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Review

Olfactory receptors

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Abstract. Olfaction is an ancient sensory system allowing an organism to detect chemicals in its environment. The first step in odor transduction is mediated by binding odorants to olfactory receptors (ORs) which belong to the heptahelical G-protein-coupled receptor (GPCR) superfamily. Mammalian ORs are disposed in clusters on virtually all chromosomes. They are encoded by the largest multigene family (~1000 members) in the genome of mammals and *Caenorhabditis elegans*, whereas *Drosophila* contains only 60 genes. Each OR specifically recognizes a set of odorous molecules that share common molecular features. In mammals, signal transduces through the G-protein-dependent signal pathway in the olfactory sensory neurons that synapse ultimately in the glomeruli of the olfactory bulb, and is finally processed in higher brain structures. The expression of a given OR conditions neuron and glomerulus choices. To date, the processes which monitor OR expression and axon wiring have emerged but are not completely elucidated.

Key words. Olfaction; odorant receptors; G-protein coupled receptors; genomics; ligand recognition; evolution.

Introduction

The olfactory system has the remarkable capacity to discriminate a wide range of odor molecules. In humans, smell is rather considered to be an esthetic sense in contrast to most other species, which rely on olfaction to detect food, predators and mates. Terrestrial animals, including humans, smell air-borne molecules, whereas aquatic animals smell water-soluble molecules with low volatility, such as amino acids.

Humans are thought to have a poor olfactory ability compared with other animals such as dog or rodents, and yet they can perceive a vast number of volatile chemicals. Of the millions of volatile molecular species that have been catalogued by chemists, hundred of thousands of distinct odors can be detected by the human nose. Odorants, typically small organic molecules of less than 400 Da, can vary in size, shape, functional groups and charge [1]. They include a set of various alcohols, aliphatic acids, aldehydes, ketones and esters; chemicals with aromatic, alicyclic, polycyclic or heterocyclic ring structures; and innumerable substituted chemicals of each of these types, as well as combinations of them. However, subtle differences in the structure of an odorant, even between two enantiomers, can lead to pronounced modifications in odor quality.

The olfactory perception begins when odorous ligands activate odorant receptors (ORs) expressed in olfactory sensory neurons (OSNs) of the olfactory epithelium located in the posterior upper part of the nasal cavity (fig. 1). ORs belong to the G-protein-coupled receptor (GPCR) superfamily and therefore are invariably seven-transmembrane domain (7TM) proteins [2]. The transduction of chemical information into electrical impulses involves signal amplification via a G-protein-coupled activation of an adenylyl cyclase, which leads to a rise in

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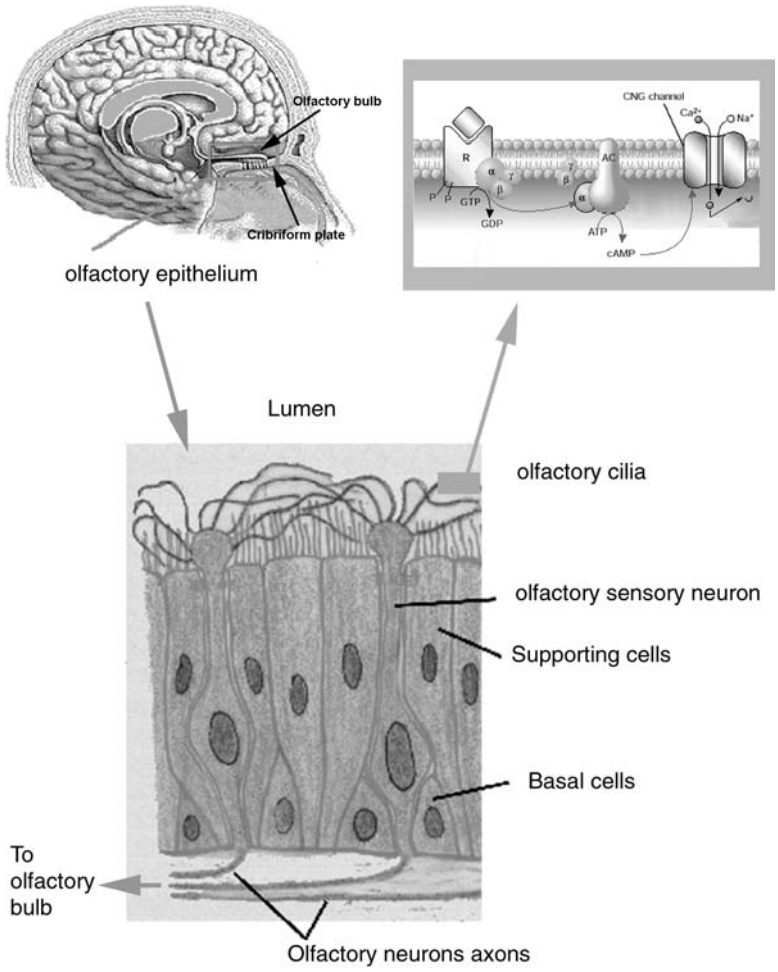

Figure 1. Functional anatomy and structure of the olfactory system. (*A*) Localization of the olfactory apparatus in a human head. The main olfactory epithelium (MOE), the cribriform plate and the olfactory bulb are squared. (*B*) Structure of the MOE tissue showing the different cell types and the wiring from axons to glomeruli of the olfactory bulb. Each neuron expresses a single OR, and the axons of neurons expressing the same OR converge onto two specific glomeruli of the olfactory bulb. (*C*) Olfactory signal transduction pathway in vertebrates. The interaction of an odorant with a specific OR triggers G protein activation, adenyl cyclase activity and opening of a cyclic nucleotide-gated channel (CNG) by elevation of the cAMP level to finally generate an action potential whose olfactory information is integrated in higher brain structures.

cyclic AMP (cAMP) and consequently the opening of cyclic nucleotide-activated, nonselective cation channels. The influx of cations through these channels depolarizes the cell membrane of the olfactory neuron, ultimately resulting in an increase in the frequency of action potentials that travel down the axons to the glomureli, which are globose structures located in the outer part of the olfactory bulb (see for review [3]) (fig. 1). The identification of specialized isoforms of Gαolf [4], adenylyl cyclase type III [5] and the cyclic nucleotide-activated channel [6] in the olfactory cilia have suggested the importance of this pathway. Moreover, gene knockout studies support that the cAMP cascade is dominant in transmitting odorant signals in the olfactory neurons [7–9], whereas the role of an inositol-1,4,5-triphosphate (IP3)-mediated pathway remains unclear [10]. Finally, the OR set activated by a particular odorant produces a combinatory signal that is integrated at the cortex level to result in the sensation of smell.

This review will describe the recent advances that have emerged concerning ORs which are responsible for the first critical step in the processing of olfactory information. Other reviews in areas covering development, higher processing, axonal wiring and other fields of olfaction have been published in recent years [3, 11–14]. Other aspects of olfaction concerning either taste or chemical communication between individuals of the same species using pheromones will not be commented on here. In general, pheromones are not considered as odorants, i.e. leading to the sensation of smell. In mammals, pheromone-specific receptors are encoded by two gene families (V1R with no introns in the coding moiety, and V2R with introns) [15–17]. The VR receptors, which belong to the 7 TM GPCR superfamily, do not share signif-

icant sequence identity with ORs and are expressed in a specialized organ, the vomeronasal organ or Jacobson's organ.

The olfactory epithelium of mammals

In mammals, the primary sensing structure is the olfactory epithelium of the nose that lines the posterior part of the nasal cavity, including the nasal septum (fig. 1). This olfactory epithelium is made up of three cell types: the OSNs, the basal cells and the sustentacular cells. Expression of ORs is restricted to the surface of OSN cilia, where they are in direct contact with inhaled odorants [18] (fig. 1). OSNs are bipolar neurons, with a dendrite end harboring the cilia in the nasal cavity, whereas a unique axon penetrates the skull through the cribriform plate and synapses in the olfactory bulb. OSNs are replaced periodically and have a short average lifetime (~70 days) throughout the entire lifespan of an individual and also have the capacity to proliferate in response to acute injury such as exposure to toxic or infectious agents. However, a study by Mackay-Sim and Kittel [19] reported that normal OSNs, i.e. nondegenerating neurons that target the olfactory bulb [20], displayed a lifespan >90 days. Such properties are possible due to the presence of multipotent neuronal precursors or globose basal cells in the olfactory epithelium [21–24], and a particular cell type of the olfactory bulb that forms the OSN sheath, the olfactory ensheathing glial cells [25]. These multipotent progenitors are capable of producing nonneuronal cells as well as neurons [22]. Sustentacular cells, which originate from a different type of progenitor [22], are nonneuronal support cells that resemble glia. These cells secrete detoxifying enzymes into mucus and isolate OSNs electrically. Moreover, in adult neuroepithelium, they produce neuropeptide Y, which is a neuroproliferative factor of basal cells [26].

OR genes

OR genes were first detected in rat [2] 12 years ago and were since identified in other species mainly by polymerase chain reaction (PCR) amplification with degenerate oligonucleotide primers derived from conserved motifs. Because OR coding sequences are intronless, PCR amplifications were carried out directly on genomic DNA. Indeed full-length or partial coding sequences have been characterized in a number of species, including mammals [2, 27–32], birds [33, 34], amphibians [35] and fish [36]. OR genes are tandemly organized into clusters in the genome, and their genomic organization has been particularly studied in humans and mouse [27, 30, 32, 37–41]. OR clusters are widely dispersed in the human genome on all chromosomes except chromosomes 20 and Y. In mammals the OR repertoire is encoded by a multigene family of 900–1500 members that ensure a high level of diversity. In ancient vertebrates as well as in some invertebrates, the size of the OR gene family is significantly reduced (100 to a few hundred members) but is relatively heterogeneous to ensure sufficient diversity [35, 42].
Originally, it was estimated that the complexity of the OR repertoire was ~1000 ORs in mouse and rat, ~500–750 in humans and ~100 in fish [2, 32, 36, 43–45]. Since the release of the human genome sequence, a complete image of the OR gene repertoire has emerged (fig. 2). About 950 ORs are distributed in ~100 locations throughout the genome, and 63–70% of them are pseudogenes, leading to ~350 intact and potentially functional genes [27, 46–48]. In parallel, the recent release of the mouse genome sequence has allowed different labs also to assemble a complete catalogue of OR genes. About 1500 OR genes have been identified in clusters of varying size throughout 18 of the 20 mouse chromosomes (except 12 and Y) in locations that are syntenical to that of humans [40, 41]. About 20% are pseudogenes leading to ~1200 functional genes, so that mouse contains >3 times more functional OR genes than humans. It seems that additional local duplications of OR genes in the mouse lineage could be responsible for the size difference between the mouse and human repertoires. Moreover, deletion of OR genes in the human lineage has exacerbated the differences between the two species [49].

OR gene structure

The common structure of OR genes includes an intronless coding region of ~1 kb that is preceded by a variable number of upstream exons and terminated by a polyadenylation signal 0.15–1.5 kb downstream from the stop codon [2, 33, 50–53]. The total distance from the initial exon to the coding exon ranges between ~1 to ~11 kb and the number of 5′ noncoding exons varies from 1 to 4. Interestingly, the sequence of the initial non-coding exon is conserved in the same OR gene subfamily, suggesting that OR genes within a subfamily were generated by duplication of an entire gene rather than by retrotranscription processes. These noncoding exons were found to be alternatively spliced, giving rise to different isoforms of OR messenger RNAs (mRNAs) [49, 51, 54], and by comparison with other GPCRs, alternative transcription might regulate OR expression in a tissue-specific manner [55]. In a cluster, the intergenic distances vary from <5 to >67 kb (average ~21 kb) [40], and each OR gene appears to have its own, independent TATA-less promoter region with a pyrimidine:purine tract and an O/E (olf-1)-like site. In some cases, regulatory motifs upsteam of OR genes have been identified [56]. Furthermore, among the numerous transcription factor binding sites identified, no ob-

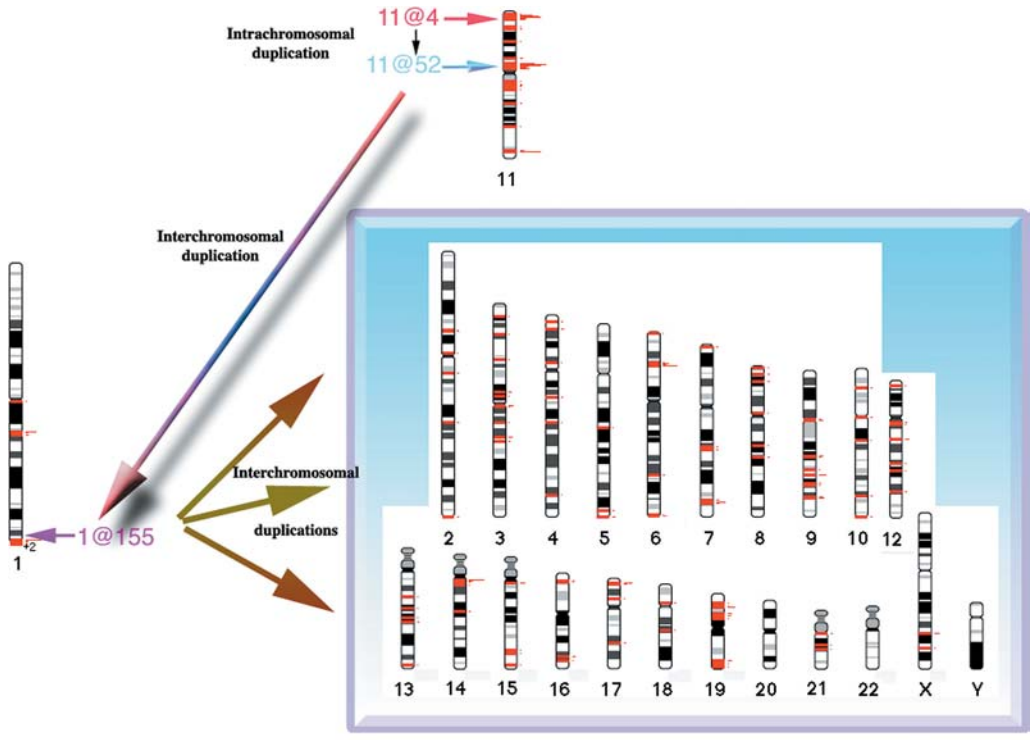

Figure 2. Distribution of the OR multigene family in the human genome. The ancestral clusters are named according to the nomenclature defined by Glusman et al. [46], i.e. distance in megabase pairs from the tip of the chromosome p arm. The chromosome 11 class I cluster (11@4) first duplicated the ancestral class II cluster in 11@52 that in turn duplicated in 1@255. This latter cluster was then duplicated in multiple sites of the genome to generate the present OR repertoire.

vious patterns were established to explain the complex mode of OR gene expression/regulation.

Two main OR classes

Sequence analysis in fish revealed the existence of an unambiguous division (class I ORs) which represents an OR repertoire that is ~10% of that of mammalian species [36], whereas class II genes comprise the majority of mammalian OR genes. Class I ORs were first discovered in fish and then found to be intermixed with class II ORs in amphibian species [35, 37]. In *Xenopus laevis*, class I and class-II ORs were shown to be expressed differentially in water or air-accessible cavities, respectively [57]. This observation and others suggested that class I ORs are activated by water-soluble compounds, whereas class II ORs are activated by volatile odorants, and hence class I ORs in terrestrial vertebrates were first considered as useless evolutionary relics. Surprisingly, genome database mining revealed a large cluster of human class I ORs which includes 102 genes on chromosome 11 (11@4, i.e. localizing 4 Mb from the p telomere on 11p15) [46] (fig. 2). Half of OR class I genes are apparently functional, since only 52% of them have been identified as pseudogenes [46]. This low pseudogene percentage compared with that of class II genes indicates a positive selection process for class I genes [37]. In the mouse OR genome, 147 class I OR genes have also been described in a supercluster on chromosome 7 (cluster 7–3) in a region syntenical to human chromosome 11, and 18% of them are pseudogenes [40, 41]. Originally, class I ORs were thought to recognize water-soluble odorants, but it is known that water-soluble compounds are generally of low volatility and are considered as poor odorants. However, volatile ligands of class I ORs have been identified in mouse [58]. Although the importance of class I ORs in mammals is now established, one can wonder what their role is. A current hypothesis suggests that class I ORs could recognize moderately hydrophobic volatile odorants, whereas class II ORs would be dedicated to hydrophobic compounds. Interestingly, expression data of mouse and rat class I ORs revealed that these ORs are expressed in the most dorsal zone of the olfactory epithelium [58–60].

Genomic organization

In humans, ORs are organized in clusters with a bias for the pericentromeric and subtelomeric regions, on all chromosomes except chromosomes 20 and Y, as first described by fluorescence in situ hybridization (FISH) approaches [27] and then by database mining [46, 48].

The total length of the olfactory subgenome is estimated to be ~30 Mb, which represents ~1% of the total genomic DNA. Similarly, the ~350 (human) to 1200 (mouse) potentially functional OR genes that have been scored represent 1–5% of the mammalian genome [61]. OR genes are not evenly distributed and in humans 6 chromosomes (1, 6, 9, 11, 14, 19) contain ~73% of the OR gene repertoire, whereas chromosome 22 contains a single OR gene [46]. About 80% of the OR genes are organized into clusters of 6–138 genes, the rest being scattered in clusters of <6 members and singletons. At least 64 clusters of >2 ORs can been identified, among which 24 clusters represent about 78% of the ORs. A major outcome of the OR mapping findings is that chromosome 11 contains >40% of all human ORs distributed in 9 clusters, suggesting that this chromosome played a central role in the evolution of the OR repertoire [46, 48]. Chromosome 11 contains the two largest OR clusters, each comprising more than 100 OR sequences. One cluster, 11@4 on 11p15 contains all 102 class I OR genes, whereas the other, 11@52 contains class-II genes [46]. A cluster composition analysis suggested that these two clusters represent the ancestral clusters of the present OR gene repertoire. Cluster 11@4 (class I) first duplicated to 11@52, which then evolved to generate a class II OR cluster (fig. 2). Then this cluster duplicated to chromosome 1 (1@255), from which ORs were then dispersed on the other chromosomes by multiple duplications [46].

Pseudogenes and evolution

Approximately 20% of mouse OR genes are pseudogenes [40, 41]. However, the human gene family contains many more pseudogenes (>63%) [27, 46]. This marked difference suggests a greater selective pressure in mouse to maintain a large functional OR repertoire. The difference in family size and pseudogene fraction means that the functional OR repertoire of mouse is three times larger than that of human [40]. This is consistent with the fact that humans are thought to display relatively poor olfactory performances (microsmates) compared with other mammals such as dog or rodents (macrosmates). Different studies have highlighted the prevalance of pseudogenes in humans [27, 46, 48] and in nonhuman primates [28, 62]. These studies showed that the increase of the pseudogene fraction parallels the evolution tree, i.e. hominoids possess the highest pseudogene content (50–70%), while only 25–30% of ORs are pseudogenes in old-world monkeys, and the OR repertoire in New World monkeys is similar to that of mouse. The high incidence of OR pseudogenes in the primate genome compared with other species such as mouse suggests a time-dependent evolutionary process of OR gene loss and is consistent with the recent decline of the olfactory sensory function [28, 62].

OR proteins

OR protein structure

OR proteins are 300–350 amino acids long and are devoid of N-terminal signal sequences. Sequence analyses reveal that they contain structural features common to all GPCRs [63–66] such as seven hydophobic stretches (19–26 amino acids each), which represent the transmembrane domains, a potential disulfide bond between the highly conserved cysteines in extracellular loops 1 and 2, a conserved NXS/T consensus for glycosylation in the N-terminal region, several potential phosphorylation sites in intracellular regions and numerous conserved short sequences (fig. 3). Nonetheless, there are certain characteristics specific to ORs such as an unusually long second extracellular loop, two conserved cysteines in this loop and conserved amino acid motifs which are sufficient to classify a vertebrate 7TM sequence as OR [67]. These consensus motifs include LHTPMY in intracellular loop1, MAYDRYVAIC at the end of TM3 and the beginning of intracellular loop 2, SY at the end of TM5, FSTCSSH at the beginning of TM6 and PMLNPF in TM7. In contrast, the transmembrane domains 3, 4 and 5 are hypervariable regions. They are part of the odorant-binding pocket, by analogy with other 7TM proteins such as β2 adrenergic receptors [47]. Seventeen hypervariable residues identified within these domains [47, 68] are likely to be involved in the diversity of ligand recognition by the odorant-binding pocket.

A complete analysis of mammalian consensus motifs in ORs present in the databases has been performed recently [69]. By analyzing 1332 ORs, the authors could charac-

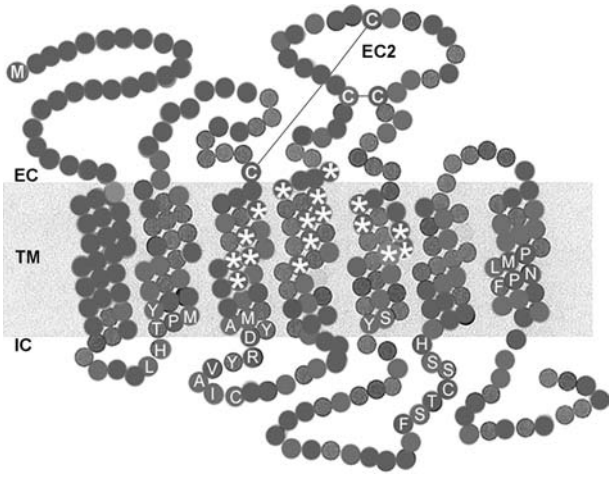

Figure 3. Schematic representation of an olfactory receptor. The seven transmembrane domains (TM) are shown embedded in the membrane. The second extracellular loop (EC2), especially long in ORs, is indicated. The main conserved motifs cited in the text are represented, and the two extracellular disulfide bonds are indicated. The most hypervariable residues are indicated by asterisks. EC, extracellular; IC, intracellular.

terize 86 specific motifs, among which are the motifs cited above. For example, it emerged that the submotif MAY (TM3) constitutes a sequence signature for the mammalian OR family, whereas motif FYVPAIFLSLTHRFGKHVPPLV (TM6) is specific for class I ORs (90%), and motifs MSPRVCVLLVAGSW (intracellular loop 2-TM4) and IFYGTAIFMY (TM6) are specific for class II (90%). Interestingly, motifs MAFLEDG and IFPPLILGL are specific for the mouse subgroup, whereas no motif specific to human ORs was found, suggesting that these two motifs survived in mice, whereas they disappeared in humans after the separation of humans and mice.

ORs and odorant molecules: the combinatorial receptor code

The principal function of ORs is to bind odorant ligands and to transduce a signal. However, functional evidence that ORs mediate responses to particular odorants has encountered difficulties. Indeed, OR proteins produced in heterologous systems seem to be trapped in the endoplasmic reticulum, Golgi and endosomal compartments with little or no membrane addressing [70]. It is known that both cis-acting and trans-acting factors may be involved in the localization of GPCRs, and in particular that a requirement for cell-specific chaperones may explain why cloned 7TM receptors expressed in heterologous cell types are often not targeted to the plasma membrane [71, 72]. Studies with *Caenorhabditis elegans* have shown the importance of the ODR-4 protein to mediate OR localization to the sensory structures of olfactory neurons [73]. Moreover, rat OR5 truncation experiments have also suggested that intramolecular interactions between N- and C-terminal domains joined by the third cytoplasmic loop appear to be responsible for the retention of olfactory receptors in heterologous cells [74].

The first demonstration of an odorant-receptor interaction was delivered in vivo by adenovirus-mediated gene transfer of the cloned rat OR I7. Electrophysiological recordings showed that overexpression of this gene was sufficient to generate responses to C7–C10 aliphatic aldehydes [75]. In parallel, Malnic et al. developed another approach which allowed analysis of responses from individual OSNs exposed to a series of odorants before the identification of the OR gene expressed by each odorant-responding OSN [58, 76]. Further evidence was provided by overexpression of chimeric ORs in human embryonic kidney cells (HEK 293) along with G proteins [77–79]. Odorant screenings were performed, and specific odorants were identified by measuring the transient elevations in intracellular calcium.

Together, these approaches have permitted the association of specific odorous agonists with particular ORs. The conclusions from these studies are that an OR recognizes multiple odorants, an odorant is recognized by multiple receptors and different odorants are recognized by distinct combinations of receptors [58] (fig. 4). In addition, an OR is specifically activated by multiple odorants which must share common molecular determinants although presenting a tolerance for other molecular features [58, 75, 77, 79–81]. For example, various studies have shown the importance of both the nature of the functional group and the molecular length of aliphatic odorants in OR recognition. Moreover, it has been shown that the receptor code for an odorant may change with odorant concentration [58, 79, 81, 82]. Therefore, the set of ORs activated by a particular odorant may change as a function of the concentration of that odorant and hence may result in the perception of a different odor. In others words, the conscious perception of an odor is an image of the combinatory code of activated ORs.

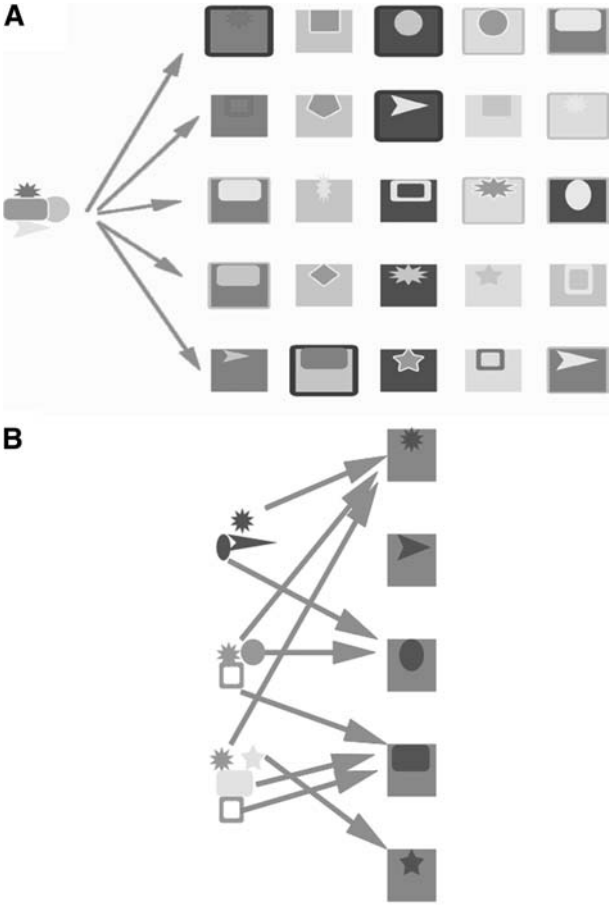

Figure 4. Combinatorial receptor code. (*A*) One odor molecule is recognized by several receptors (ORs). An odorant (left) may contain different structural features or odotopes (as indicated by colors and shapes) that can be recognized by different receptors with different affinities (right). (*B*) Most ORs (right) recognize several odorants (left) that share common motifs. As illustrated in this scheme, the perception of an odor is the result of the activation of a particular combination of receptors.

Expression of vertebrate OR genes

In the olfactory epithelium, a particular vertebrate OR gene is expressed in a tiny subset of OSNs that are interspersed with OSNs expressing other OR genes [83]. Single cell RT-PCR (reverse transcriptase-polymerase chain reaction) data and other aproaches indicate that only one OR is expressed per OSN [58, 76]. Moreover, a single OSN not only expresses an exclusive OR gene, but it also appears that only one allele is expressed in any given neuron even though each gene is represented by two alleles, [84]. This expression pattern was termed 'allelic exclusion' ; OSNs expressing either the maternal or the paternel allele that coexist in an approximately equal number in the olfactory epithelium. Moreover, in mouse, the epithelium is divided into four zones, such that each OR gene is expressed in only one zone. Each zone occupies ~25% of the surface of the epithelium [83, 85], and within the appropriate zone, each OR allele is expressed in a small fraction of neurons, in an apparently random distribution, suggesting that the final selection step occurs through a stochastic process. In rodents these zones have complex topographies but are generally oriented in the anterior-posterior direction as stripes along different regions of the turbinates [86]. To date, this spatial organization has not been confirmed in humans, and its biological significance remains unclear.

OR expression has also been clearly detected in another adult tissue, the testis. About 10% of mammalian ORs are transcribed during the maturation of male germ cells and in mature spermatozoa [51, 87]. Some ORs are known to be transcribed in the olfactory epithelium as well as in the testis [88, 89]. In contrast, a subset of OR genes is specifically expressed in testis [90]. ORs expressed in testis seem more conserved than those expressed in OSNs, especially in certain domains (see for review [91]). The physiological significance of OR expression in testis is unknown, but it has been hypothesized that it could have a role in sperm chemotaxis towards the oocyte or in sperm maturation.

Convergence of the olfactory axons

In addition to their role in binding odorant molecules and mediating olfactory signal transduction, ORs may have other functions. Whereas the neurons expressing a given receptor are restricted to one of four zones in the olfactory epithelium, the axons from OSNs that express a particular OR converge with extraordinary precision onto defined glomeruli within the olfactory bulb to create a topographic map of odor quality [92–95] (fig. 5). Each OSN projects its axon to a specific glomerulus in the olfactory bulb. In mouse, there are ~1800 glomeruli which are globose neural structures of ~100 μm in diameter covering the surface of the olfactory bulb. The pattern of

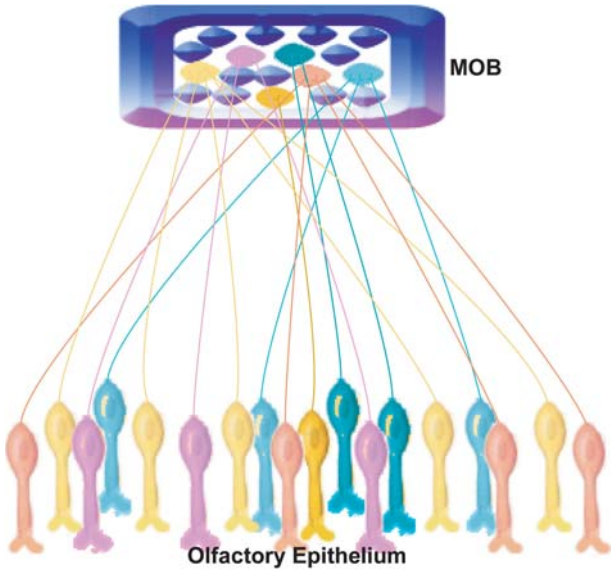

Figure 5. Schematic axonal projection pattern of the olfactory sensory neurons from the olfactory epithelium to the main olfactory bulb (MOB). The mouse olfactory epithelium can be subdivided in four zones of equal surface area. A single zone of the epithelium is represented here. Olfactory sensory neurons (OSNs) expressing the same OR gene (indicated by the same color) are widely distributed within this zone. However, despite their wide distribution, OSNs expressing the same OR project their axons to a single glomerulus in each half of the MOB.

convergence is absolute, and there is a high degree of conservation in the position of individual glomeruli in all animals of the same species. How do neurons expressing a given receptor know which glomerulus to target in the olfactory bulb? The question is unanswered, but evidence suggests that ORs are instructive in axon guidance of OSNs [92, 93]. Deletion of the OR coding region destroys glomerular convergence and the labeled axons wander on the surface of the olfactory bulb [94]. Indeed, the fact that one glomerulus is a site of axonal convergence of an OSN expressing a specific OR suggests that odor quality is encoded by a specific combination of activated glomeruli and that different spatial patterns of activated glomeruli may correspond to distinct odors [96, 97].

However, the mechanisms underlying OR gene choice, expression and glomerular convergence remain enigmatic. Sequence analysis of OR loci [49, 56] and transgenesis with tagged mouse OR genes have been performed [52, 98–100], and no coherent view has emerged. More recently, Vassalli et al. [101] showed that transfected MOR23 and M71 OR minigenes (9 kb and as short as 2.2 kb) are selectively expressed in a monoallelic fashion by neurons that do not coexpress the endogenous gene but coproject their axons to the same glomeruli. Deletion of a 395-bp upstream region in the minigene abolishes expression in the olfactory epithelium. This region contains sequence motifs such as the O/E (Olf-1/EBF-like

transcription factor) and homeodomain binding sites which are known to be key elements for OR gene expression [102]. This observation suggests that zonal expression of OR genes does not necessarily involve distant control elements. Also, they found that neurons expressing an OR transgene allele in ectopic epithelial zones form ectopic glomeruli, which also receive input from OSNs expressing the cognate endogenous receptor allele. The authors conclude that this abnormal targeting might be due to homotypic interactions among axons of neurons expressing the two alleles, and they underline the role of such interactions in axonal targeting. These data are consistent with the notion that ORs mediate both axon guidance and odorant responsiveness.

Olfaction in other organisms

Olfaction in other organisms such as invertebrates has been extensively studied because these animal models, especially insects, exhibit complex behaviors that are controled by an olfactory system that is far simpler than that of vertebrates. In this section we summarize the present knowledge for the two main model organisms that are the insect *Drosophila melanogaster* and the worm *Caenorhabditis elegans*. A third model, the zebrafish *Danio rerio* will not be commented on here.

The fruit fly *Drosophila melanogaster*

Olfaction in insects has been studied for many years in moths and honeybees concerning behavioral and physiological aspects. However, molecular approaches have been made possible only recently by the knowledge of the genome sequence and genetics of *Drosophila*.

Drosophila possesses two olfactory organs: the third segment of the antenna and the maxillary palp [103–105]. In both organs ORNs (Olfactory Receptor Neurons) are expressed in sensory hairs called sensilla [106]. There are ~1200 ORNs in the antenna distributed in 16–30 different classes on the basis of electrophysiological criteria, and 120 ORNs in the maxillary palp that are distributed in 6 classes [105, 107 and references therein]. There are 3 types of sensory hairs on the antennae, each innervated by 1–4 ORNs [106]. ORNs extend their axons to the antennal lobe (the equivalent of the olfactory bulb), the first relay of olfactory information, where they synapse with the antennal lobe neurons that in turn project axons to higher brain structures that are the mushroom body and lateral horn of the protocerebrum [108, 109].

After the characterization of the first OR genes in mammals [2], almost a decade has been necessary to identify the olfactory receptor genes (DOR genes) of *Drosophila*, mainly because searches for DOR genes using sequence similarities from other organisms have been unsuccessful. In 1999–2000, DOR genes were finally identified by database mining of the genome sequence of *Drosophila* and antennal complementary DNA (cDNA) library sequencing [107, 110–112]. A total of 57–60 genes have been identified. Unlike mammals ORs, DOR genes contain introns. Compared with rodents, the repertoire of odors detected by *Drosophila* is probably small and may reflect the diminished importance of olfaction in insects with a highly developed visual system, and/or alternatively with a very specialized OR repertoire. The encoded receptors are 369–403 amino acid proteins. They share 9–73% sequence identity (average 15–25%) [110], and share no significant sequence identity with class I and II ORs, nor with *C. elegans* chemical receptors (see below). The most divergent region is a stretch of 30 amino acids overlapping part of the first extracellular loop and the third transmembrane domain [107]. In situ hybridizations using fluorescent or digoxigenin-labeled antisense RNA probes and RT-PCR experiments allowed different labs to characterize the expression of the DOR genes [105, 107, 111, 112]. Most of them are expressed in the antenna; only seven (among 57–60) are expressed in the maxillary palp, and very few in both organs. DOR genes are not expressed in other tisssues such as the proboscis or mouth, the insect organs devoted to taste which are the sites of expression of gustatory receptors (about 60 members) [113, 114]. This observation suggests that the DORs are dedicated to the perception of volatile odorants. The two systems are quite different since olfactory information is transmitted to the antennal lobe, whereas gustatory information is transmitted to the subesophageal ganglion. However, gustatory receptors are also expressed in regions of the antenna that do not express DOR genes, resulting in a spatial segregation of the two receptor families [114]. As in mammals, ORNs express a single receptor [111] in a spatially invariant population of cells of the olfactory organs, thus defining a functional map of ORNs on the antennal surface [105, 114]. Similarly, ORNs expressing a given DOR gene converge upon 1 or 2 spatially invariant glomeruli of the antennal lobe [112]. The antennal lobe contains 41–43 glomeruli [115] for ~39 distinct neuronal cell types within the olfactory organs, suggesting that olfactory neurons expressing a given DOR converge on a single glomerulus. Different approaches using transgenic flies (DOR gene promoter driving the expression or either LacZ or green fluorescent protein, GFP), in situ hybridization and immunochemistry have allowed characterization of the map of receptor activation in the *Drosophila* brain. Indeed, the antennal lobe retains a two-dimensional map that reflects the different spatial patterns encoding odors. As in mammals, a single odorant could activate different glomeruli, resulting in a combinatorial code that would be decoded in higher brain centers [112]. Similarly, as a second-order level of complexity, tracing experiments using the FLP-out technique [116, 117] re-

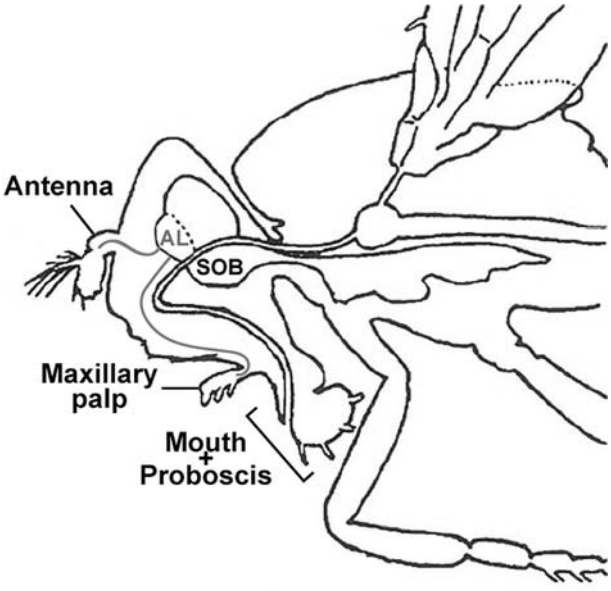

Figure 6. Schematic sagittal view of a *Drosophila* head showing the olfactory organs and the antennal lobe (AL, olfactory bulb in mammals). *Drosophila* olfactory receptors (DOR) are expressed in olfactory receptor neurons (ORN) that innervate hairs (sensilla) on the surface of the third antennal segment and the maxillary palp. ORNs project their axons to the antennal lobe of the protocerebrum whereas gustatory neurons (expressed in the mouth and proboscis) target the subesophageal ganglion (SOB). Adapted from [114].

vealed that neurons innervating the same glomerulus display similar projection patterns in the protocerebrum (mushroom body and lateral horn). These observations demonstrate that the olfactory information is processed through at least three olfactory maps (on the surface of the olfactory organs, in the antennal lobe and in the protocerebrum) before integration as a sensation of smell [112, 118]. Finally, it appears that the scheme for odor discrimination has been maintained from the separation of insects and mammals, about 500 millions years ago.

The worm *C. elegans*

The nematode *C. elegans* has also been widely used to study chemodetection because (i) its genome is more compact than mammals (~10^8 bp vs. 3.10^9 bp) and has been entirely sequenced, (ii) its anatomy and cellular organization are well known and (iii) behavioral assays (attraction or repulsion) are easy to perform.

C. elegans can detect light, temperature and touch, but the responses to chemicals are the most diverse in its behavioral repertoire [119]. *C. elegans* eats bacteria, and the chemicals produced by bacteria stimulate chemotaxis as well as feeding, defecation and egg laying [120 and references therein]. In *C. elegans* the two main types of sensory organs are involved in chemodetection: two symmetric amphids in the head (considered as the nose of the worm) that contain the endings of 12 types of neuron pairs, and two symmetric phasmids in the tail that contain the endings of 2 types of neurons (for review see [121, 122]). Thus, among the 302 neurons (that are invariant in number and position) of *C. elegans*, 32 (14 types) are devoted to chemosensation [123–125]. The function of these chemosensory neurons has been determined by individual neuron ablation using a laser beam and observation of the behavior of the treated animals [126–129]. Using this approach, it has been determined that most volatile odorants are sensed by three pairs of neurons called AWA, AWB and AWC, with AWA and AWC neurons dedicated to attractive odorants and AWB to repulsive odorants [122, 127, 130]. *C. elegans* chemoreceptors were first identified in 1995 by analysis of the 15% of the genome sequence available at that time [120]. As for chemoreceptors from other species, *C. elegans* chemoreceptors are 7 TM GPCRs that are expressed in sensory neurons. Most of them are arranged in clusters and, as in *Drosophila*, they contain multiple introns. The *odr-10* gene has been extensively studied. It encodes a receptor for the attractive odorant diacetyl and is expressed in the AWA neurons [131]. Its expression is regulated by the transcription factor gene *odr-7*. Furthermore, if ODR-10 is expressed by trangenesis only in AWB neurons (detection of repellents) rather than in AWA neurons, transgenic animals avoid diacetyl, suggesting that the attractive or repulsive nature of an odorant is encoded by the type of neuron that is stimulated rather than by the molecular sequence/structure of the activated receptor [132, 133].

At the present time, database analysis has revealed that the complete *C. elegans* genome contains 900–1000 chemoreceptor genes [42, 122, 134 and references therein] that are distributed in different families: the *odr-10* family (~700 members) containing subfamilies str (300 genes among which *odr-10*), stl, srd and srh (~300); the sra family (120 genes) containing subfamilies sra, srb, sre (40 genes each); the sro family (~80 genes) and the srg family (~40 genes), which is related to the opsin subfamily as for the vertebrate ORs. The nucleotide sequence identity between subfamilies is low (10–30%), and the similarity with receptors of other species is also very low, so that for example ODR-10 and vertebrate ORs share only 10% of protein identity, a level not significant enough to suggest that they could derive from a common ancestor. The organization of these genes is typically in tandem arrays, and the repertoire evolved by multiple duplications as in mammals. Among these genes, 30% are pseudogenes, leading to 500–550 potentially functional genes [42, 134]. In summary, the 800–1000 chemosensory genes represent >6% of the genome sequence, and the ~550 encoded receptors represent ~4% of the protein complement. The size of this repertoire is very large and resembles those of vertebrates. However, the repertoire of odors detected by *C. elegans* is smaller than those de-

tected by mammals. In contrast to mammals, each sensory neuron in *C. elegans* expresses multipe receptors (20–30 chemosensory neurons and ~500 receptors, leading to a ratio of 15–25 receptors per neuron) and recognizes a variety of chemicals, suggesting that regulatory events in processing the odorant information could contribute to odorant recognition [120]. Also, the signal transduction cascade presents some specificities with respect to mammals (reviewed in [122]). For example, the Gi-like Gα protein ODR-3 [130, 135] is required to trigger responses from AWC neurons. A number of other G proteins are probably involved in chemodetection in *C. elegans*, and it has been estimated that about 20 G proteins are encoded in the genome, 15 of which are expressed in chemosensory neurons [136]. A second example is that a cGMP messenger is involved in AWC neurons. Indeed, *odr-1* that encodes a transmembrane guanylyl cyclase is necessary for AWC olfaction to provide a cGMP input that opens TAX channels [137]. Another important difference with mammals is that *C. elegans* receptors do not play a significant role in neuronal wiring [132]. A synthetic comparison underlining the differences between the number of chemosensory cell types and the different chemoreceptor families of *C. elegans*, *Drosophila* and mammals has been made previously [138]. However, the molecular conservation over 550 Myrs of the nervous system of nematode and vertebrates makes *C. elegans* a valuable tool to study chemodetection [139].

OR nomenclature

A large annotated set of human OR genes is available in online databases such as HORDE (http://bioinformatics.weizmann.a.c.il/HORDE/) [46] and ORDB (http://senselab.med.yale.edu/senselab/ordb/) [140], while a mouse OR database containing orthology information is available at http://www.fhcrc.org/labs/trask/OR/ [40]. Various nomenclatures have been used to classify the OR sequences. Glusman and Lancet have grouped OR genes into families and subfamilies such that the members of a given family share a protein sequence identity >40% (PID) and the subfamily members a PID >60% [46, 141]. Using this definition, 17 families have been identified in the human genome, four of which contain more than 100 members. Zozulya et al. proposed a new nomenclature based on chromosomal localization and phylogenetic analysis [48]. Recently, Zhang and Firestein introduced a different naming scheme which combines phylogenic relationships and protein identity to classify the mouse OR sequences [41]. Although databases attempt to provide the correspondences of a given OR in the different nomenclatures, the situation is still confused and should be clarified in the near future. *Drosophila* and *C. elegans* information can be found repectively at http://flybase.bio.indiana.edu/.and http://www.sanger.ac.uk/Projects/C_elegans/

Conclusion

Our understanding of sensory systems has grown impressively in recent years as a result of intense effort to characterize the mechanisms underlying olfaction. Together with the use of model organisms, the release of the complete sequence of human and mouse opened the way to decipher the olfactory system. In particular, we now know that odor perception is the result of a combinatorial code, and that ORs govern the formation of a topographic map in the bulb and reflect odor quality by the convergence of OSN axons towards precise glomeruli. Netherveless, the subtle mechanisms monitoring these processes are not completely elucidated, and many questions remain open. In particular, the regulation of OR expression leading to the expression of a single OR allele per neuron which will then synapse within two specific and spatially defined glomeruli is a key element required to understand the fine tuning of mammalian olfaction.

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