Mol Neurobiol (2007) 35:175–181
DOI 10.1007/s12035-007-0013-2

Searching for the Ligands of Odorant Receptors

Bettina Malnic

Received: 15 August 2006 / Accepted: 9 November 2006 / Published online: 19 June 2007

Abstract Through the sense of smell mammals can detect and discriminate between a large variety of odorants present in the surrounding environment. Odorants bind to a large repertoire of odorant receptors located in the cilia of olfactory sensory neurons of the nose. Each olfactory neuron expresses one single type of odorant receptor, and neurons expressing the same type of receptor project their axons to one or a few glomeruli in the olfactory bulb, creating a map of odorant receptor inputs. The information is then passed on to other regions of the brain, leading to odorant perception. To understand how the olfactory system discriminates between odorants, it is necessary to determine the odorant specificities of individual odorant receptors. These studies are complicated by the extremely large size of the odorant receptor family and by the poor functional expression of these receptors in heterologous cells. This article provides an overview of the methods that are currently being used to investigate odorant receptor–ligand interactions.

Keywords Odorant receptor (OR) · Guanine nucleotide exchange factor (GEF) · Receptor transporting protein (RTP) · G-protein coupled receptors (GPCRs) · Endoplasmic reticulum (ER) · Olfactory sensory neurons · Heterologous expression · Ligands · Odorants

B. Malnic (✉)
Departamento de Bioquímica, Instituto de Química,
Universidade de São Paulo,
Av. Prof. Lineu Prestes, 748,
CEP 05508-000 São Paulo, São Paulo, Brazil
e-mail: bmalnic@iq.usp.br

Introduction

Mammals can discriminate between a vast number of odorants. The mechanisms involved in this task have been the subject of intense investigation during the past few years. The discovery of a large family of genes, which codes for approximately 1,000 odorant receptors (ORs) in rodents [1], led to major insights into the molecular mechanisms of olfaction. Odorant receptors belong to the superfamily of G-protein coupled receptors (GPCRs) and are extremely diverse in their amino acid sequences, consistent with the ability to recognize a large variety of odorants [2].

Odorant receptors are specifically expressed in specialized cell types, the olfactory sensory neurons (OSNs) located in the olfactory epithelium of the nose. Each neuron expresses one single odorant receptor type [3–6]. The axons from neurons that express the same OR type converge onto a few identical glomeruli in the olfactory bulb [7–9] so that the information provided by the different ORs in the nose is organized into a sensory map in the olfactory bulb. In the olfactory cortex, which receives input from the bulb, there is another map of OR inputs that is different from the bulb. In the cortex, spatial representations of odorant receptor inputs are more complex and distributed over relatively larger areas [10], but still, odorant representations are similar among individuals [11, 12]. Thus, a given odorant will bind to a specific group of olfactory neurons in the nose and activate a characteristic combination of glomeruli in the bulb. The signals will then be transmitted to specific regions of the brain leading to odorant perception.

Despite all of the recent progress in olfactory research, several important questions remain unanswered: how is the OR family used to generate diverse odorant perceptions? Is there a direct correlation between ORs that are activated in the nose and specific odorant perceptions?

A logical approach to understand how the olfactory system utilizes its highly organized architecture to discriminate odorants would be to determine the odorant specificities of individual receptors. However, only a few ORs have been linked to odorants they recognized to date because they cannot be efficiently expressed in heterologous cells [13, 14] (Fig. 1). Odorant receptors are usually retained in the endoplasmic reticulum and cannot reach the plasmatic membrane [15–19].

The olfactory cilia of olfactory neurons, where ORs are expressed in vivo, are highly specialized sensory organelles that contain molecules that are unique to the olfactory cells and are necessary for olfactory signal transduction [20, 21]. It is thus likely that specific molecular mechanisms, which are absent in heterologous cells, are required for proper OR functioning. These mechanisms could be involved in several steps that ultimately determine the level of receptor available at the cell membrane, such as folding, intracellular trafficking, export from the endoplasmic reticulum, and degradation. In addition, olfactory-specific accessory molecules could stabilize receptor expression at the cell surface, and even change its ligand specificity, as was shown for other types of GPCRs (for reviews on GPCR interacting proteins, see Bockaert et al. [22] and Tan et al. [23]).

Accessory Proteins for Odorant and other Chemosensory Receptors

A number of unrelated accessory proteins that work as chaperones for chemosensory GPCRs have already been described. ODR-4, a membrane protein that shows an uncommon membrane topology, facilitates surface delivery of a subset of odorant receptors to cilia in *Caenorhabditis elegans* chemosensory neurons [24]. ODR-4 is specifically expressed in intracellular membranes of the chemosensory neurons, and not in the cilia, and therefore may be involved in receptor folding or vesicle transport [24]. Like odorant receptors, members of the V2R family of putative pheromone receptors do not traffic to the plasma membrane of heterologous cells. V2Rs are normally coexpressed with members of the M10 family of MHC class Ib proteins and β2-microglobulin in vomeronasal neurons located in the basal half of the vomeronasal epithelium [25, 26]. It was demonstrated that cotransfection with a specific M10 member and β2-microglobulin promoted surface expression of one V2R in cultured cells [26].

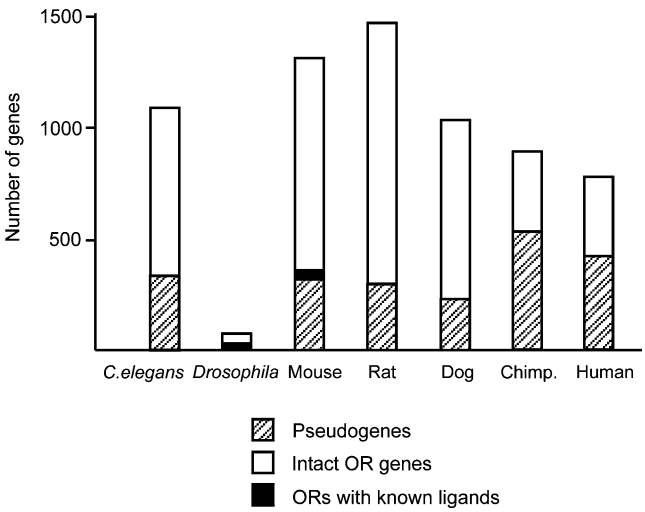

Fig. 1 The vast majority of odorant receptors are orphan (their ligands are unknown). The numbers of intact OR genes and pseudogenes were determined from *C. elegans* [71–73], *Drosophila* [74], mouse [75–78], rat [79], dog [79, 80], chimpanzee [81], and human [82–85] complete genomic sequences. Numbers of ORs with known ligands are indicated in *black*. Although there are 60 *Drosophila* OR genes [74], only ~40 of them are expressed in the adult fly sensory organs [48, 86]. Twenty nine out of these ORs (72%) have been matched with ligands [44, 46, 47]

Accessory proteins that aid cell surface expression of mammalian ORs were also identified. Interestingly, it was demonstrated that the receptor transporting proteins RTP1, RTP2, and REEP1, which are specifically expressed in OSNs, promote functional expression of ORs in HEK293T cells [27]. Also, a testis-enriched variant of the Hsp70 family of heat shock proteins that is specifically expressed in testis and in the olfactory epithelium was shown to increase surface expression of ORs in heterologous cells [28]. The exact mechanisms through which all of these accessory proteins exert their functions remain unknown.

Evidence has been rapidly accumulating that many GPCRs act as homodimers or heterodimers [29]. This seems to be the case for some chemosensory GPCRs as well. Taste receptors T1R1, T1R2, and T1R3 are not responsive when expressed by themselves in heterologous cells. However, coexpression of T1R2 and T1R3 results in the formation of sweet receptors [30], whereas coexpression of T1R1 with T1R3 produces umami receptors [31]. Heterodimerization of *Drosophila* odorant receptors with a conserved "chaperone" GPCR, denominated OR83b was shown to be required for the functional expression of all *Drosophila* ORs [32–34]. The silk moth *Bombyx mori* ORs are also coexpressed with OR83b, indicating that OR83b works as an accessory molecule for ORs from several insect species [35]. The *Drosophila* ORs, however, differ from conventional GPCRs and other chemosensory receptors because they show a distinct membrane topology, with the N terminus located intracellularly [34]. Thus, it seems that some chemosensory receptors must form heterodimers to be properly targeted to the cell surface and also to be functionally active. In the case of the mammalian ORs, because only one OR type is expressed per olfactory neuron [3–6], no OR heterodimer is expected to occur in vivo. Yet, it was recently demonstrated that coexpression with a different type of GPCR, the β2-adrenergic receptor, enhances surface expression of OR M71 in HEK293 cells [36]. The

β2-adrenergic receptor is widely expressed in OSNs and therefore could play a general role in OR trafficking. However, coexpression with the β2-adrenergic receptor does not promote cell surface expression of rat I7 and human 17–40 ORs [36], indicating that this GPCR does not work as an accessory molecule for all members of the OR family.

It is important to note that, although all of the receptors mentioned above (insect ORs, mammalian taste and pheromone receptors, and nematode chemosensory receptors) share a common basic structure of seven transmembrane domains, they are completely different in amino acid sequence. Therefore, it is not surprising that different receptors require different mechanisms in trafficking to the cell surface.

Analysis of OR Ligand Specificities Using Homologous Systems

Since the discovery of ORs in 1991 [1], a series of alternative methods using homologous cells have been used to circumvent the problems encountered with OR expression in heterologous cells. Only in 1998 was the first OR linked to an odorant it recognized [37]. In this case, an adenovirus vector was used to drive the expression of the rat OR I7 in an increased number of OSNs in vivo. Electrophysiological recordings showed increased responses to octanal, indicating that octanal is a ligand for this odorant receptor.

In a different approach, a combination of calcium imaging and single cell RT-PCR was used to identify the ORs expressed by OSNs that responded to different aliphatic odorants [4], to lyral [38], or to eugenol [39]. The specificities of the eugenol and lyral receptors were checked using the adenovirus system as described above [38, 39]. Altogether, these experiments showed that one given OR can recognize multiple odorants, but that different odorants are recognized by different combinations of receptors [4, 38, 39]. Thus, the olfactory receptor family is used in a combinatorial manner to discriminate odorants. Mizrahi and colleagues [40] developed a variant approach, where intrinsic signal imaging in the olfactory bulb is first used to locate glomeruli that have been activated by specific odorants, followed by retrograde tracing to label the corresponding OSNs in the olfactory epithelium. Single cell RT-PCR is then used to identify the ORs expressed in the labeled OSNs.

In different types of experiments, gene targeting was used to produce mice that express GFP along with a given OR, which is endogenously expressed in a subset of OSNs. GFP labeled neurons were then analyzed using calcium imaging or other techniques to determine their responses to different odorants [41–43].

A large-scale analysis of the *Drosophila* ORs was accomplished using a mutant neuron that lacks endogenous ORs as an in vivo expression system [44–47]. The mutant neuron (denominated the "empty neuron") is unresponsive to odorants, so individual ORs can be expressed in these neurons and their responses to odorants can be measured in vivo by electrophysiological recordings. By using this approach, nearly all of the ORs expressed in the *Drosophila* antenna [48] have been deorphanized [44, 47].

Homologous in vivo systems such as the ones described above are likely to be reliable and authentic because the ORs are analyzed in their endogenous expression system. However, these methods are extremely laborious and cannot be easily applied to the study of such a large family of receptors and ligands. For this purpose, a robust and high-throughput heterologous expression system for ORs is needed.

Analysis of OR Ligand Specificities Using Heterologous Systems

Functional heterologous expression has successfully been achieved for several members of the GPCR superfamily [49, 50], but chemosensory GPCRs are, in general, refractory to expression in heterologous cells [13, 14]. Consequently, only ~40 mouse ORs (4% out of total repertoire) [4, 27, 38, 39, 42, 51–54], 5 human ORs [28, 55–58], and 2 rat ORs [37, 59] have been linked to odorants they recognize to date (Fig. 1). Only one *C. elegans* chemosensory receptor has been matched with a ligand [60, 61]. In an exceptional case, 24 out of the 32 *Drosophila* ORs expressed in the antenna and 5 out of the 7 *Drosophila* ORs expressed in the maxillary palps have been matched with ligands [44, 46, 47] (Fig. 1).

As explained above, it is believed that the major reason for the inefficient functional expression of ORs in heterologous cells is the fact that the receptors do not reach the plasma membrane. Thus, the technical strategies used to date to deorphanize ORs in heterologous cells are based on tricks that should contribute to increased amounts of receptors on the cell surface. Examples of these strategies are summarized in Fig. 2.

It has been demonstrated that fusion of the 20 N-terminal amino acids of rhodopsin or serotonin receptor to the N-terminal region of ORs facilitates cell surface expression of some ORs [53, 57] (Fig. 2a). To date, the vast majority of ORs that have been successfully expressed in mammalian cell lines are rho-tagged ORs [14]. Using cotransfection, ORs with an N-terminal segment of rhodopsin (rho-tagged ORs) can be expressed in heterologous cells together with the $G\alpha_{15/16}$ subunits, which can promiscuously couple receptors to the phospholipase C pathway [53]. Receptor activation by odorants results in increased intracellular Ca^{2+}, which can be measured at the single-cell level using Ca^{2+}-sensitive dyes, compensating for the low number of OR expressing cells.

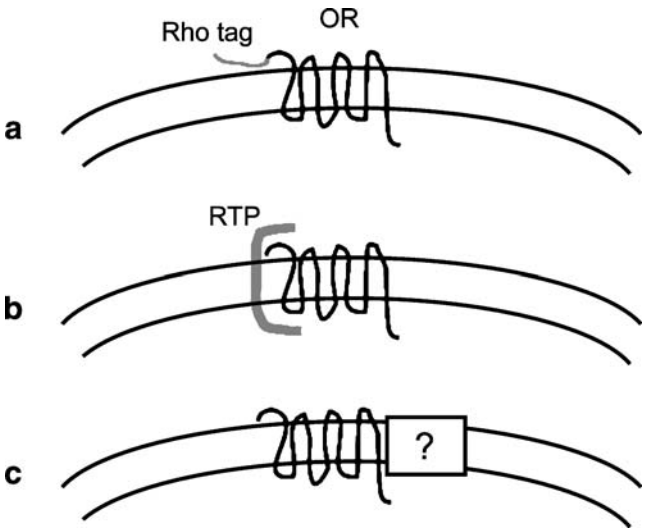

Fig. 2 Strategies used to express odorant receptors on the surface of heterologous cells. **a** Fusion of the 20 N-terminal amino acids of rhodopsin to the N-terminal region facilitates cell surface expression of ORs. **b** Coexpression with the olfactory specific RTPs promotes OR surface expression. The actual role of RTPs in OR trafficking is still not understood, but it is possible that they act as a coreceptor with ORs at the plasmatic membrane, as illustrated here. **c** Molecules yet to be identified may be required for functional OR targeting to the cell surface

Coexpression with the olfactory specific RTP and REEP promotes OR surface expression in HEK293T cells [27]. The RTPs and REEP1 are transmembrane proteins and were shown to directly interact with ORs in coimmunoprecipitation assays [27]. The exact roles of these accessory proteins in OR trafficking are unknown, but because it was demonstrated that cotransfection of RTP1 and OR also enhances surface expression of RTP1, it is possible that they work as coreceptors with ORs (Fig. 2b). They could also be involved in different functions, such as OR folding, export from the endoplasmic reticulum, or vesicle transport [27]. HEK293T cells stably expressing Gαolf (the G protein α subunit that couples to ORs in vivo, [62, 63]), RTP1, RTP2, and REEP1 were established (named Hana3A cell line) and can now be used to investigate the specificities of a large number of ORs [27].

It is also possible that a general role in OR trafficking to the plasma membrane is played by other types of GPCRs or proteins expressed in the OSNs, yet to be identified (Fig. 2c).

Heterologous Expression of ORs Using Olfactory Signaling Proteins

It is well known that the efficacy and fidelity of signal transduction through GPCRs depends on interactions among the multiple components that constitute the GPCR signaling pathways [64]. Thus, the understanding of the detailed molecular determinants required for odorant signal transduction in OSNs may contribute to the establishment of an efficient heterologous system for OR expression.

Odorant receptors expressed in heterologous cells can couple to Gαolf (the natural partner of ORs), leading to odorant-induced increases in cAMP [39, 54]. The increases in cAMP can be monitored using the luciferase reporter gene assay [65], which is more sensitive and therefore allows analysis of ORs that are expressed at low levels or reach the surface in only a small percentage of cells. Shirokova and colleagues used a cell line that stably expresses the olfactory signal transduction molecules Gαolf and CNGA2 (named HeLa/Olf cell line) to functionally express ORs [54]. Importantly, they noted that coupling of ORs to nonolfactory Gα subunits (such as Gα15 or Gα16) may lead to altered response profiles. Thus, heterologous systems that use endogenous olfactory transduction molecules are more likely to reproduce OR physiological responses.

Ric-8B, an olfactory specific putative guanine nucleotide exchange factor (GEF), which is normally expressed in OSNs and interacts with Gαolf, was identified [66]. GEFs catalyze the exchange of GDP for GTP to generate an activated form of Gα, which is then able to activate a variety of effectors. Consistent with this function, it was demonstrated that Ric-8B can amplify signal transduction through Gαolf in HEK293T cells [66]. These results indicate that Ric-8B functions as an up regulator for Gαolf and therefore may be critical in determining the efficacy and potency of the Gαolf signaling cascade. Recently it was demonstrated that coexpression with Ric-8B and Gαolf results in functional expression of ORs in HEK293T cells [67]. Importantly, it was shown that Ric-8B promotes functional expression of untagged (without a rho tag) ORs, what is advantageous because it is possible that receptor protein modifications interfere with the ligand specificities. The mechanisms through which Ric-8B enhances functional expression of ORs remain unknown. Ric-8B could act as a GEF to amplify initially low levels of Gαolf stimulation. If this is the case, this would be an alternative method to enhance OR functional expression: instead of increasing the amounts of receptor at the cell surface, Ric-8B could be used to increase the probability of G protein activation, even if only a small number of receptors can reach the cell surface.

Altogether, the results described above indicate that the employment of endogenous olfactory transduction molecules or other accessory proteins should enhance the ability to identify OR ligand specificities in heterologous expression systems. It is important to note, however, that all the strategies described above are still not equally applicable to all members of the OR family. For unknown reasons, some ORs cannot be satisfactorily expressed in any of the conditions described, whereas others are easier to express. Therefore, the OR expression systems can still be further

improved to allow analysis of the OR family members in a comprehensive manner.

Conclusions

The olfactory system constitutes an excellent model for the study of how percepts are generated in the brain. In the last decade, because of the discovery of the odorant receptors, fundamental progresses in the olfactory research area have been achieved. The structure of the olfactory system has been determined at the molecular level, revealing an extremely organized architecture, where information from the ~1,000 ORs is first segregated in the olfactory bulb and then assembled into overlapping regions in the cortex. To understand how the olfactory system utilizes this architecture to discriminate odorants, it is necessary to determine the odorant specificities of individual ORs.

The determination of OR/ligand pairs should reveal how the OR family is used to generate diverse odorant perceptions. Different aspects of perception can be addressed, such as why some odorants are differently perceived by different populations or why some individuals are anosmic for (unable to smell) specific odorants. Also, although we know that there are thousands of odorants that can be detected by the olfactory system, we do not know the extent of what we can smell. Different species may be able to detect different odorant "spaces." The analysis of the recognition properties of receptors belonging to different species will allow us to determine how different (or not) the odorant spaces are among different species. In addition, because recent studies indicate that pheromone-induced behaviors can be mediated by the main olfactory system as well [68, 69], the analysis of ORs that are specific to one given species should also contribute to the identification of pheromones that are involved in species-specific behaviors.

Recently, a new family of chemosensory receptors expressed in the main olfactory epithelium was identified [70]. These receptors, called "trace-amine-associated receptors" (TAARs), show amino acid sequence identity with receptors for biogenic amines, such as the serotonin and dopamine receptors, and are present in human, mouse, and fish. It was shown that at least three mouse TAARs recognize volatile amines found in urine, indicating they may be involved in the detection of pheromones [70].

A number of other crucial questions still remain unanswered: Are some odorants recognized by a higher number of ORs than others? Are there odorants that are recognized by a very small number of ORs? Are odorants that elicit similar perceptions or emotions recognized by overlapping sets of ORs? Or are odorants that elicit opposite behaviors (attraction vs repulse) recognized by completely different sets of ORs? As the methods to deorphanize ORs improve, more and more answers to these important questions will be obtained, providing insights into the molecular mechanisms of odorant perception.

Acknowledgements The author is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

References

1. Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65:175–187
2. Buck LB (2004) Olfactory receptors and odor coding in mammals. Nutr Rev 62:S184–S188
3. Chess A, Simon I, Cedar H, Axel R (1994) Allelic inactivation regulates olfactory receptor gene expression. Cell 78:823–834
4. Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96:713–723
5. Ressler KJ, Sullivan SL, Buck LB (1993) A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell 73:597–609
6. Vassar R, Ngai J, Axel R (1993) Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. Cell 74:309–318
7. Mombaerts P, Wang F, Dulac C, Chao S, Nemes A, Mendelsohn M, Edmondson J, Axel R (1996) Visualizing an olfactory sensory map. Cell 87:675–686
8. 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 79:1245–1255
9. Vassar R, Chao S, Sitcheran R, Nunez J, Vosshall L, Axel R (1994) Topographic organization of sensory projections to the olfactory bulb. Cell 79:981–991
10. Zou Z, Horowitz L, Montmayeur JP, Snapper S, Buck L (2001) Genetic tracing reveals a stereotyped sensory map in the olfactory cortex. Nature 414:173–179
11. Zou Z, Buck L (2005) Odor maps in the olfactory cortex. Proc Natl Acad Sci U S A 102:7724–7729
12. Zou Z, Buck L (2006) Combinatorial effects of odorant mixes in olfactory cortex. Science 311:1477–1481
13. McClintock TS, Sammeta N (2003) Trafficking prerogatives of olfactory receptor. Neuroreport 14:1547–1552
14. Mombaerts P (2004) Genes and ligands for odorant, vomeronasal and taste receptors. Nat Rev Neurosci 5:263–278
15. Gimelbrant A, Haley S, McClintock T (2001) Olfactory receptor trafficking involves conserved regulatory steps. J Biol Chem 276:7285–7290
16. Gimelbrant A, Stoss T, Landers T, McClintock T (1999) Truncation releases olfactory receptors from the endoplasmic reticulum of heterologous cells. J Neurochem 72:2301–2311
17. Lu M, Echeverri F, Moyer BD (2003) Endoplasmic reticulum retention, degradation, and aggregation of olfactory G-protein coupled receptors. Traffic 4:416–433
18. Lu M, Staszewski L, Echeverri F, Xu H, Moyer B (2004) Endoplasmic reticulum degradation impedes olfactory G-protein coupled receptor functional expression. BMC Cell Biol 5:34
19. Katada S, Tanaka M, Touhara K (2004) Structural determinants for membrane trafficking and G protein selectivity of a mouse olfactory receptor. J Neurochem 90:1453–1463
20. Menco BPM (1997) Ultrastructural aspects of olfactory signaling. Chem Senses 22:295–311

21. Menco BPM, Bruch RC, Dau B, Danho W (1992) Ultrastructural localization of olfactory transduction components: The G protein subunit Golf and type III adenylyl cyclase. Neuron 8:441–453
22. Bockaert J, Fagni L, Dumuis A, Marin P (2004) GPCR interacting proteins (GIP). Pharmacol Ther 103:203–221
23. Tan C, Brady A, Nickols H, Wang Q, Limbird L (2004) Membrane trafficking of G protein-coupled receptors. Annu Rev Pharmacol Toxicol 44:559–609
24. Dwyer ND, Troemel ER, Sengupta P, Bargmann CI (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell 93:455–466
25. Ishii T, Hirota J, Mombaerts P (2003) Combinatorial coexpression of neural and immune multigene families in mouse vomeronasal sensory neurons. Curr Biol 13:394–400
26. Loconto J, Papes F, Chang E, Stowers L, Jones E, Takada T, Kumánovics A, Lindahl K, Dulac C (2003) Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. Cell 112:607–618
27. Saito H, Kubota M, Roberts RW, Chi Q, Matsunami H (2004) RTP family members induce functional expression of mammalian odorant receptors. Cell 119:679–691
28. Neuhaus E, Mashukova A, Zhang W, Barbour J, Hatt H (2006) A specific heat shock protein enhances the expression of mammalian olfactory receptor proteins. Chem Senses 31:445–452
29. Prinster S, Hague C, Hall R (2005) Heterodimerization of G protein-coupled receptors: Specificity and functional significance. Pharmacol Rev 57:289–298
30. Nelson G, Hoon M, Chandrashekar J, Zhang Y, Ryba N, Zuker C (2001) Mammalian sweet taste receptors. Cell 106:381–390
31. Nelson G, Chandrashekar J, Hoon M, Feng L, Zhao G, Ryba N, Zuker C (2002) An amino-acid taste receptor. Nature 416:199–202
32. Larsson M, Domingos A, Jones W, Chiappe M, Amrein H, Vosshall L (2004) Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43:703–714
33. Neuhaus E, Gisselmann G, Zhang W, Dooley R, Stoertkuhl K, Hatt H (2005) Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. Nat Neurosci 8:15–17
34. Benton R, Sachse S, Michnick S, Vosshall L (2006) Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo. PLoS Biol 4:e20
35. Nakagawa T, Sakurai T, Nishioka T, Touhara K (2005) Insect sex-pheromone signals mediated by specific combinations of odorant receptors. Science 307:1638–1642
36. Hague C, Uberti MA, Chen Z, Bush CF, Jones SV, Ressler KJ, Hall RA, Minneman KP (2004) Olfactory receptor surface expression is driven by association with the b2-adrenergic receptor. Proc Natl Acad Sci U S A 101:13672–13676
37. Zhao H, Ivic L, Otaki J, Hashimoto M, Mikoshiba K, Firestein S (1998) Functional expression of a mammalian odorant receptor. Science 279:237–242
38. Touhara K, Sengoku S, Inaki K, Tsuboi A, Hirono J, Sato T, Sakano H, Haga T (1999) Functional identification and reconstitution of an odorant receptor in single olfactory neurons. Proc Natl Acad Sci U S A 96:4040–4045
39. Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H, Touhara K (2001) Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants. J Neurosci 21:6018–6025
40. Mizrahi A, Matsunami H, Katz L (2004) An imaging-based approach to identify ligands for olfactory receptors. Neuropharmacology 47:661–668
41. Belluscio L, Lodovichi C, Feinstein P, Mombaerts P, Katz L (2002) Odorant receptors instruct functional circuitry in the mouse olfactory bulb. Nature 419:296–300
42. Bozza T, Feinstein P, Zheng C, Mombaerts P (2002) Odorant receptor expression defines functional units in the mouse olfactory system. J Neurosci 22:3033–3043
43. Grosmaitre X, Vassali A, Mombaerts P, Shepherd G, Ma M (2006) Odorant responses of olfactory sensory neurons expressing the odorant receptor MOR23: A patch clamp analysis in gene-targeted mice. Proc Natl Acad Sci U S A 103:1970–1975
44. Hallem E, Ho M, Carlson J (2004) The molecular basis of odor coding in the Drosophila antenna. Cell 117:965–979
45. Kreher S, Kwon J, Carlson J (2005) The molecular basis of odor coding in the Drosophila larva. Neuron 46:445–456
46. Goldman A, Van der Goes van Naters W, Lessing D, Warr C, Carlson J (2005) Coexpression of two functional odor receptors in one neuron. Neuron 45:661–666
47. Hallem E, Carlson J (2006) Coding of odors by a receptor repertoire. Cell 125:143–160
48. Vosshall L, Wong A, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102:147–159
49. Civelli O (2005) GPCR deorphanizations: the novel, the known and the expected transmitters. Trends Pharmacol Sci 26:15–19
50. Civelli O, Saito Y, Wang Z, Nothacker HP, Reinscheid R (2006) Orphan GPCRs and their ligands. Pharmacol Ther 110:525–532
51. Abaffy T, Matsunami H, Luetje C (2006) Functional analysis of a mammalian odorant receptor subfamily. J Neurochem 97:1506–1518
52. Gaillard I, Rouquier S, Pin JP, Mollard P, Richard S, Barnabe C, Demaille J, Giorgi D (2002) A single olfactory receptor specifically binds a set of odorant molecules. Eur J Neurosci 15:409–418
53. Krautwurst D, Yau KW, Reed RR (1998) Identification of ligands for olfactory receptors by functional expression of a receptor library. Cell 95:917–926
54. Shirokova E, Scmiedeberg K, Bedner P, Niessen H, Willecke K, Raguse JD, Meyerhof W, Krautwurst D (2005) Identification of specific ligands for orphan olfactory receptors. J Biol Chem 280:11807–11815
55. Levasseur G, Persuy MA, Grebert D, Remy JJ, Salesse R, Pajot-Augy E (2003) Ligand-specific dose-response of heterologously expressed olfactory receptors. Eur J Biochem 270:2905–2912
56. Spehr M, Gisselman G, Poplawski A, Riffel JA, Wetzel CH, Zimmer RK, Hartt H (2003) Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science 299:2054–2058
57. Wetzel CH, Oles M, Wellerdieck C, Kuczkowiak M, Hatt H (1999) Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and Xenopus laevis oocytes. J Neurosci 19:7426–7433
58. Matarazzo V, Clo-Faybesse O, Marcet B, Guiraudie-Capraz G, Atanasova B, Devauchelle G, Cerutti M, Etiévant P, Ronin C (2005) Functional characterization of two human olfactory receptors expressed in the baculovirus Sf9 insect cell system. Chem Senses 30:195–207
59. Murrel J, Hunter D (1999) An olfactory sensory neuron line, odora, properly targets olfactory proteins and responds to odorants. J Neurosci 19:8260–8270
60. Sengupta 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
61. Zhang Y, Chou JH, Bradley J, Bargmann CI, Zinn K (1997) The Caenorhabditis elegans seven-transmembrane protein ODR-10 functions as an odorant receptor in mammalian cells. Proc Natl Acad Sci U S A 94:12162–12167
62. Belluscio L, Gold GH, Nemes A, Axel R (1998) Mice deficient in G(olf) are anosmic. Neuron 20:69–81
63. Jones DT, Reed RR (1989) Golf: An olfactory neuron-specific G-protein involved in odorant signal transduction. Science 244:790–795
64. Pierce K, Premont R, Lefkowitz R (2002) Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3:639–650

65. Katada S, Nakagawa T, Kataoka H, Touhara K (2003) Odorant response assays for a heterologously expressed olfactory receptor. Biochem Biophys Res Commun 305:964–969
66. von Dannecker LEC, Mercadante AF, Malnic B (2005) Ric-8B, an olfactory putative GTP exchange factor, amplifies signal transduction through the olfactory-specific G-protein Gaolf. J Neurosci 25:3793–3800
67. Von Dannecker L, Mercadante A, Malnic B (2006) Ric-8B promotes functional expression of odorant receptors. Proc Natl Acad Sci U S A 103:9310–9314
68. Mandiyan V, Coats J, Shah N (2005) Deficits in sexual and aggressive behaviors in Cnga2 mutant mice. Nat Neurosci 8:1637–1638
69. Wang Z, Sindreu C, Li V, Nudelman A, Chan GK, Storm D (2006) Pheromone detection in male mice depends on signaling through type 3 adenylyl cyclase in the main olfactory epithelium. J Neurosci 26:7375–7379
70. Liberles S, Buck L (2006) A second class of chemosensory receptors in the olfactory epithelium. Nature 442:645–650
71. Robertson H (2001) Updating the str and srj (sti) families of chemoreceptors in Caenorhabditis nematodes reveals frequent gene movement within and between chromosomes. Chem Senses 26:151–159
72. Robertson HM (1998) Two large families of chemoreceptor genes in the nematodes Caenorhabditis elegans and Caenorhabditis briggsae reveal extensive gene duplication, diversification, movement, and intron loss. Genome Res 8:449–463
73. Robertson HM (2000) The large srh family of chemoreceptor genes in Caenorhabditis nematodes reveals processes of genome evolution involving large duplications and deletions and intron gains and losses. Genome Res 10:192–203
74. Robertson H, Warr C, Carlson J (2003) Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proc Natl Acad Sci U S A 100:14537–14542
75. Godfrey PA, Malnic B, Buck LB (2004) The mouse olfactory receptor gene family. Proc Natl Acad Sci U S A 101:2156–2161
76. Niimura Y, Nei M (2005) Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods. Proc Natl Acad Sci U S A 102:6039–6044
77. Young JM, CF, Williams EM, Ross JA, Tonnes-Priddy L, Trask BJ (2002) Different evolutionary processes shaped the mouse and human olfactory receptor gene families. Hum Mol Genet 11:535–546
78. Zhang X, Rodriguez I, Mombaerts P, Firestein S (2004) Odorant and vomeronasal receptor genes in two mouse genome assemblies. Genomics 83:802–811
79. Quignon P, Giraud M, Rimbault M, Lavigne P, Tacher S, Morin E, Retout E, Valin AS, Lindblad-Toh K, Nicolas J, Galibert F (2005) The dog and rat olfactory receptor repertoires. Genome Biol 6:R83
80. Olender T, Fuchs T, Linhart C, Shamir R, Adams M, Kalush F, Khen M, Lancet D (2004) The canine olfactory subgenome. Genomics 83:361–372
81. Gilad Y, Man O, Glusman G (2005) A comparison of the human and chimpanzee olfactory receptor gene repertoires. Genome Res 15:224–230
82. Glusman G, Yanai I, Rubin I, Lancet D (2001) The complete human olfactory subgenome. Genome Res 11:685–702
83. Malnic B, Godfrey PA, Buck LB (2004) The human olfactory receptor gene family. Proc Natl Acad Sci U S A 101:2584–2589
84. Niimura Y, Nei M (2003) Evolution of olfactory receptor genes in the human genome. Proc Natl Acad Sci U S A 100:12235–12240
85. Zozulya S, Echeverri F, Nguyen T (2001) The human olfactory receptor repertoire. Genome Biol 2:18.1–18.2
86. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron 22:327–338