

## CHAPTER

## 52

## Molecular Basis of Olfaction and Taste

Steven D. Munger

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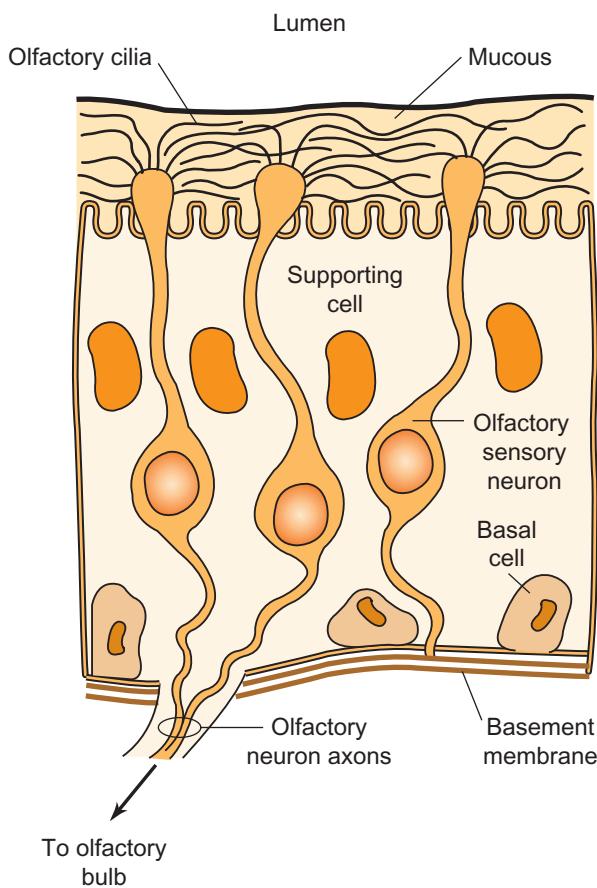
## OLFACTION

## The mammalian olfactory system possesses enormous discriminatory power

It is claimed that humans can perceive many thousands of different odorous molecules, termed odorants. Even slight alterations in the structure of an odorant can lead to profound changes in perceived odor quality. One commonly cited example is carvone, whose L- and D-stereoisomers are perceived as spearmint and caraway, respectively. However,

more subtle molecular alterations can also generate striking changes in perception.

The fine discriminatory power of the mammalian olfactory system is likely to derive from information-processing events that occur at several distinct anatomical sites (Firestein, 2001): the olfactory epithelium of the nasal cavity, where odors are first sensed by olfactory sensory neurons (OSNs); the olfactory bulb, where information received from the sensory neurons is initially processed; and the cerebral cortex, where information received from the olfactory bulb is thought to be further refined to allow for the discrimination of thousands of different odors.



**FIGURE 52-1** A schematic diagram of the olfactory epithelium. Odorants enter the nasal cavity, diffuse through the nasal mucus and interact with specific receptors on the dendritic cilia of olfactory sensory neurons. The signals initiated by this receptor binding are transduced into electrical signals within the cilia and are transmitted along the sensory neuron axons to the olfactory bulb in the brain.

### The initial events in olfaction occur in a specialized olfactory neuroepithelium

In mammals, this structure lies within the posterior nasal cavity. The olfactory epithelium contains three predominant cell types: the OSN; the supporting, or sustentacular, cell; and the basal cell (Figure 52-1). OSNs turn over throughout life. They are continuously replaced from progenitor populations within the basal cells.

The OSN is a bipolar neuron that extends a single dendrite to the apical surface of the epithelium. Numerous nonmotile cilia project from the end of the dendrite into the layer of mucus that lines the nasal cavity. These cilia contain the molecular machinery of olfactory transduction, including receptors, effector enzymes and ion channels. The details of odorant detection and transduction will be discussed in a later section.

Each OSN projects an unbranched axon to the olfactory bulb, where it forms synapses within specialized regions of neuropil, called glomeruli. OSNs form synapses with both projection neurons and local interneurons. The major output neurons of the bulb, the mitral and tufted cells, project to higher olfactory areas in the brain, including the olfactory

cortex. Olfactory bulb interneurons such as periglomerular cells and granule cells help to shape sensory input and olfactory bulb output in several ways before this information is sent to higher centers in the brain, which include the anterior olfactory nucleus and the piriform cortex.

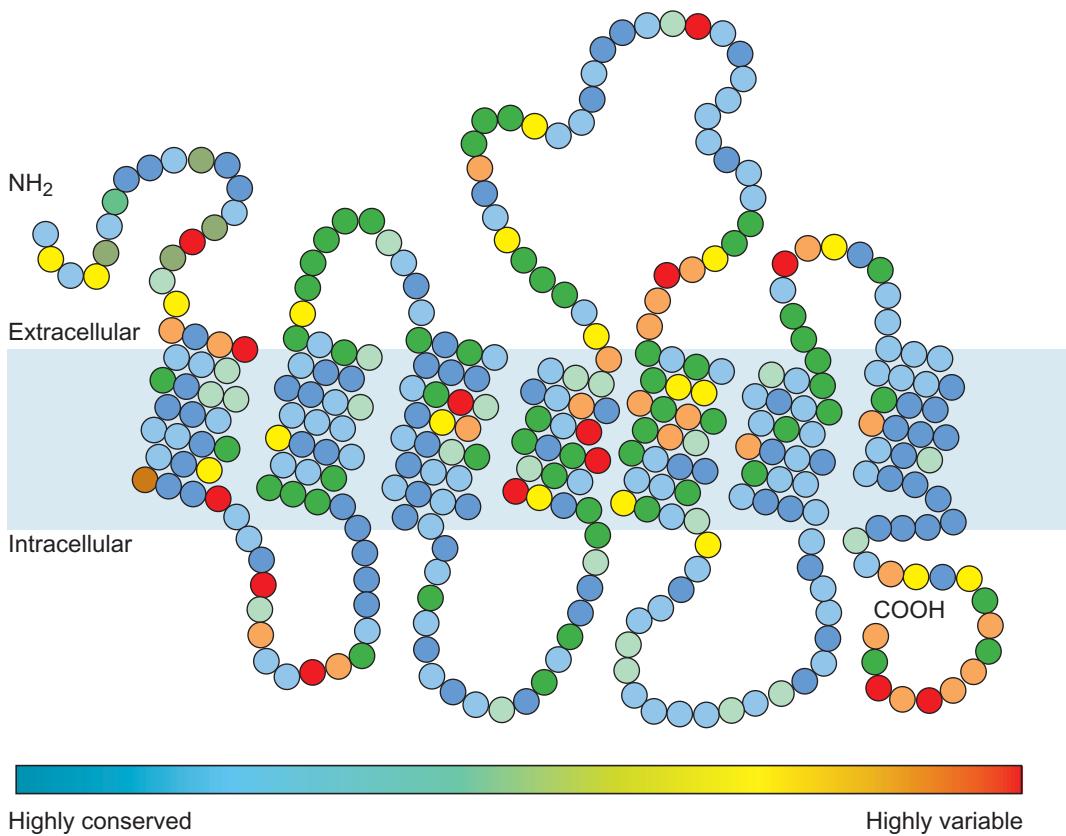
### The identification and cloning of genes encoding odorant receptors helped to reveal organizational principles of odor coding

Electrophysiological experiments demonstrate that most OSNs recognize multiple odorants. It is thought that the patterns of axonal convergence in the olfactory bulb by OSNs that recognize different odorants might constitute elementary odor codes. However, for many years it was not clear how many different types of odorant receptors would be required to accurately encode a single odorant, how narrowly or broadly tuned those receptors might be, or how their expression might be organized to achieve a high level of sensory discrimination. The identification by Buck and Axel of the first members of a large multigene family encoding hundreds of diverse mammalian odorant receptors (ORs) (Buck & Axel, 1991) has enabled researchers to begin to answer these questions. Indeed, the discovery of the OR gene family was so significant for our understanding of olfaction that the work of Buck and Axel was recognized with the 2004 Nobel Prize in Physiology or Medicine (see Mombaerts, 2004).

ORs are members of the G protein-coupled receptor (GPCR) superfamily, and share with other members a number of stereotypical motifs, including an apparent seven-transmembrane domain topology (Ch. 21). However, ORs exhibit unique sequence motifs not seen in other GPCRs, and which, along with their expression in OSNs, help to define them as a distinct group of receptor proteins (Spehr & Munger, 2009). Analyses of the human and mouse genomes has revealed ~350 functional OR genes in humans (with an equivalent number of OR pseudogenes) and ~1200 in mice, making this the largest mammalian gene family. OR genes are found in clusters within the genome on nearly every chromosome, suggesting a large number of local tandem duplications during evolution (Young et al., 2002). The large number of mammalian ORs has enormous implications for the way odorants are detected and encoded by the olfactory system.

A striking feature of ORs is that some regions of the protein are highly variable, while others are relatively conserved. As seen in Figure 52-2, transmembrane domain 4 (TM4), TM5 and parts of TM3 are highly variable across the OR repertoire. As it would be expected that variation in amino acid sequence would be a hallmark of a ligand-binding pocket, enabling divergent ORs to recognize chemically diverse odorants, these regions have been proposed as the site of ligand interaction (Buck & Axel, 1991). Indeed, many Class A GPCRs, which include rhodopsin (Chapter 51) and the  $\beta$ -adrenergic receptor (Chapter 14) as well as the ORs, have a ligand-binding pocket formed by the transmembrane helices. Structure-function studies of several ORs in which specific transmembrane-localized amino acids have been mutated *in vitro* support this location for the ligand-binding pocket.

Somewhat surprisingly, ORs have been found in a number of tissues outside of the olfactory system. In some cases, these



**FIGURE 52-2** Amino acid sequence conservation across mammalian odorant receptors. ORs pass through the plasma membrane (blue box) seven times, with the N-terminus located extracellularly and the C-terminus intracellularly. The degree of conservation of each amino acid in this consensus OR is indicated by a colored ball, with dark blue being most highly conserved and red most highly variable. Source: Modified from Liu et al. (2003) *Genomics*, 81: 443–456 with permission.

ectopically expressed ORs may have been classified as ORs based only on sequence similarity, and would be more accurately referred to as “OR-like” receptors. However, some true ORs function as both olfactory and non-olfactory chemosensory receptors. One of the best examples is a human OR that is expressed in both the olfactory epithelium, where it mediates olfactory sensitivity to the compound bourgenal, and in sperm, where it has been implicated in chemotaxis (Spehr et al., 2003).

### Odor discrimination involves a very large number of different odorant receptors, each responsive to a small set of odors

Although technical hurdles slowed progress for many years on pairing individual receptors with their cognate odor ligands, recent advances in heterologous expression and screening strategies are now permitting more systematic structure-function analysis of ORs. Receptor “deorphaning” has been accomplished using a number of approaches including viral-mediated overexpression of individual receptors *in vivo*, isolation of receptor cDNAs from single OSNs with characterized responses to a panel of odors or the coupling of expressed receptors to endogenous cellular signaling pathways in cultured cell lines or *Xenopus* oocytes (Spehr & Munger, 2009). Even so, researchers have still identified ligands for only a

minority of either mouse or human ORs. However, even this small number has revealed some important features of OR function. First, ORs can have a broad receptive range; that is, they respond to more than one odorant, and often to odorants of more than one chemical class (e.g., aldehydes vs. alcohols). Second, more than one OR can be activated by the same odorant. Third, not every OR activated by a particular odor responds with the same efficacy to that odor. Together, these observations indicate a repertoire of ORs with overlapping odorant profiles. The idea that the identity of an individual odor is encoded by several differentially tuned ORs has been called a combinatorial odor code (Malnic et al., 1999). As will be discussed in the next section, studies of odor coding in the olfactory bulb largely support this model.

### The information generated by hundreds of different receptor types must be organized to achieve a high level of olfactory discrimination

The visual, auditory and somatosensory systems (Chs. 51, 53, 54) localize environmental information in space and possess neural topographical maps of that spatial information. The olfactory system does not perceptually localize environmental information in external space. However, it could use spatial determinants within the nervous system to encode

information. If so, topographical maps or spatial codes for odors might be evident within the olfactory epithelium or olfactory bulb. For example, olfactory neurons that express a particular OR gene, and therefore recognize the same odorants, might be clustered in one region of the olfactory epithelium or might all form synapses at a discrete site in the olfactory bulb. On the other hand, neurons that express the same OR gene could be broadly distributed in the olfactory epithelium or form synapses at many different bulbar sites, encoding information by a nontopographical strategy. The identification of ORs has provided an important tool for dissecting how olfactory information is coded in neural space.

### **Zonal Expression of Olfactory Receptors**

In the olfactory epithelium, different OR genes are expressed within restricted anatomical zones (Ressler et al., 1993; Vassar et al., 1993). These zones exhibit bilateral symmetry in the two nasal cavities and are virtually identical across individuals, although the number of distinct zones is unclear and individual zones may overlap. Within each zone, many different members of the OR gene family are expressed. However, each individual gene may be expressed only within a single zone. Although the zonal assignment of each gene seems to be strictly regulated, within each zone OSNs that express a particular receptor gene are broadly distributed. It thus appears that when an OSN or its progenitor chooses which OR gene(s) to express, it is restricted to a single zonal gene set; the cell then chooses the specific OR gene to express from among that set via a stochastic mechanism. Studies from several groups indicate the presence of a poorly understood negative feedback mechanism for a phenomenon that is accepted, but not proven: in most circumstances, OSNs express only one OR gene (Serizawa et al., 2004).

### **Convergence of Sensory Neurons Onto a few Glomeruli in the Olfactory Bulb**

Comparisons of the locations of the expression zones with the topography of projections between the olfactory epithelium and olfactory bulb indicate that the organization of OR gene expression in the epithelium is preserved in the axonal projection to the olfactory bulb. Indeed, the map is even more specific than this. Axons from OSNs expressing the same receptor gene converge on just a few glomeruli in the bulb, usually two (Ressler et al., 1994; Vassar et al., 1994). Thus, it appears that all of the cells expressing a particular OR are somehow targeted to just a few among approximately 1,900 glomeruli in the mouse olfactory bulb. Interestingly, the OR itself seems to play a critical role in this specific pattern of axonal targeting, though whether this is a direct or indirect role is unclear. These results suggest that an initial organization of olfactory sensory information occurs in the olfactory epithelium and that this organization is maintained in the patterns of signals transmitted to the olfactory bulb.

This pattern of organization in the bulb argues that the glomerulus, not the OR, is the fundamental unit of olfactory coding. Given the overlapping odor specificities of individual olfactory receptors, one would then expect that an individual odor would activate more than just one or two glomeruli, an expectation that has been supported by a number of functional studies. It is now believed that some version of a combinatorial code for odors is present in the bulb, where the

identity of a particular odorant is represented by the pattern of glomerular activity in the whole bulb. As more researchers turn their focus to higher brain centers, such as the olfactory cortex, it will be interesting to see the extent to which the glomerular pattern of odor activity is maintained.

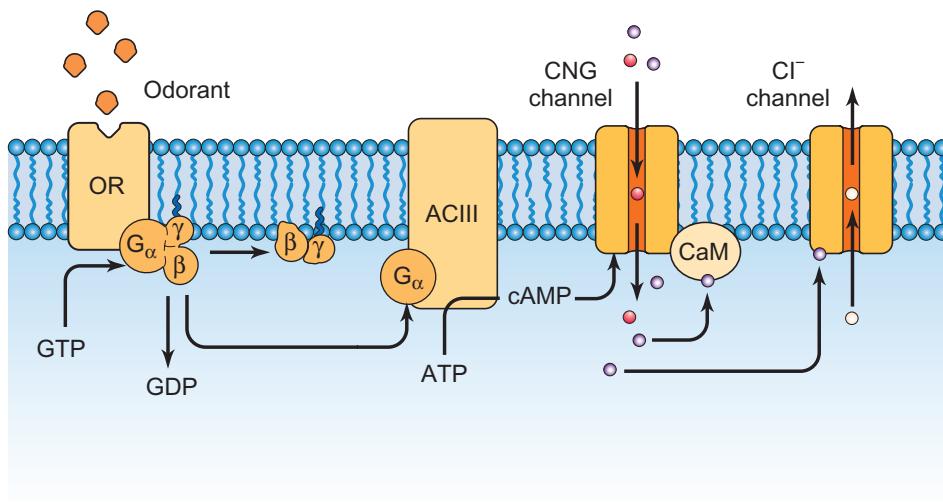
### **The sensitivity of the olfactory system is likely to derive from the capacity of the olfactory transduction apparatus to effectively amplify and rapidly terminate signals**

The olfactory system responds to extremely low concentrations of odorants, and olfactory perception is believed to be extremely sensitive, although the degree of sensitivity is controversial. For example, human olfactory thresholds of  $10^{-11} M$  have been measured by some psychophysical methods. Even so, the behavioral sensitivities of mammals to odors appear to be several orders of magnitude less than the apparent affinities of the ORs for their ligands. It can be shown from the law of mass action that with significant amplification mechanisms, such that occupation of only a few receptors would be sufficient to elicit a response, thresholds as much as five or six orders of magnitude lower than the  $K_d$  can be attained. Thus,  $K_d$  values of  $10^{-6}$  could, in theory, produce threshold responses at odor concentrations as low as  $10^{-11}$ , permitting the olfactory system to maintain the broad specificity seen in physiological recordings or receptor activation assays and the high threshold sensitivity measured in psychophysical experiments. Several amplification mechanisms that could serve this purpose are described below.

### **Odorant recognition initiates a second-messenger cascade leading to the depolarization of the neuron and the generation of action potentials**

Stimulation of single OSNs with odorants causes the membrane to depolarize, leading to the generation of action potentials. Underlying this initial depolarization is the influx of cations through a conductance in the specialized cilia extending from the distal end of the cell. The biochemical elements coupling odorant receptor binding to the opening of a cation channel are now understood in some detail (Munger et al., 2009). A consensus view is presented in Figure 52-3.

The odor-dependent second-messenger cascade is a classic cyclic nucleotide-based system with some interesting modifications. A G protein (Ch. 21) that is likely to couple odorant receptors to other intracellular elements of the cascade,  $G_{\alpha\text{olf}}$ , has been identified (Jones & Reed, 1989). An isoform of  $G_{\alpha\text{s}}$ , it is highly enriched in OSN cilia. Deletion of this gene in mice abolishes nearly all odor responses in OSNs and odor-mediated behaviors, confirming its central role in olfactory transduction (Belluscio et al., 1998). OSNs also contain an adenylyl cyclase isoform (Ch. 22), type III (ACIII), that is highly enriched in olfactory cilia (Bakalyar & Reed, 1990). An important characteristic of this isozyme is that, when expressed in a mammalian cell line, its basal activity is extremely low, while in its stimulated state it has a catalytic rate higher than other known cyclases. These properties could confer a high signal-to-noise ratio on the system, being quiescent in the absence of stimulus



**FIGURE 52-3** A model for the transduction of odors in canonical OSNs. The individual steps are detailed in the text. Note that several feedback loops modulate the odor response, including inhibition of the CNG channel by  $\text{Ca}^{2+}$  (purple balls) that permeate the channel, and a  $\text{Ca}^{2+}$ /calmodulin (CaM)-mediated desensitization of the CNG channel that underlies rapid odor adaptation. Several other mechanisms have also been described, including phosphodiesterase-mediated hydrolysis of the second messenger cAMP and phosphorylation of the OR by various kinases.

but able to rapidly generate large amounts of the second messenger adenosine 3'5'-cyclic monophosphate (cAMP) (Ch. 22) upon odor exposure. Deletion of the gene encoding ACIII elicits a general anosmia in mice (Wong et al., 2000). ACIII is also inhibited by  $\text{Ca}^{2+}$ , providing an opportunity for negative feedback upon intracellular elevations of this signaling molecule.

In 1987, Nakamura and Gold (Nakamura & Gold, 1987) recorded a cAMP-activated conductance from OSN cilia. This was the final link between the biochemical cascade and the electrical signal. It was subsequently determined that the channel responsible is comprised of three distinct olfactory-specific subunits, CNGA2, CNGA4 and CNGB1b (Kaupp & Seifert, 2002); all are members of the cyclic nucleotide-gated (CNG) channel family (Ch. 22) that also contains the guanosine 3'5'-cyclic monophosphate (cGMP)-activated channel found in photoreceptors (Ch. 51). These channels are homologous with voltage-sensitive channels, such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  channels (Kaupp & Seifert, 2002). CNGA2 is obligatory for channel function both *in vitro* and *in vivo*, while CNGA4 and CNGB1b serve important modulatory roles (Kaupp & Seifert, 2002). The olfactory CNG channel is selective for cations; is sensitive to both cAMP and cGMP, with a  $K_d$  of  $20\mu\text{M}$  or  $K_d$  of  $5\mu\text{M}$ , respectively; requires at least three molecules of cyclic nucleotide to bind for activation; and is required for odor signaling in OSNs (Brunet et al., 1996; Kaupp & Seifert, 2002).

The activation of tens to hundreds of these channels and the subsequent influx of cations, including both  $\text{Na}^+$  and  $\text{Ca}^{2+}$ , leads to depolarization of the cell membrane. OSNs have one additional level of amplification that is rather unusual. A  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  conductance is also present on the cilia and is opened during the odor response by the  $\text{Ca}^{2+}$  ions flowing into the cilia through the cAMP-gated channel; this channel was recently identified as anoctamin 2 (ANO2) (Stephan et al., 2009). Curiously, olfactory neurons maintain a very high intracellular  $\text{Cl}^-$  concentration, perhaps as high as  $125\text{ mM}$ , so that the driving force for  $\text{Cl}^-$  ions is outward. Thus, activation of this  $\text{Cl}^-$

conductance further depolarizes the cell. This additional current may be important for preserving ionic driving forces across a membrane in a compartment that is not highly regulated; changing ion concentrations in the mucus could affect the driving forces on ions, so the olfactory neuron supplies its own driving force by maintaining a high intracellular  $\text{Cl}^-$  concentration.

The olfactory transduction pathway provides several amplification steps between odorant binding and signal generation. Due to the electrically compact structure of the cell, it is possible for the activation of only a few tens of channels to drive the membrane to threshold for action potential generation. Thus, it is theoretically possible that the limit of olfactory detection is the single molecule.

### Negative feedback processes mediate adaptation of the olfactory transduction apparatus to prolonged or repetitive stimulation

Upon application of a sustained odor stimulus to an OSN, the current response is transient, falling back to baseline within 4 to 5 sec. The termination of a response in the continued presence of an agonist is characteristic of many signaling systems and is variously known as adaptation or desensitization, depending on the putative site of the off mechanism. Typically, a negative feedback process is at work such that accumulation of a product of the agonist response serves to turn off an upstream link in the signal-generating cascade. In the OSN, two feedback messengers have been identified and, as might be expected, they are  $\text{Ca}^{2+}$  and cyclic nucleotides.

The CNG channel is permeable to cations, especially  $\text{Ca}^{2+}$ . Thus, increased channel activation results in influx of  $\text{Ca}^{2+}$  and a transient rise in the intracellular  $\text{Ca}^{2+}$  concentration. Intracellular  $\text{Ca}^{2+}$  concentrations of 1 to  $3\mu\text{M}$  have been found to lead to a decrease in the open probability of the ion channel, even in the presence of high concentrations of cAMP.

Mediated by the intermediate  $\text{Ca}^{2+}$ -binding protein calmodulin, this  $\text{Ca}^{2+}$ -dependent desensitization of the channel is a major contributor to short-term adaptation and is dependent on the presence of the CNGA4 subunit, which increases the kinetics of  $\text{Ca}^{2+}$ /calmodulin-mediated desensitization of the native channel nearly 70-fold (Munger et al., 2001). This is an attractive mechanism for mediating a rapid but short-lasting form of adaptation since it is dependent on the influx of  $\text{Ca}^{2+}$  during the response to an odor.

Other mechanisms have also been implicated in odor adaptation, including phosphodiesterase-mediated hydrolysis of cAMP; cAMP-dependent phosphorylation of ciliary proteins via protein kinase A; G protein receptor kinase activity (GRK3), possibly via phosphorylation of the OR;  $\text{Ca}^{2+}$ /calmodulin kinase II (CaMKII) phosphorylation of ACIII; cGMP; and carbon monoxide (Zufall & Leinders-Zufall, 2000). These latter three mechanisms have been particularly linked to longer-lasting forms of adaptation, on the order of tens of seconds (for CaMKII) or minutes (CO, cGMP). Together with the short-term adaptation described previously, these various molecular mechanisms provide the OSN with a number of ways to fine-tune odor responses over time.

### Subpopulations of OSNs use alternative olfactory transduction mechanisms

The importance of ORs and cAMP in olfactory transduction is well established. However, alternative receptors and

signaling pathways also play important roles in mammalian olfactory transduction. For example, trace amine-associated receptors (TAARs) are expressed in a small subpopulation of OSNs, where they seem to act as olfactory receptors for biogenic amines and related stimuli (Liberles & Buck, 2006). TAAR-expressing OSNs do express at least one key component of the olfactory cAMP cascade,  $G\alpha_{olf}$ , but do not express ORs.

Another small subset of OSNs clearly does not utilize cAMP to transduce olfactory stimuli. These cells, which may represent less than 0.1% of the OSNs in the epithelium, do not express  $G\alpha_{olf}$ , ACIII or CNGA2, and are unlikely to express ORs. They do, however, express components of a cGMP signaling mechanism: a transmembrane isoform of guanylyl cyclase called GC-D (Ch 22); a splice variant of the cGMP-sensitive channel subunit CNGA3, the principal subunit found in cone photoreceptors; and cGMP-sensitive phosphodiesterase 2A; and type II carbonic anhydrase (Zufall & Munger, 2010). Surprisingly, these OSNs are responsive to diverse chemostimuli including the natriuretic peptides uroguanylin and guanylin, the gas carbon dioxide and the volatile carbon disulfide. Although the receptor mechanisms for these various stimuli remain unclear, all compounds appear to elevate cGMP levels through the direct or indirect regulation of GC-D. Thus, in contrast to mammalian photoreceptors, GC-D-expressing OSNs utilize an excitatory cGMP-mediated cascade to transduce sensory stimuli. Recently, it was determined that these specialized OSNs play a critical role in food-related social learning in mice through their sensitivity to carbon disulfide (Munger et al., 2010), a social odor present in the breath of mammals (see Box, below).

### OLFACTION AND SOCIAL COMMUNICATION

**Steven D. Munger**

When you sit down with a friend for dinner, you rarely just eat a meal. The experience is filled with opportunities for social communication. Obviously, language is immensely useful for communicating both simple and complex ideas. However, gestures, facial expressions and tone of voice carry critical information, as well, and can help us to relate to each other in a more nuanced way. For example, if your companion smiles, takes a big bite of food, leans back in his chair, looks around and says, "I'm glad we decided to come here," he is likely commenting on the quality of the restaurant and his appreciation for your selecting it. However, if she smiles, leans forward, looks directly at you and says those same words, she is more likely showing her appreciation of you than for the venue.

The importance of these messages for humans may be best illustrated by individuals for whom social communication is the most challenging. A hallmark of autism spectrum disorders (Chapter 59) is a deficit in social communication. Individuals on the autism spectrum often have difficulties in recognizing tone of voice or facial expressions and so fail to grasp the full context of their communication with others. This impairment of social communication can negatively affect the formation of relationships and interactions with peers in social or work environments.

Of course, most animals do not use spoken language to communicate. Even so, they depend on the ability to recognize, interpret and respond to social signals in order to effectively reproduce, establish social hierarchies and find food. In many mammals, the production and recognition of olfactory cues is a primary means of communicating critical social information. A number of volatile, peptide and protein compounds found in breath, urine or glandular secretions can act as olfactory stimuli in the recipient and can elicit learned (e.g., acquisition of a food preference) or innate responses (e.g., the promotion of mating behaviors) (Stowers & Logan, 2010). They can even communicate genetic identity, fitness or compatibility.

Many of our insights into the molecular basis of olfactory-mediated social communication come from studies of specialized olfactory subsystems found in mice (and many other lower mammals). Vomeronasal sensory neurons (VSNs) respond to a number of compounds, produced by a conspecific, that elicit stereotyped behaviors or physiological changes in the recipient animal. These include mating and aggression behaviors as well as hormonal changes that accelerate puberty onset or promote estrus synchronization. For example, VSNs expressing the vomeronasal receptor V2Rp5 are activated by a peptide, exocrine gland-secreted peptide 1 (ESP1), secreted from the lacrimal

## OLFACTION AND SOCIAL COMMUNICATION (cont'd)

glands of male mice. V2Rp5-dependent detection of this peptide by VSNs of female mice enhances lordosis behaviors, a response to male mounting that facilitates successful copulation (Haga, et al., 2010). In this species, at least, successful olfactory-mediated social communication provides a clear reproductive advantage.

The main olfactory system also detects social stimuli. For example, olfactory sensory neurons (OSNs) that express the guanylyl cyclase GC-D are highly sensitive to the social odor carbon disulfide (Munger et al., 2010), which is found in the breath of mammals. This compound, when paired with a food odor, lets the recipient animal know that the food he just smelled on his friend's breath is safe to eat (as the friend is obviously still breathing).

Interestingly, many genes that are important for olfactory-mediated social communication in lower animals, including most vomeronasal receptor genes, as well as the genes encoding GC-D and the vomeronasal transduction channel TRPC2, became pseudogenes in the primate line. This suggests that vomeronasal function became less important as mammals moved into the trees, where they are often physically separated and forced to rely on visual and auditory cues for communicating across distances. Do these genetic and olfactory differences between rodents and primates mean that olfactory-mediated social communication is irrelevant for studying human social communication? This seems unlikely. We rely heavily on our

sense of smell when it comes to interacting with our fellow humans, as the fragrance industry knows well. But more importantly, many of the neural circuits that process information about social odors are highly conserved across species. Thus, understanding the molecular and neural mechanisms by which mammals use olfaction and other sensory modalities to communicate important social information should help elucidate the way humans recognize and process critical sensory cues that enrich our lives and help us to relate to others.

## References

- Haga, S., Hattori, T., Sato, T., Sato, K., Matsuda, S., Kobayakawa, R., et al. (2010). The male mouse pheromone ESP1 enhances female sexual receptive behaviour through a specific vomeronasal receptor. *Nature*, 466(7302), 118–122.
- Munger, S. D., Leinders-Zufall, T., McDougall, L. M., Cockerham, R. E., Schmid, A., Wandernoth, P., et al. (2010). An olfactory subsystem that detects carbon disulfide and mediates food-related social learning. *Current Biology*, 20(16), 1438–1444.
- Stowers, L., & Logan, D. W. (2010). Olfactory mechanisms of stereotyped behavior: On the scent of specialized circuits. *Current Opinion in Neurobiology*, 20(3), 274–280.

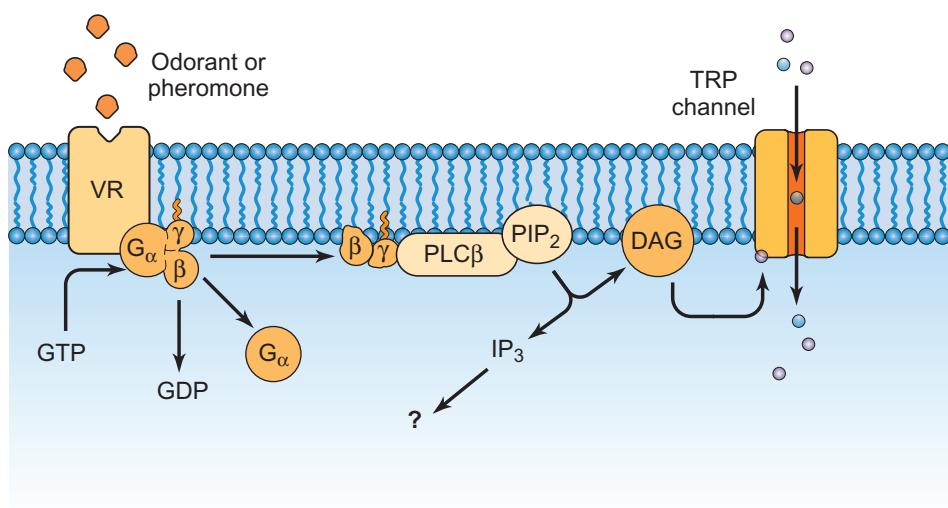
Several groups have reported that certain odorants can elicit an increase in the second messenger inositol 1,4,5-trisphosphate ( $IP_3$ ) (Ch. 23). However, there is no clear evidence that  $IP_3$  directly mediates an electrical response in mammalian OSNs, nor is there a clear rationale for two parallel excitatory odor transduction cascades. However, more recent data supports the idea that phosphoinositides or enzymes related to their metabolism may play a modulatory role, shaping the OSN output by interactions with the cAMP signaling cascade or the CNG channel. Furthermore, phosphoinositide signaling may be important in other cell types within the olfactory epithelium. As will be seen below, phosphoinositides certainly play an important role in chemosensory transduction in another olfactory tissue, the vomeronasal organ (Ch. 23).

### **The vomeronasal organ is an accessory chemosensing system that plays a major role in the detection of semiochemicals**

This functional class of odorants, which includes both conspecific and interspecific cues, can convey important information such as social or mating status, genetic identity, food safety or the presence of disease. Although both the main and accessory olfactory systems are sensitive to some semiochemicals, the vomeronasal organ (VNO) seems particularly attuned to

this class of olfactory stimuli. The mammalian VNO is a thin epithelial tissue within a bony capsule on the floor of the nasal cavity and is separate from the main olfactory epithelium. It is vestigial in humans. The VNO epithelium contains two major populations of microvillar chemosensory neurons; one is in the more apical aspects of the epithelium, while the other is more basal. These two populations of vomeronasal sensory neurons (VSNs) are defined by the differential expression of different G protein subunits (Munger et al., 2009): apical VSNs express  $G_{o1}2$ , while the basal neurons express  $G_{o0}$ . Most apical and basal VSNs also express one of two chemosensory receptor families, the V1Rs and V2Rs, respectively (Munger et al., 2009). There are ~150 V1Rs, and a similar number of V2Rs. These receptors, while both GPCRs, are otherwise unrelated to each other: V1Rs resemble Class A GPCRs, while V2Rs are members of Class C (similar to metabotropic glutamate receptors; Ch. 17). Neither shares any sequence homology with the ORs. Some basal VSNs also express several major histocompatibility complex class Ib genes, the specific function of which is still unclear.

More recently a third class of GPCRs, members of the formyl peptide receptor (FPR) family, has been identified in a subpopulation of VSNs (Riviere et al., 2009). Unlike either the V1Rs or V2Rs, FPR family members are not restricted to either the apical or basal layer: four family members are found in apical VSNs, while one is expressed in cells of the basal layer. FPR-expressing VSNs do not appear to express either V1Rs or V2Rs, indicating that there are at least three different VSN populations.



**FIGURE 52-4** A model for chemosensory transduction in vomeronasal sensory neurons. The individual steps are detailed in the text. In contrast to the transduction cascade in OSNs, the mechanism of vomeronasal transduction is less well characterized. Vomeronasal sensory neurons express V1R, V2R or FPR receptors and either G<sub>αi2</sub> or G<sub>αo</sub>. The TRPC2 channel subunit is expressed in all VSNs, and may be part of a multimeric channel complex. Ca<sup>2+</sup> ions are represented as purple balls; Na<sup>+</sup> ions as blue balls. VR, vomeronasal receptor (V1R, V2R or FPR); PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; IP<sub>3</sub>, inositol 1,4,5-trisphosphate. DAG, diacylglycerol.

### Most vomeronasal sensory neurons are narrowly tuned to specific chemical cues, and utilize a unique mechanism of sensory transduction

Unfortunately, technical challenges have slowed progress towards pairing most vomeronasal receptors with their cognate ligands. However, some themes are emerging (Munger et al., 2009). V1R-expressing neurons respond largely to volatile stimuli, including several compounds found in rodent urine and that have been implicated as pheromones (odorants released by one member of a species that elicit a behavioral or hormonal response in another member). In contrast V2R-expressing neurons seem to be sensitive to peptide or protein stimuli found in urine or glandular secretions, including stimuli that can elicit mating behaviors in female conspecifics or fear of a predator. Finally, FPRs are responsive to a small group of peptides that are associated with inflammation or pathogens and that may communicate critical information about health status.

Physiological studies of VSNs have suggested some functional properties, likely a consequence of receptor selectivity, that differentiate these sensory neurons from OSNs (Munger et al., 2009). First, VSNs are extremely sensitive to their ligands, with activation thresholds as low as 10–11M. Second, in contrast to the broadly tuned OSNs, VSNs are highly selective, seemingly responsive to only single stimuli even at high stimulus concentrations. These properties suggest a different coding strategy for these two types of olfactory neurons: broadly tuned OSNs that can respond to novel odorants, and narrowly tuned, highly sensitive VNs that specifically respond to conspecific odorants of particular behavioral relevance. Interestingly, FPR-expressing VSNs may be an exception to this dichotomy: these neurons show overlapping ligand selectivities reminiscent of the broad stimulus tuning seen in OSNs.

The biochemical cascade responsible for the transduction of VSN stimuli is not fully understood, but the key components

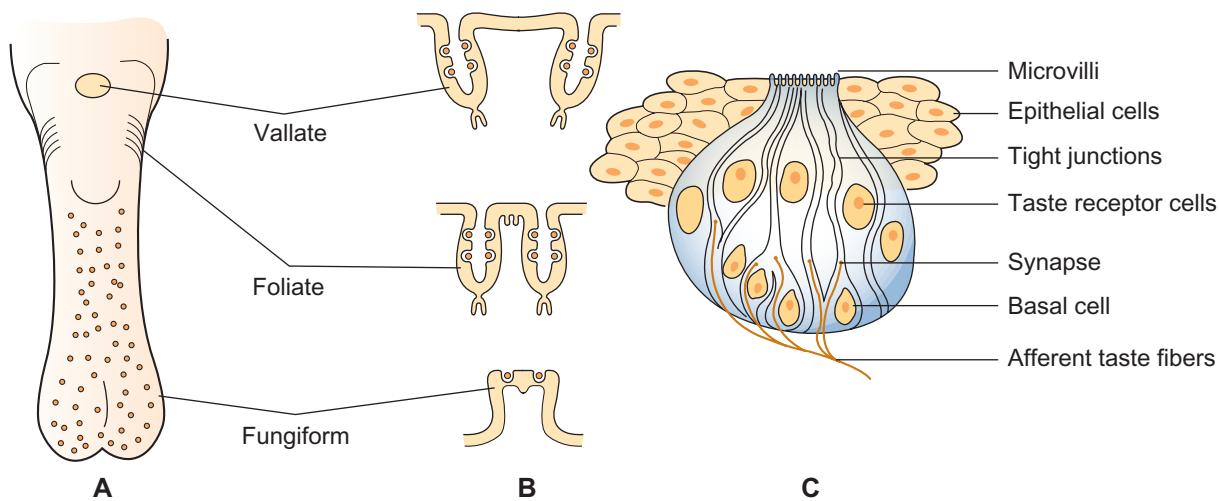
of a phospholipid signaling pathway are shared by VSNs (Figure 52-4). All VSNs appear to express an ion channel subunit, TRPC2, that is a member of the transient receptor potential (TRP) family (Liman et al., 1999). The essential role of TRPC2 in VSN function, and the critical role of VNs in some pheromone-dependent behaviors, was demonstrated when the *Trpc2* gene was deleted in mice: VSN activity in response to pheromones was nearly or completely abolished, and null mice exhibited altered sexual and social behaviors (Leybold et al., 2002; Stowers et al., 2002). The native VSN channel containing TRPC2 is directly gated by the lipid messenger diacylglycerol in a pheromone-dependent manner (Munger et al., 2009). However, the activation of vomeronasal receptors has still not been directly linked to diacylglycerol production via any G protein or phospholipase isoforms.

### TASTE

#### Multiple senses, including taste, contribute to our total perception of food

Our perception of the flavor of food is a complex experience based upon multiple senses: taste per se, which includes sweet, sour, salty, bitter and umami (the savory taste of glutamate); olfaction, which includes food aromas; touch, also termed “mouth feel,” provided by the texture of food; and thermoreception and nociception, caused by pungent spices and irritants.

The chemical complexity of taste stimuli suggests that taste receptor cells utilize multiple molecular mechanisms to detect and distinguish among these compounds. Our sense of taste can detect and discriminate among various ionic stimuli—for example, Na<sup>+</sup> as salty, H<sup>+</sup> as sour, sugars as sweet and alkaloids as bitter. While a number of recent studies have begun to



**FIGURE 52-5** Tongue, taste papillae and taste buds. (A) Surface of the rat tongue showing the location of the taste papillae. (B) Cross-section of the three main types of taste papillae: fungiform, foliate and (circum)vallate. (C) Taste buds contain taste receptor cells (TRCs) and basal cells. Taste receptor proteins present on the microvilli of TRCs respond to tastants in the oral cavity, initiating a transduction cascade that results in the release of neurotransmitter onto afferent cranial nerve fibers, which carry taste information back to the brainstem.

identify the molecular players in taste transduction, the relative inaccessibility of the sensory cells, the diversity of sensory cells across the epithelium, and a number of functional and anatomical differences between animal models, even among rodents, mean that the mechanisms of taste transduction are still poorly understood.

### Taste receptor cells are organized into taste buds

The chemical detection of taste stimuli resides in specialized epithelial cells, taste receptor cells (TRCs). In vertebrates, TRCs are mostly found within an ovoid cluster of 50 to 100 cells called taste buds (Figure 52-5). The taste buds are embedded within the nonsensory lingual epithelium of the tongue and are housed within connective tissue specializations called fungiform, foliate and circumvallate papillae. TRCs are also found in the palate, pharynx and upper portion of the esophagus. The taste bud is a polarized structure with a narrow apical opening, termed the taste pore, and basolateral synapses with afferent nerve fibers. Solutes in the oral cavity make contact with the apical membranes of the TRCs via the taste pore. There is a growing understanding of extensive chemical and electrical communication between TRCs within the taste bud, suggesting that significant processing of taste information may occur prior to synaptic transmission to afferent nerves.

### Sensory afferents within three cranial nerves innervate the taste buds

The sensory fibers that innervate the taste buds travel in cranial nerves VII, IX and X. The chorda tympani branch of VII innervates taste buds on the anterior portion of the tongue, within the fungiform papillae and the anterior foliate

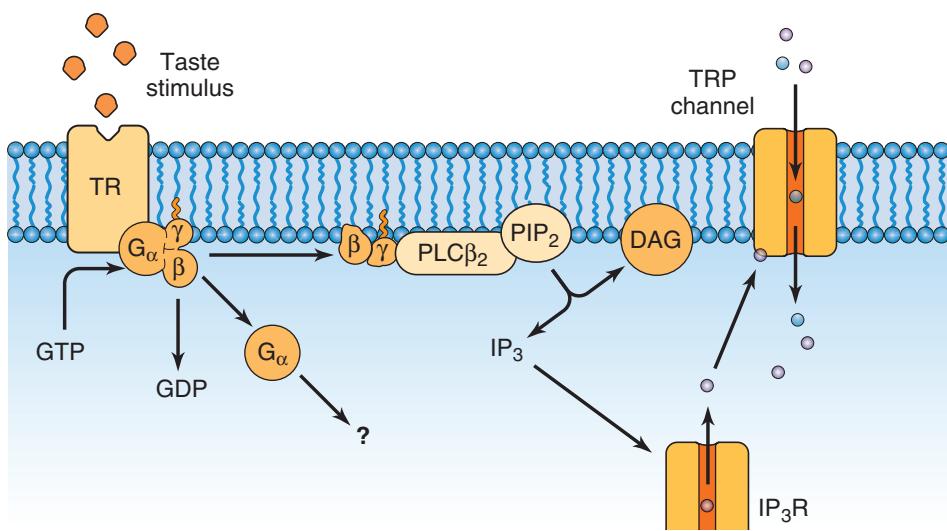
papillae. The lingual branch of the glossopharyngeal nerve (IX) innervates buds within the circumvallate papillae and the posterior foliate papillae, while the superior laryngeal branch of the vagus (X) innervates taste buds of the epiglottis and the esophagus. These nerves relay taste information both to brainstem taste areas and to circuits involved in oromotor reflexes.

### Sweet, bitter and umami taste involve G protein-coupled receptors

Research on the biochemistry and molecular biology of taste long lagged behind that on vision or olfaction because of the relative inaccessibility of TRCs and the lack of high-affinity ligands specific for taste receptors. However, significant progress has been made in identifying key players in the transduction of sweet, umami and bitter taste. It is now clear that G protein-coupled signaling cascades transduce these tastants (Figure 52-6).

#### Type 1 Taste Receptors (T1Rs) Recognize Sweet and Umami Stimuli

The first taste-specific GPCRs were identified in 1999 (Hoon et al., 1999) but remained orphan receptors (i.e., their physiological ligands were unknown) for several years. Now called T1R1 and T1R2, these receptors are Class C GPCRs and contain a large extracellular N-terminus that contains the orthosteric ligand-binding site. (Several allosteric sites are found on other parts of the proteins.) (Vigues et al., 2009). Subsequently, several groups utilized a combination of molecular biological and genetic approaches to identify the gene encoding a third family member, T1R3 (Vigues et al., 2009). The T1Rs are differentially distributed across the gustatory epithelium: T1R1 and T1R2 are expressed in distinct populations of TRCs (T1R1 is found predominantly on the anterior tongue and T1R2 mostly on the posterior tongue), but are always coexpressed with T1R3. This



**FIGURE 52-6** A model for the major signaling mechanisms for the transduction of sweet, bitter and umami stimuli. The individual steps are detailed in the text. Note that stimuli of each of these taste qualities interact with GPCRs: bitter stimuli with T2Rs, and sweet and umami stimuli with T1Rs.  $\alpha$ -Gustducin has been implicated in the transduction of all three types of stimuli, but other  $\alpha$ -subunits likely also couple to T1Rs or T2Rs in some TRC populations. PLC- $\beta$ 2 and the  $\text{Ca}^{2+}$ -activated TRP channel subunit TRPM5 are essential for normal sweet, bitter and umami taste. The role of IP<sub>3</sub> and the IP<sub>3</sub>R in the stimulus-dependent increase in intracellular  $\text{Ca}^{2+}$  as depicted are speculative.

differential pattern of expression suggested varied functions of T1R1 and T1R2, a possibility born out by subsequent *in vitro* and *in vivo* studies (see below).

Certain mutations in the mouse *Tas1r3* gene (which encodes T1R3) correlate with a reduced sensitivity to sweet compounds, including saccharin and many natural sugars. The identification of *Tas1r3* as the saccharin-sensitivity gene *Sac* provided the first evidence that the T1Rs might be involved in sweet taste. The T1Rs function as heteromeric receptors (likely dimers), with T1R2 and T1R3 combining to form a receptor for sweet-tasting compounds including sugars, sweeteners, and some D-amino acids (Li et al., 2002; Nelson et al., 2001). In contrast, T1R1/T1R3 heteromers are insensitive to sweet-tasting stimuli but do respond to umami stimuli, including some L-amino acids (Li et al., 2002; Nelson et al., 2002). Furthermore, T1R1/T1R3 responses are potentiated by 5'-ribonucleotides, a characteristic of umami taste.

#### Type 2 Taste Receptors (T2Rs) Mediate Responses to Bitter-Tasting Stimuli

A distinct receptor family is involved in the detection of bitter tastants. Bitter agents are structurally diverse, suggesting a multiplicity of receptors and/or detection pathways. Many bitter compounds are lipophilic and membrane permeant, and may act on intracellular or integral membrane targets. However, it is clear that GPCRs play a central role in the detection of bitter stimuli. The T2R family contains ~30 GPCRs whose genes (*the Tas2rs*) were first identified based on their proximity to known genetic loci involved in bitter taste (Adler et al., 2000; Matsunami et al., 2000). T2Rs are most often grouped as a separate class of GPCRs, and they share no sequence homology with T1Rs. Most human T2Rs, as well as many rodent homologues, have been paired to one or more bitter-tasting ligands, confirming the role of T2Rs in bitter taste. It appears that individual T2R-containing TRCs express

most *Tas2r* genes. This overlapping pattern of expression is consistent with bitter taste behavior, as animals do a poor job of discriminating bitter compounds and may be more dependent on high taste sensitivity to these compounds, which are often toxic.

#### T1Rs and T2Rs also Have Important Functions Outside the Gustatory System

More recently it has been recognized that both T1Rs and T2Rs are expressed in numerous tissues, where they may serve disparate roles (Dotson et al., 2010). For example both receptor types are expressed in enteroendocrine cells of the gastrointestinal tract, where it appears they sense ingested nutrients, metabolites or other factors present in the gut lumen and they influence hormonal responses and/or nutrient assimilation. Therefore, while both receptor families certainly play critical roles in taste, it may be too simplistic to refer to them as "taste" receptors.

#### Sweet, bitter and umami tasting stimuli are transduced by a G-protein–coupled signaling cascade

The taste transduction cascade is not as fully understood as is the canonical olfactory cascade, but significant progress has been made in the last decade towards defining key molecular components. (A model for the major molecular players is presented in Figure 52-6.) Even before the discovery of the T1Rs and T2Rs, it was clear from molecular and biochemical experiments that G protein-coupled signaling was important for taste transduction. The first heterotrimeric G protein subunit to be implicated in this process was  $\alpha$ -gustducin (McLaughlin et al., 1992). This novel G protein  $\alpha$  subunit was cloned from taste tissue and is expressed in about 25–30% of TRCs of the

circumvallate, foliate and fungiform papillae.  $\alpha$ -Gustducin is most closely related to transducin (Ch. 51), the photoreceptor G protein  $\alpha$  subunit that is also expressed in taste cells.  $\alpha$ -Gustducin functions in sweet, bitter and umami taste, as exemplified by the profound decrement of taste responses to these types of stimuli in mice lacking *Gnat3*, the gene encoding  $\alpha$ -gustducin (Wong et al., 1996). These animals do retain some residual responses to these stimuli, suggesting a role for other G protein isoforms. Indeed, both transducin and  $G\alpha 14$  have been implicated in taste transduction. Consistent with the GPCR-independent mechanisms that mediate salty and sour taste (see discussion below), these mice exhibit normal responses to salts and acids.

In TRCs,  $\alpha$ -gustducin associates with the G protein  $\beta$  and  $\gamma$  subunits  $G\beta 3$  and  $G\gamma 13$ . It is these subunits that activate the effector enzyme phospholipase C- $\beta 2$  (PLC $\beta 2$ ), which can produce the second IP<sub>3</sub> messengers and diacylglycerol through the cleavage of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (Ch. 23). Studies in transgenic and gene-targeted mice support an essential role for PLC $\beta 2$  in the transduction of sweet, bitter and umami stimuli. Deletion of the gene encoding PLC $\beta 2$  from mice abolishes behavioral responses to bitter, sweet or umami stimuli, while restoration of PLC $\beta 2$  expression in only T2R-expressing cells rescues bitter taste alone (Zhang et al., 2003). These interesting results suggested that different groups of taste cells are dedicated to encoding individual taste qualities, a model born out by subsequent experiments (Chandrashekhar et al., 2006).

The second messenger used by these taste cells is unclear. In many cells, phosphoinositide signaling leads to an elevation in intracellular calcium levels through the release of calcium from intracellular stores in response to IP<sub>3</sub>-dependent gating of channels in the endoplasmic epithelium (Ch. 23). It is not known if IP<sub>3</sub> plays an equivalent role in TRC transduction, but such a role would be consistent with observations that a Ca<sup>2+</sup>-activated cation channel is present in taste cells: the TRP channel type M5 (TRPM5), is essential for normal sweet, bitter and umami taste function (Perez et al., 2002; Zhang et al., 2003). Finally, cAMP has also been suggested to play a role in taste transduction, perhaps as a mediator of taste adaptation.

Many textbooks continue to incorrectly state that taste sensitivities are strictly localized on the gustatory epithelium. However, there is some regionalization of sensitivity that is consistent with the observation that taste receptors and intracellular signaling components are differentially localized across the gustatory epithelium. For example,  $\alpha$ -gustducin is only expressed in a subset of TRCs, although it is found across the epithelium.  $G\alpha 14$  is preferentially localized to the posterior tongue. T1R1, T1R2 and the T2Rs are also differentially distributed. These differential patterns of expression highlight the functional and molecular complexity of taste transduction and the challenges in understanding the subtleties of diverse signaling cascades in the gustatory system.

## Salts and acids are transduced by direct interaction with ion channels

Several salts, including both NaCl and KCl, can elicit a perception of salty taste. At least two distinct molecular

mechanisms mediate salt taste in mammals: one is Na<sup>+</sup> specific, while the other is responsive to all salty-tasting stimuli. The presumed mechanism for Na<sup>+</sup> taste had long been the direct influx of Na<sup>+</sup> through amiloride-sensitive epithelial sodium channels (ENaCs): the  $\alpha$ ,  $\beta$  and  $\gamma$  subunits of the ENaC channel have been localized to the apical membranes of TRCs (ENaCs function as heterooligomeric complexes), and the Na<sup>+</sup>-specific component of salt taste is blockable by amiloride at micromolar concentrations. This mechanism was recently confirmed with the demonstration that mice genetically engineered to lack the ENaC  $\alpha$  subunit in taste buds are deficient in Na<sup>+</sup>-specific salt taste (Chandrashekhar et al., 2010). In contrast, the molecular mechanisms underlying general salt taste remain unclear, although candidates have been proposed.

Sour taste is a function of the acidity of a solution, depending primarily on the proton concentration and to a lesser extent on the particular anion involved. Several mechanisms have been proposed to mediate sour taste, including proton or pH-dependent gating of ion channels, direct flux of protons through ion channels or intracellular acidification of ion channels or other proteins. To date only a single, very specialized sour taste mechanism has been identified: the membrane-bound carbonic anhydrase isoform Car4 is expressed on the surface of a subset of TRCs and mediates the sour taste of carbonation (Chandrashekhar et al., 2009), likely through the generation of protons through the catalysis of carbon dioxide and water.

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## References

- Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekhar, J., Ryba, N. J., & Zuker, C. S. (2000). A novel family of mammalian taste receptors. *Cell*, 100(6), 693–702.
- Bakalyar, H. A., & Reed, R. R. (1990). Identification of a specialized adenylyl cyclase that may mediate odorant detection. *Science*, 250(4986), 1403–1406.
- Belluscio, L., Gold, G. H., Nemes, A., & Axel, R. (1998). Mice deficient in G(olf) are anosmic. *Neuron*, 20(1), 69–81.
- Brunet, L. J., Gold, G. H., & Ngai, J. (1996). General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron*, 17(4), 681–693.
- Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell*, 65(1), 175–187.
- Chandrashekhar, J., Hoon, M. A., Ryba, N. J., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature*, 444(7117), 288–294.
- Chandrashekhar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J., et al. (2010). The cells and peripheral representation of sodium taste in mice. *Nature*, 464(7286), 297–301.
- Chandrashekhar, J., Yarmolinsky, D., von Buchholtz, L., Oka, Y., Sly, W., Ryba, N. J., et al. (2009). The taste of carbonation. *Science*, 326(5951), 443–445.
- Dotson, C. D., Vignes, S., Steinle, N. I., & Munger, S. D. (2010). T1R and T2R receptors: The modulation of incretin hormones and

- potential targets for the treatment of type 2 diabetes mellitus. *Current Opinion in Investigational Drugs*, 11(4), 447–454.
- Firestein, S. (2001). How the olfactory system makes sense of scents. *Nature*, 413(6852), 211–218.
- Hoon, M. A., Adler, E., Lindemeier, J., Battey, J. F., Ryba, N. J., & Zuker, C. S. (1999). Putative mammalian taste receptors: A class of taste-specific GPCRs with distinct topographic selectivity. *Cell*, 96(4), 541–551.
- Jones, D. T., & Reed, R. R. (1989). Golf: An olfactory neuron specific-G protein involved in odorant signal transduction. *Science*, 244(4906), 790–795.
- Kaupp, U. B., & Seifert, R. (2002). Cyclic nucleotide-gated ion channels. *Physiological Reviews*, 82(3), 769–824.
- Leypold, B. G., Yu, C. R., Leinders-Zufall, T., Kim, M. M., Zufall, F., & Axel, R. (2002). Altered sexual and social behaviors in trp2 mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 99(9), 6376–6381.
- Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. *Proceedings of the National Academy of Sciences of the United States of America*, 99(7), 4692–4696.
- Liberles, S. D., & Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium. *Nature*, 442(7103), 645–650.
- Liman, E. R., Corey, D. P., & Dulac, C. (1999). TRP2: A candidate transduction channel for mammalian pheromone sensory signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 96(10), 5791–5796.
- Malnic, B., Hirono, J., Sato, T., & Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell*, 96(5), 713–723.
- Matsunami, H., Montmayeur, J. P., & Buck, L. B. (2000). A family of candidate taste receptors in human and mouse. *Nature*, 404(6778), 601–604.
- McLaughlin, S. K., McKinnon, P. J., & Margolskee, R. F. (1992). Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature*, 357(6379), 563–569.
- Mombaerts, P. (2004). Genes and ligands for odorant, vomeronasal and taste receptors. *Nature Reviews Neuroscience*, 5(4), 263–278.
- Munger, S. D., Lane, A. P., Zhong, H., Leinders-Zufall, T., Yau, K. W., Zufall, F., et al. (2001). Central role of the CNGA4 channel subunit in  $\text{Ca}^{2+}$ -calmodulin-dependent odor adaptation. *Science*, 294(5549), 2172–2175.
- Munger, S. D., Leinders-Zufall, T., McDougall, L. M., Cockerham, R. E., Schmid, A., Wandernoth, P., et al. (2010). An olfactory subsystem that detects carbon disulfide and mediates food-related social learning. *Current Biology*, 20(16), 1438–1444.
- Munger, S. D., Leinders-Zufall, T., & Zufall, F. (2009). Subsystem organization of the mammalian sense of smell. *Annual Review of Physiology*, 71, 115–140.
- Nakamura, T., & Gold, G. H. (1987). A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature*, 325(6103), 442–444.
- Nelson, G., Chandrashekhar, J., Hoon, M. A., Feng, L., Zhao, G., Ryba, N. J., et al. (2002). An amino-acid taste receptor. *Nature*, 416(6877), 199–202.
- Nelson, G., Hoon, M. A., Chandrashekhar, J., Zhang, Y., Ryba, N. J., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell*, 106(3), 381–390.
- Perez, C. A., Huang, L., Rong, M., Kozak, J. A., Preuss, A. K., Zhang, H., et al. (2002). A transient receptor potential channel expressed in taste receptor cells. *Nature Neuroscience*, 5(11), 1169–1176.
- Ressler, K. J., Sullivan, S. L., & Buck, L. B. (1993). A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell*, 73(3), 597–609.
- Ressler, K. J., Sullivan, S. L., & Buck, L. B. (1994). Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell*, 79(7), 1245–1255.
- Riviere, S., Challet, L., Fluegge, D., Spehr, M., & Rodriguez, I. (2009). Formyl peptide receptor-like proteins are a novel family of vomeronasal chemosensors. *Nature*, 459(7246), 574–577.
- Serizawa, S., Miyamichi, K., & Sakano, H. (2004). One neuron-one receptor rule in the mouse olfactory system. *Trends in Genetics*, 20(12), 648–653.
- Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J. A., Wetzel, C. H., Zimmer, R. K., et al. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science*, 299(5615), 2054–2058.
- Spehr, M., & Munger, S. D. (2009). Olfactory receptors: G protein-coupled receptors and beyond. *Journal of Neurochemistry*, 109(6), 1570–1583.
- Stephan, A. B., Shum, E. Y., Hirsh, S., Cygnar, K. D., Reisert, J., & Zhao, H. (2009). ANO2 is the ciliary calcium-activated chloride channel that may mediate olfactory amplification. *Proceedings of the National Academy of Sciences of the United States of America*, 106(28), 11776–11781.
- Stowers, L., Holy, T. E., Meister, M., Dulac, C., & Koentges, G. (2002). Loss of sex discrimination and male–male aggression in mice deficient for TRP2. *Science*, 295(5559), 1493–1500.
- Vassar, R., Chao, S. K., Sitcheran, R., Nunez, J. M., Vosshall, L. B., & Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell*, 79(6), 981–991.
- Vassar, R., Ngai, J., & Axel, R. (1993). Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell*, 74(2), 309–318.
- Vigues, S., Dotson, C. D., & Munger, S. D. (2009). The receptor basis of sweet taste in mammals. *Results and Problems in Cell Differentiation*, 47, 187–202.
- Wong, G. T., Gannon, K. S., & Margolskee, R. F. (1996). Transduction of bitter and sweet taste by gustducin. *Nature*, 381(6585), 796–800.
- Wong, S. T., Trinh, K., Hacker, B., Chan, G. C., Lowe, G., Gaggar, A., et al. (2000). Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron*, 27(3), 487–497.
- Young, J. M., Friedman, C., Williams, E. M., Ross, J. A., Tonnes-Priddy, L., & Trask, B. J. (2002). Different evolutionary processes shaped the mouse and human olfactory receptor gene families. *Human Molecular Genetics*, 11(5), 535–546.
- Zhang, Y., Hoon, M. A., Chandrashekhar, J., Mueller, K. L., Cook, B., Wu, D., et al. (2003). Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways. *Cell*, 112(3), 293–301.
- Zufall, F., & Leinders-Zufall, T. (2000). The cellular and molecular basis of odor adaptation. *Chemical Senses*, 25(4), 473–481.
- Zufall, F., & Munger, S. D. (2010). Receptor guanylyl cyclases in mammalian olfactory function. *Molecular and Cellular Biochemistry*, 334(1–2), 191–197.