Olfactory receptor gene repertoires in mammals

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Abstract

In mammals, olfaction is mediated by two distinct organs that are located in the nasal cavity: the main olfactory epithelium (MOE) that binds volatile odorants is responsible for the conscious perception of odors, and the vomeronasal organ (VNO) that binds pheromones is responsible for various behavioral and neuroendocrine responses between individuals of a same species. Odorants and pheromones bind to seven transmembrane domain G-protein-coupled receptors that permit signal transduction. These receptors are encoded by large multigene families that evolved in mammal species in function of specific olfactory needs.

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1. Introduction

Chemodetection is achieved in mammals by olfaction (detection of odorants and pheromones) and taste (gustatory perception) (Fig. 1). These three functions enable an animal to detect chemicals in the external environment and to identify chemical cues from conspecifics. In the present review, we will focus on the evolution of the olfactory receptor gene repertoires devoted to olfaction (sniffing of chemical compounds permitting the detection of odorants and pheromones).

The sense of smell is an ancient sensory system that is present in most species (worms, insects, fish, birds and mammals). Although in humans, olfaction is viewed rather as an esthetic sense, it is essential for survival in other species such as mouse for locating food, mates or predators.

Surprisingly, until 1991, olfaction was poorly characterized at the gene level. In 1991, Buck and Axel discovered the olfactory receptor (OR) gene family in rat [1]. This founder paper opened the door for deciphering the mechanisms of olfaction. For this major discovery, Linda Buck and Richard Axel won the Nobel Prize in Physiology or Medicine in October 2004. Fifteen years after this initial discovery, OR genes have been found in most species, and since this date, the public release of the complete sequence of numerous species (>12 in mammals) has allowed different teams to characterize the complete OR gene repertoires of these species and to trace their evolutionary story. Similarly, pheromone receptors (VR) were described more recently as two different receptor families (V1R and V2R) in mouse [2–4]. Searches for VR in different species, particularly the V1R family, have also permitted to follow the evolution of pheromone detection. These past 10 years, a number of data about odorant and pheromone receptor genes have been accumulated and various reviews have been written on the principles of odor/pheromone detection. Here, we will focus on the recent data concerning the evolution of these gene repertoires in mammals with a comparison of odor and pheromone detection.
2. Odor detection

2.1. Background

Odor detection is achieved in every species by the binding of odorants by olfactory receptors (OR). This interaction induces a transduction pathway that ultimately transmits a signal to the central nervous system that results in a sensation of smell. In vertebrates, OR are mainly expressed on the cilia of the dendrites of olfactory sensory neurons that emerge in the nasal olfactory epithelium and for some of them in mature male germ cells. ORs are heptahelical G-protein-coupled receptors (GPCR) that share a significant homology in vertebrates, particularly in conserved domains. However, although OR are invariably GPCR, they do not share any significant homology when comparing those of worms (*C. elegans*), insects and vertebrates.

Some years ago, we demonstrated for the first time that the OR gene repertoire in humans was largely distributed in the genome and that the number of functional genes was very low (∼30%), providing a possible explanation for the reduced sense of smell of humans compared with that of other species such as dog or mouse [5]. Later the release of the complete sequence of the human genome permitted to precise these results [6]. Briefly, the human genome contains ∼1000 OR genes dispersed in >50 chromosomal locations and organized mostly in clusters. About 65% of them have incurred deleterious mutations during evolution through a pseudogenization process, leading to only ∼300–350 potentially functional OR genes. ORs are distributed in two main classes: class I that corresponds to fish-like receptors that bind water-soluble odorants, and class II that contains mammal-like receptors dedicated to binding volatile odorants. Actually, sequence analysis of the human genome revealed that the complete OR gene repertoire originated from a class I OR cluster located on chromosome 11. This cluster duplicated first in another location of chromosome 11 to generate a class II cluster that was in turn duplicated on chromosome 1. From this latter location the repertoire expanded by multiple duplications throughout the genome to generate the present OR repertoire [6].

Starting from the observations that humans have a reduced functional OR repertoire, we then asked the question whether this pseudogenization was specific of the hominization process. To answer this question we sampled the genomes of various primate species [7]. In summary, we found that there is an acceleration in the pseudogenization process from New World monkeys (NWM, low pseudogene content as in mouse) to Old World monkeys (OWM, ∼30% pseudogenes) and apes (∼45% pseudogenes) with humans having the highest pseudogene content (∼65%). It was therefore tempting to speculate that during evolution, primates lost a part of their olfactory ability because, on the contrary to mouse, they do not rely anymore on olfaction for survival.
2.2. Recent advances

2.2.1. Primates

Recent and more detailed analyses have precised these data. Gilad et al. [8] analysed the orthologs of a set of 50 OR genes in apes and rhesus macaque. They found that humans have accumulated deleterious mutations leading to pseudogenes about four-fold faster than any other primate species sampled. As a consequence, the OR pseudogene content in humans is twice as high as in the other species. This observation suggests that there is a human-specific process of OR gene pseudogenization, probably due to reduced olfactory needs relative to non-human primates. More recently they have also analysed a set of 100 ORs randomly chosen in 19 primate species [9]. In this study they found that New World monkeys and lemur (prosimian) have the lowest pseudogene content (∼15–20%), similar to that of mouse, whereas Old World monkeys and apes have the highest (∼30% and 35%, respectively), with humans >50% as previously described. Furthermore, this study reported a striking observation, i.e. among NWM, the howler monkey contains ∼30% of pseudogenes as OWM. The howler monkey is the only NWM to have full trichromatic vision as OWM and apes. Although this observation could be a coincidence, the authors suggest that the reduction of the OR gene repertoire is parallel to the acquisition of trichromatic vision, meaning that the development of vision was detrimental to olfaction. Furthermore, it seems that the divergence NWM/OWM represents the starting point of the OR gene repertoire decline. Sequence analysis of the first release of the chimpanzee genome allowed Gimelbrant et al. [10] to rule out a general positive selection in humans and chimpanzee, consistent with the diminishing importance of olfaction in these species, while they found a weak purifying selection over half of the repertoire. However, very recently Gilad et al. [11] refined the analyses of the human and chimpanzee repertoires. They found that (1) at the exclusion of the large OR pseudogene family 7E, the pseudogene count of humans and chimpanzee is 51% and 41%, respectively; (2) the number of OR genes in chimpanzee is ∼26% higher than in humans; (3) several subfamily expansions are either specific to human or chimpanzee, suggesting that some OR genes are under positive selection. As for other mammal species, it therefore appears that the functional OR repertoire of both species has been shaped by specific sensory requirements. Another study from Linda Buck’s group focused on the relationship between genomics and function of the human OR repertoire [12]. They identified 339 intact OR genes and 297 pseudogenes from the human genome database. As previously described, OR genes are unevenly distributed among ∼50 different chromosomal loci. Most subfamilies are encoded by a single locus and most loci encode a single or very few subfamilies. By analyzing ORs with known odorant activities, the authors found that ORs of a single locus recognize structurally related odorants, suggesting that different parts of the genome are involved in the detection of different odorant types.

2.2.2. Mouse

In contrast to human, mouse is considered as a macrosmatic animal that relies on the sense of smell. Its OR gene repertoire is organized similarly to humans. Indeed, after discovering OR genes in most mammals including mouse, a first work describing the mouse OR repertoire [13] reported that mouse ORs are organized in paralogous clusters generated by duplications. More recent works [14,15] evidenced very important differences with humans, probably shaped by specific olfactory needs: (1) the mouse genome contains about 1500 OR sequences (versus ∼960 in humans); (2) this OR gene repertoire contains only 20% of pseudogenes, leading to a functional repertoire ∼3 times as high as in humans. Therefore, mouse possesses 1000–1200 potentially functional OR genes versus 300–350 in humans. Also, the mouse OR gene repertoire is more compact (reduced divergence between genes), an observation that correlates with the rapid pseudogenization of the human repertoire. Similar data were obtained by the Buck’s group [16], i.e. a repertoire containing ∼1200 genes and 24% pseudogenes. However, they also found 22 subfamilies that are specific to humans versus 84 in mouse, suggesting a refined specialization in rodents.

2.2.3. Dog

Dog strains have the particularity to have derived recently (∼10–15,000 years ago) from a common ancestor through domestication and selective breeding. They present a spectrum of very different physical phenotypes and behaviors but all of them are thought to be populated by macrosmatic animals. Dogs are known to display a better olfactory sensitivity than humans and that is why they are trained and used for finding either hidden substances such as explosives or drugs, or victims of natural disasters. Up to now the basis for the difference in olfactory ability between micro- and macro-smates is not clearly established since the human OR repertoire theoretically permits to detect the same classes of chemicals than those of dog or rodents. However, several points could explain this difference: (1) dogs have a larger surface of olfactory epithelium (up to 20 times),
suggesting that the number of olfactory neurons and the density of ORs are higher than those of humans; (2) the brain structures involved in the olfactory function such as the olfactory bulb are larger; (3) the number of functional OR genes is higher, as in the case of mouse (at least three times), and consequently the number of specific subtypes/subfamilies is also higher, permitting a finer tuning. The complete sequencing of the dog genome allowed different teams to fully characterize the OR repertoire. Two main studies [17,18] established that the canine OR repertoire contains \( \sim 1300 \) OR genes whose genomic organization is similar to those of humans and mouse, and the pseudogene fraction is in the range 12–18% as in mouse. It seems therefore that in good smellers animals (dog, rodents) the high number of functional OR genes correlates with better olfactory performances. A recent work [19] characterized more accurately the dog and rat OR gene repertoires. In this analysis, uncomplete genes were sorted out and not scored. Hence, the dog genome contains \( \sim 1100 \) genes distributed in \( \sim 300 \) subfamilies, and 20.3% pseudogenes, whereas the rat genome contains \( \sim 1500 \) genes with 19.5% of pseudogenes. It is the largest mammal OR gene repertoire that has been characterized to date, but it is distributed in only \( \sim 280 \) subfamilies. Although the rat repertoire is larger, it is less polymorphic, suggesting that the dog repertoire presents a higher level of diversification. However, it is not clear if these observations correlate with the olfactory ability of the two species, since many dog breeds were selected for their olfactory performances. To investigate this hypothesis, Galibert’s group sampled 16 OR genes in 95 dogs pertaining to 20 different breeds [20]. They observed a high level of allelic polymorphism with up to 11 single nucleotide polymorphism (SNP) sites between two alleles of some genes, whereas some others are breed-specific. OR genes are thus highly polymorphic with >50% SNPs leading to amino-acid changes. Some other mutations are deleterious and give birth to pseudogenes. As observed before in humans, different populations, or breeds for dogs, have different subsets of pseudogenes. Although preliminary, this study found that the percentage of pseudogenes is 20.3% in boxer versus 18% in poodle, a breed that is known to possess a more acute sense of smell.

3. Olfactory receptors in spermatozoa

Just after the discovery of OR genes, several articles described that a number of specific ORs were expressed predominantly if not exclusively in spermatozoa (mature male germ cells) of mammals [21,22]. These ORs are mostly expressed on the sperm flagellar midpiece. At that time, when nothing was known about sperm guidance, the authors hypothesized that sperm chemoreceptors could be involved in sperm maturation and especially in sperm chemotaxis, i.e. how spermatozoa actively swim towards the egg (ovula) for fertilization in following a gradient of attractants released by the egg or secreted along the female genital tract. More than a decade after this finding, a lot of data have been accumulated about sperm guidance (for review see [23]). Concerning the role of ORs in sperm cells, in 2003, Spehr et al. [24] found that a particular human receptor, hOR17-4 was expressed in spermatozoa and activated by floral scents such as bourgeonal at very dilute concentrations \((\sim 10^{-8} \text{ M})\) with a rise of intracellular \(\text{Ca}^{2+}\) as in typical OR responses to odorants. These authors also demonstrated \(\text{in vitro}\) that most of the motile spermatozoa swam up a gradient of bourgeonal and that about one third of these cells expressed hOR17-4. Thus, bourgeonal stimulates chemotaxis (directed movement in a chemical concentration gradient) and chemokinesis (change in swim speed in the concentration gradient). In addition, undecanal was identified as a potential inhibitor of bourgeonal. Moreover hOR17-4 is also expressed in the olfactory epithelium to play a role in odor detection [25], showing that this receptor is not sperm-specific. Of course, the importance of OR in sperm chemotaxis (“the sperm’s nose”) led to speculate about potential applications of hOR17-4 as a therapeutic target in fertility treatment or in contraception approaches [26]. However, despite these data provide evidence for an implication of ORs in sperm-egg communication, it seems that ORs expressed in sperm may contribute to species specificity in fertilization as any bourgeonal-activated calcium responses were never observed in pig or mouse sperm.

Similarly, in mouse, MOR23, an OR expressed in the olfactory epithelium and testis, plays also a role in sperm chemotaxis in allowing spermatozoa to swim up a concentration gradient of lyral odorant [27]. Various chemoattractants non-OR-dependent have been identified so far [23], but in the case of hOR17-4 and MOR23, the physiological analogues of bourgeonal and lyral have not been identified yet. Given that there is multiple sources of chemoattractants and that a number of different ORs are expressed in mature sperm cells, it is likely that sperm guidance goes through different steps to guide the spermatozoa from one chemoattractant to the next one along the genital tract. In addition one could imagine that this multistep chemoattraction could also promote sperm selection.

In summary, this past decade has opened a new field in sperm guidance and fecundation. However, the signal-transduction pathways involved in sperm chemotaxis in
responses to the spectrum of chemotactants are still poorly known although it was described that hOR17-4 activation is coupled to a cAMP-dependent pathway and that the receptor-ligand binding activates membrane adenylate cyclases such as mAC III and/or mAC VIII via the G_{olf} protein [28].

4. Pheromone detection

4.1. Background

It is known for a long time that substances called pheromones drive chemical communication between individuals of a same species (conspecifics) that leads mainly to sexual and social changes in the behavior and physiology of the recipient. Pheromones are still not well defined in mammals, but in contrast to odorants are mostly non-volatile chemicals that require a direct contact with the sensory cells for detection. Pheromones are found in bodily fluids such as urine, sweat, saliva and other secretory glands. A number of pheromone-driven effects have been documented particularly for puberty, pregnancy, copulation, intermale aggression and protection of the newborns (for review see [29] and references therein). Pheromone communication is achieved in vertebrates by a second specialized olfactory organ called vomeronasal organ (VNO) or organ of Jacobson or accessory olfactory system. VNO resides on the bottom of the nasal cavity, and is therefore separated from the main olfactory epithelium. It is a bilateral organ that communicates with the nasal cavity and/or the mouth via a small duct. It contains sensory neurons that project towards a specialized part of the olfactory bulb, the accessory olfactory bulb. The pheromone information is then transmitted to the amygdala and the hypothalamus (limbic system) and results in modifications of the endocrine status of the recipient.

4.2. Pheromone receptors

Two pheromone receptor families have been successively identified. As for ORs, they both belong to the G-protein-coupled receptor family (Fig. 1). First, the V1R family [2] comprises receptors that are encoded by multiexonic genes whose coding part, as for ORs, is contained in a single exon. Due to the easy access in silico to the protein sequence from genomic DNA, the V1R family has been used as the pilot family for studying the function and evolution of pheromone communication. In mouse, V1Rs are contained in 12 protein families that are encoded by a compact gene repertoire dispersed in a few genomic locations (four main clusters) and expressed in the apical layer of the VNO. Despite V1R genes are reminiscent to OR genes, the encoded receptors do not share any sequence homology with ORs. V1Rs are only related to the T2R taste receptor family with only 15–20% amino-acid sequence identity (for review see [30] and references therein). Second, another family, called V2R has been described later by three different groups [3,4,31]. V2Rs are readily different from V1Rs. The protein sequences are encoded by multiple exons. V2Rs possess a long extracellular N-terminal end and are related to Ca^{2+}-sensing and metabotropic glutamate receptors as well as to the T1R taste receptors (sweet) [29]. V2Rs are expressed in the basal layer of the VNO. Another difference between V1Rs and V2Rs is that they are expressed in different sets of neurons that coexpress G proteins G_{olf} and G_{q}, respectively. Also, recent studies [32,33] have shown that nine MHC class Ib genes (M1 and M10 subclasses) are specifically expressed by the VNO. Each of these genes is expressed in a subset of VNO neurons that coexpress one V2R gene. Together with β2-microglobulin, they form a complex with V2R that is essential for a correct trafficking of V2Rs to the neuron membrane. However, V1Rs and V2Rs have in common a transduction pathway that involves an ion channel of the transient receptor potential family named TRP2 or TRPC2 [34]. TRP2 is specifically expressed in the VNO, and it has been shown that TRP2 depletion impaired pheromone detection and induced changes in sexual and aggressive behaviors.

4.3. Evolution of the VR repertoires

Given that VR genes were characterized a few years ago, the evolution of the pheromone receptor repertoire in mammals has been investigated only recently. The first extensive study on mouse V1R gene repertoire was performed in 2002 [35]. In this work the authors showed by screening the complete mouse genome that mouse possesses at least 137 V1R genes encoding receptors distributed in 12 families with a high degree of sequence diversity, and about 100 V2R genes. The same team also showed that analysis of the different mammal V1R families indicated divergences and specializations making some of these families species-specific [36]. Despite VNO is vestigial and probably non-functional in human adult and Old World monkeys, five potentially functional V1R have been described in human. Nevertheless a study of one these genes (V1RL1) in 13 different primate species [37] showed that it is a pseudogene in most of them originating from different mutations and suggesting that the human gene could be also non-functional. One of the first reviews on the VR genes [30] compared
Fig. 2. Evolution of the olfactory systems (odor and pheromone detection) in mammals. Left panel, schematic phylogenetic tree showing the time scale of separation of the different clades in million years (MYA). Right panel, comparison of the number of olfactory receptor (OR) genes and pheromone receptor (VR) genes with an intact open reading frame (potentially functional) in the different clades. An horizontal thick line between New World and Old World monkeys separates catarrhines from the other species. Catarrhines show the highest degree of decimation of both olfactory systems (pseudogenization of OR and VR genes, of TRP2, and absence of a functional vomeronasal organ (VNO)). Mouse and dog have the highest count of functional OR genes. However, although dog kept intact its OR repertoire, its VNO-mediated VR repertoire is decimated in contrast to mouse. At the present time, VR genes seem essentially specific to rodents.

The VR repertoire in human and mouse: 137 potentially functional V1R genes in mouse versus five in human; 140 V2R genes in mouse versus zero in human, and nine MHC 1b genes that are associated with the expression of V2Rs, in mouse versus zero in human (Fig. 2). It seems therefore that despite the difficulty to pull out V2R coding sequences from genome sequence analysis, humans do not possess any functional V2R, whereas this VR class is well represented in rodents. In parallel, Zhang and Webb followed the evolution of the V1R repertoire and TRP2 in catarrhine primates (Old World monkeys and hominoids) [38]. They showed that TRP2 is a pseudogene in catarrhines, whereas it retains an intact open reading frame (ORF) in New World monkeys. This observation reinforced the idea that the five human V1R genes with an intact ORF are probably non-functional. Furthermore, an examination of these five sequences in apes (chimpanzee, gorilla and orangutan) indicated that they are pseudogenes. Similarly, ORF-containing V2R genes were found in rodents, whereas only pseudogenes were found in humans, suggesting that it is probably also the case in Old World monkeys since adult hominoids and Old World monkeys are devoid of functional VNO, whereas New World monkeys do have one. It seems therefore that VNO-mediated pheromone detection was lost in the ancestor of hominoids and Old World monkeys about 23 million years (MY) ago, a date that corresponds to the acquisition of trichromatic color vision in catarrhines after they were separated from New World monkeys. Grus and Zhang [39] then compared the V1R gene repertoire in mouse and rat. They characterized 95 rats V1R genes distributed in 10 families that are common to mouse and two families that are specific to rat. They showed that the evolution of the V1R repertoire is characterized by a rapid turnover due to gains and losses of VR genes, suggesting important changes in pheromone communication between species. The number of genes per family is different between mouse and rat because different duplication and pseudogenization events occurred independently. The authors also showed that most of the V1R families emerged 90–140 MY ago, an era that corresponds to the radiation of placental mammals (~80–110 MY ago) (Fig. 2). Another study compared the complete OR and V1R repertoires in two mouse genome assemblies to show that a high level of single-nucleotide polymorphisms (SNPs) are present in both repertoires. The authors show that V1R genes are likely subjected to positive selection, an observation in accordance with the apparition of species- or strain-specific pheromone communication. Two reviews [29,40] compiled most of these data. In one of them, Rodriguez [29] pointed out that human V1RL1 is expressed in the olfactory epithelium, suggesting that pheromone receptors may be expressed in...
non-VNO tissues in some species and that the barrier between olfactory and pheromone detection is probably not so clear-cut than previously thought. Also, as for ORs, some V1Rs are expressed in rodent testis but up to now no role has been attributed to this expression. Finally, VR are also involved in axonal guidance into the VNO.

More recently, Young and colleagues performed a detailed analysis of the V1R repertoire in five species by genome mining. The authors scored a total of 364, 220, 65, 116, and 171 V1R sequences in mouse, rat, dog, chimpanzee and human, respectively (with at least 165, 110, 54, 102 and 115 pseudogenes and 165, 102, 8, 0 and 2 V1R genes with an intact ORF). Important information emerged from these observations: (1) mouse has a functional V1R repertoire that is ∼50% larger than rat (165 versus 106). The rodent repertoire is compact with respect to primates, i.e. distributed on six chromosomes in rat and 8 in mouse versus 22 and 21 chromosomes in human and chimpanzee, respectively; (2) surprisingly, dog has a very reduced functional V1R repertoire with only eight intact V1R genes. Dogs have a functional VNO and are known for their olfactory performances as well as for using a social hierarchy when living in group that presumably requires pheromone communication. However, dog VNO is thin and not well developed as well as the accessory olfactory bulb, observations that correlate with the small number of intact V1Rs. Nonetheless, in contrast to catarrhines, TRP2 seems functional. It is therefore possible that V1R pheromone detection was kept intact in rodents, whereas it was decimated in dogs that overdeveloped the main olfactory system to communicate. The champions in olfaction are rodents that both have overdeveloped olfactory and VNO-based pheromone systems. Dogs have also an overdeveloped sense of smell but seem to have lost VNO-mediated pheromone communication suggesting that they may use olfaction as an alternative system. These past 15 years olfaction has revealed many of its secrets but a lot of things are still unknown, i.e. what are the bases of olfactory performances between animals? Does dog use VNO? Does it exist other receptor classes and systems for pheromone/chemical communication? It is likely than unexpected findings are still to be made.

5. Conclusion

Although human enjoys smelling perfumes and consuming fine cuisine and good wines, it appears that its sense of smell is probably the weaker in mammals since the gene repertoire and the structures devoted to olfaction have been decimated during evolution. The same process is observed for pheromone detection. During primate evolution the separation of New World monkeys from catarrhines (Fig. 2) marks the deterioration of the two olfactory systems (main and accessory), i.e. high rate of pseudogenization for OR and VR genes, complete pseudogenization of TRP2, size reduction of olfactory epithelium and olfactory bulb, and absence of functional VNO. This limit coincides with the apparition of trichromatic color vision suggesting that vision became more important than chemical communication in these clades to communicate. The champions in olfaction are rodents that both have overdeveloped olfactory and VNO-based pheromone systems. Dogs have also an overdeveloped sense of smell but seem to have lost VNO-mediated pheromone communication suggesting that they may use olfaction as an alternative system. These past 15 years olfaction has revealed many of its secrets but a lot of things are still unknown, i.e. what are the bases of olfactory performances between animals? Does dog use VNO? Does it exist other receptor classes and systems for pheromone/chemical communication?

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