. Dhallan RS, Yau KW, Schrader KA, Reed RA. 1990. Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature* 347:184–87
232. Baimbridge KG, Celio MR, Rogers JH. 1992. Calcium-binding proteins in the nervous system. *Trends Neurosci.* 15: 303–8
233. Bastianelli E, Polans AS, Hidaka H, Pochet R. 1995. Differential distribution of six calcium-binding proteins in the rat olfactory epithelium during postnatal development and adulthood. *J. Comp. Neurol.* 354:395–409
234. Anholt RRH, Rivers AM. 1990. Olfactory transduction: cross-talk between second-messenger systems. *Biochemistry* 29:4049–54
235. Hsu Y-T, Molday RS. 1993. Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. *Nature* 361:76–79
236. Kurahashi T, Shibuya T. 1990. Ca²⁺-dependent adaptive properties in the solitary olfactory receptor cell of the newt. *Brain Res.* 515:261–68
237. Kurahashi T, Yau K-W. 1993. Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature* 363:71–74
238. Iino S, Kobayashi S, Okazaki K, Hidaka H. 1995. Neurocalcin-immunoreactive receptor cells in the rat olfactory epithelium and vomeronasal organ. *Neurosci. Lett.* 191:91–94
239. Bastianelli E, Pochet R. 1995. Calmodulin, calbindin-D28k, calretinin and neurocalcin in rat olfactory bulb during postnatal development. *Brain Res. Dev. Brain Res.* 87:224–27
240. Miwa N, Kobayashi M, Takamatsu K, Kawamura S. 1998. Purification and molecular cloning of a novel calcium-binding protein, p26olf, in the frog olfactory epithelium. *Biochem. Biophys. Res. Commun.* 251:860–67
241. Boekhoff I, Braunewell KH, Andreini I, Breer H, Gundelfinger E. 1997. The calcium-binding protein VILIP in olfactory neurons: regulation of second messenger signaling. *Eur. J. Cell. Biol.* 72:151–58
242. Kleene SJ. 1993. Origin of the chloride current in olfactory transduction. *Neuron* 11:123–32
243. Sibley DR, Benovic JL, Caron MG, Lefkowitz RJ. 1987. Regulation of transmembrane signaling by receptor phosphorylation. *Cell* 48:913–22
244. Haganir RL, Greengard P. 1990. Regulation of neurotransmitter receptor desensitization by protein phosphorylation. *Neuron* 5:555–67
245. Hausdorff WP, Caron MG, Lefkowitz RJ. 1990. Turning off the signal: desensitization of β -adrenergic receptor function. *FASEB J.* 4:2881–89
246. Benovic JL, Bouvier M, Caron MG, Lefkowitz RJ. 1988. Regulation of adenylyl

- cyclase-coupled β -adrenergic receptors. *Annu. Rev. Cell Biol.* 4:405–28
247. Benovic JL, DeBlasi A, Stone WC, Caron MG, Lefkowitz RJ. 1989. β -adrenergic receptor kinase: primary structure delineates a multigene family. *Science* 246:235–40
248. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. 1990. β -arrestin: a protein that regulates β -adrenergic receptor function. *Science* 248:1547–50
249. Dawson TM, Arriza JL, Lefkowitz RJ, Jaworsky DE, Ronnett GV. 1993. Beta-adrenergic receptor kinase-2 and beta-arrestin-2: mediators of odorant-induced desensitization. *Science* 259:825–29
250. Schleicher S, Boekhoff I, Arriza J, Lefkowitz RJ, Breer H. 1993. A β -adrenergic receptor kinase-like enzyme is involved in olfactory signal termination. *Proc. Natl. Acad. Sci. USA* 90:1420–24
251. Poppel K, Boekhoff I, McDonald P, Breer H, Caron MG, Lefkowitz RJ. 1997. G protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of OR desensitization. *J. Biol. Chem.* 272:25425–28
252. Boekhoff I, Breer H. 1992. Termination of second messenger signaling in olfaction. *Proc. Natl. Acad. Sci. USA* 89:471–74
253. Hill CS, Treisman R. 1995. Transcriptional regulation by extracellular signals: mechanisms and specificity. *Cell* 80:199–211
254. Hirotsu T, Saeki S, Yamamoto M, Iino Y. 2000. The Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. *Nature* 404:289–93
255. Watt WC, Storm DR. 2000. Odorants stimulate the Erk/MAP kinase pathway and activate CRE-mediated transcription in olfactory sensory neurons. *J. Biol. Chem.* 276:2047–52