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Odorant receptor-mediated signaling in the mouse

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In the mouse olfactory system, there are ∼1000 types of odorant receptors (ORs), which perform multiple functions in olfactory sensory neurons (OSNs). In addition to detecting odors, the functional OR protein ensures the singular gene choice of the OR by negative-feedback regulation. ORs also direct the axonal projection of OSNs both globally and locally by modulating the transcriptional levels of axon-guidance and axon-sorting molecules. In these latter processes, the second messenger, cAMP, plays differential roles in the fasciculation and targeting of axons. In this review, we will discuss how ORs differentially regulate intracellular signals for distinct functions.

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Introduction

The mouse olfactory system is capable of detecting and discriminating a large number of volatile odorants with approximately 1000 different receptors [1]. Inhaled odorants are dissolved in the olfactory mucosa in the olfactory epithelium (OE). Olfactory sensory neurons (OSNs) in the OE extend a single dendrite into the lumen of the nasal cavity. The dendrite gives rise to 20–30 ciliary processes that contain odorant receptors (ORs). Mammalian ORs are seven-transmembrane receptors (7-TMRs), which are also referred to as G-protein coupled receptors (GPCRs). By contrast, insect ORs are heteromeric ligand-gated ion channels [2••,3••]. OR genes were first discovered in rodents by Buck and Axel in 1991 [1]. In the mouse, there are approximately 1400 predicted OR genes and pseudogenes, which are clustered at ∼40 loci throughout the genome [4]. Each OSN expresses only one functional OR gene in a mono-allelic manner, following the one neuron–one receptor rule (reviewed in [5]).

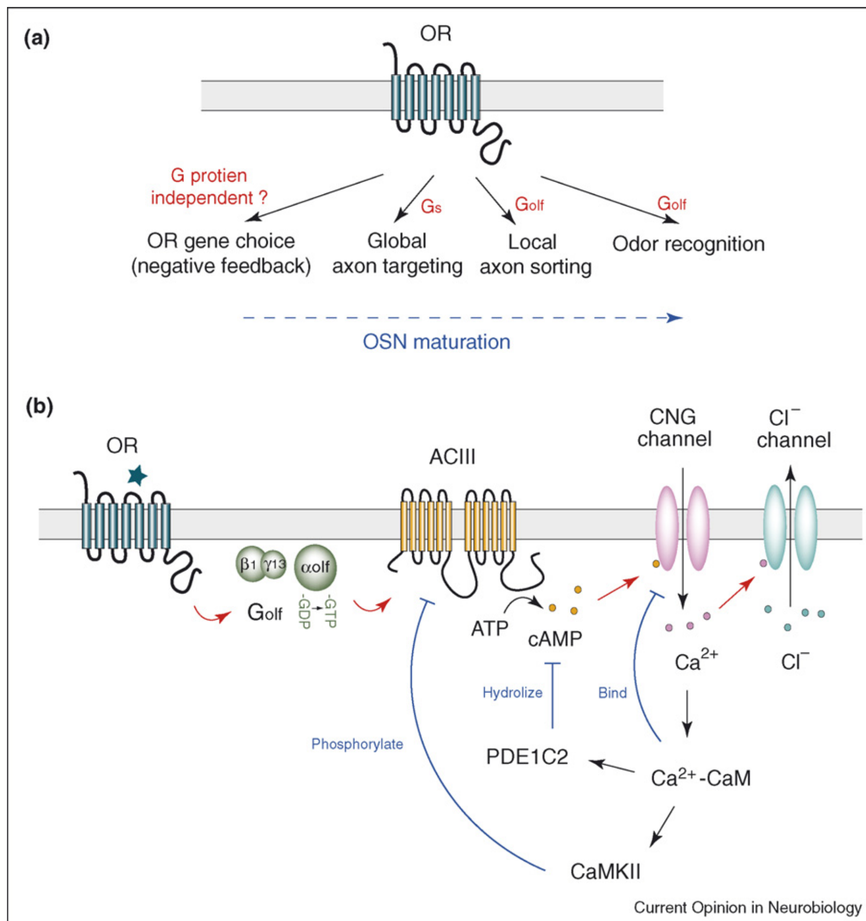
Each OSN extends a single unbranched axon to the olfactory bulb (OB), a part of the forebrain, where it forms synapses with second-order neurons, that is, mitral/tufted cells. OSNs expressing a given type of OR converge their axons in a specific pair of glomeruli in each OB, one on the lateral and one on the medial side of the OB. This is referred to as the one glomerulus–one receptor rule (reviewed in [6••]). Thus, the odor information detected in the OE is topographically represented as a set of activated glomeruli in an odor map in the OB (reviewed in [7]). Recent studies in mice demonstrated that fearful odors, such as those of predators (e.g. TMT), are detected by separate sets of ORs those dedicated for innate and those for memory-associated learning responses [8••]. These observations indicate that glomeruli have both chemical and behavioral specificities.

Since the discovery of OR genes, singular OR gene choice and OR-instructed axonal projection have been the most challenging issues in the study of the mammalian olfactory system. Recent studies revealed that the OR protein itself plays essential roles in regulating not only the choice of OR genes [5,9,10], but also the fasciculation and targeting of OSN axons [6••,11]. Thus, ORs are multifunctional receptors that mediate odor detection, OR gene choice, and axon guidance (Figure 1a). Here, we summarize the recent progress in the study of OR signaling in the mammalian main olfactory system and we discuss how ORs might differentially utilize OR-derived signals for different functions during the development. For a review of signaling by other mammalian chemosensory receptors, see [12,13].

OR protein localization

In mammals, ORs are one of the most abundant proteins in OSNs: OR mRNA accounts for ∼1% of total transcripts in mice [14••]. Localization of ORs to the cilia is essential for odor detection, because loss of olfactory cilia causes severe anosmia [15]. Antibody staining of OSNs revealed that ORs are present not only in the dendrites, cilia, and cell bodies, but also in axons [16,17]. The difficulty in expressing functional ORs in heterologous systems suggests that the transport of ORs to the plasma membrane requires escort/chaperone molecules. Matsunami and colleagues searched for molecules that enhance the cell-surface expression of ORs in HEK293 cells [18,19•]. They identified RTP1, RTP2, and REEP1, which are specifically expressed in OSNs, interact with ORs and facilitate the functional expression of ORs in HEK293 cells. The RTP proteins have since been useful in deorphanizing ORs using heterologous systems [20]. However, it is yet to be studied how RTP proteins function in the mammalian olfactory system. Studies in knockout animals will help elucidate their *in vivo* functions.

Figure 1



Signal transduction in the mouse olfactory system. **(a)** Multiple functions of mammalian ORs. In addition to odor recognition, ORs regulate the singular gene choice of OR and axonal projection at both global and local levels. **(b)** Canonical signal transduction pathway for odor recognition. cAMP plays a major role in odor recognition. Cation influx through CNG channel and chloride efflux through chloride channel together results in the depolarization of membrane potentials. Calcium influx leads to the desensitization by regulating at least three different molecules: CNG channel, ACIII, and PDE1C2. Stimulatory and inhibitory actions are indicated in red and blue arrows, respectively.

Like other 7-TMRs [21], glycosylation is essential for the cell-surface expression of ORs. Most ORs possess *N*-glycosylation sites (Asn-X-Ser/Thr) at their N-termini. For MOR-EG, in HEK293 cells, the disruption of the N-terminal glycosylation site abolishes cell-surface expression, whereas the addition of the N-terminal rhodopsin glycosylation site facilitates cell-surface expression [22]. It has also been reported that the disruption of the glycosylation site in OR-M71 perturbs OSN maturation *in vivo* [23].

Structural basis of odor recognition

Convincing evidence for ligand–OR interaction was first provided by Firestein and colleagues [24]. They infected the rat OE with recombinant adenovirus carrying OR-I7 and recorded the field potential in the OE (electro-olfactogram: EOG) during odorant exposure. OR-I7 recognizes octanal and related molecules with similar carbon chain lengths [24,25]. Although most ORs still remain as orphan receptors, ligands have been identified for a few dozen ORs to date. These studies, together with calcium imaging of OSNs, revealed the logic of odor coding in the main olfactory system. Unlike antigen receptors in the immune system, the ligand–receptor interaction is not strictly one to one. Each OR is broadly tuned to odorants and each odorant can be detected by a combination of ORs [26].

ORs are rhodopsin-like, type-A 7-TMRs and crystal structures of bovine rhodopsin [27] and human β2-adrenergic receptor (reviewed in [28•]) have provided insights for understanding the structural basis of odor recognition by ORs. On the basis of the three-dimensional structure of bovine rhodopsin, Touhara and colleagues reported a structural model for MOR-EG and predicted the amino acid residues that form a putative ligand-binding pocket [29••]. Site-directed mutagenesis revealed that nine amino

acids in TM3, TM5, and TM6 are essential for the recognition of the odorant ligand, eugenol. A similar study with MOR42-3 also revealed essential amino acid residues for ligand binding in TM3, TM5, and TM6 [30••]. 7-TMRs are assumed to have two different conformations, inactive and active, and are believed to spontaneously transit between the two [31]. This is probably why 7-TMRs possess basal signaling activity in the absence of ligands [31,32]. Although the inactive conformation is maintained by the intramolecular electrostatic interactions (e.g. 'ionic lock' between the cytoplasmic ends of TM3 and TM6), agonist binding disrupts such linkages and changes the conformation to an active form (reviewed in [31]). Odorants can function as competitive antagonists for some ORs, reducing their responses to agonists [33]. Thus, the response to odorant mixtures may not necessarily equal the sum of responses to individual ligands.

Although the precise mechanism of G-protein activation is poorly understood, it is known that the conserved Asn-Arg-Tyr (DRY) motif at the cytoplasmic end of TM3 is essential for G-protein activation. The DRY motif is found in most type-A 7-TMRs and is involved in the ionic interaction between TM3 and TM6. Mutations in the DRY motif often cause constitutive activity or abolish G-protein coupling [34]. Other intracellular domains of 7-TMRs are differentially involved in the binding of different types of G proteins [22,35].

G-protein activation and action potentials

ORs couple to heterotrimeric guanine nucleotide binding proteins, Gαs, Gαolf, and Gα15 *in vitro* [36]. Although various Gα genes are expressed in OSNs (e.g. Gαolf, Gαs, Gαo, Gαi2, Gα11, and Gα13) [37,38], it is well established that Gαolf plays a major role in detecting odorants *in vivo* [39]. Odor-activated ORs generate signals through Gαolf, converting GDP to GTP and releasing Gβ1γ13 dimer, with the aid of guanine nucleotide exchange factor, Ric8B [40]. The GTP-bound Gαolf, then stimulates adenylyl cyclase type III (ACIII), leading to the cAMP production [41]. cAMP binds and opens the calcium permeable, tetrameric cyclic nucleotide gated (CNG) channel, comprising 2-CNGA2, 1-CNGA4, and 1-CNGB1b subunits [42]. Mice deficient for CNGA2 are anosmic [43]. Although CNGA2 is essential for the function of the channel, CNGA4 and CNGB1b serve modulatory roles. Calcium influx through the CNG channel results in the opening of chloride channels, for example, mBest2 [44•], and the efflux of chloride. The Na^+–K^+–$2Cl^-$ cotransporter, NKCC1, is required for the chloride current [45•]. Together, the calcium influx and chloride efflux result in the depolarization of membrane potentials (Figure 1b).

Mechanisms of desensitization

Desensitization is an important feature of sensory systems in general. In the mouse olfactory system, calcium influx through the CNG channel is essential for the rapid desensitization of OSNs (reviewed in [46]). Calcium regulates the desensitization through at least three different molecules: CNG channel, ACIII, and phosphodiesterase, PDE1C2 (Figure 1b).

Calcium and calcium-binding protein, calmodulin (CaM), together, bind and desensitize the CNG channel. CNGA4 and CNGB1b play essential roles in the desensitization process [47–50]. ACIII is also negatively regulated by calcium. Ca^{2+}/CaM-dependent protein kinase II (CaMKII) phosphorylates ACIII and thereby reduces cAMP production [51]. Ca^{2+}/CaM-dependent phosphodiesterase (PDE1C2) is enriched in olfactory cilia, and hydrolyzes cAMP in a calcium-dependent manner [52]. Phosphorylation and β-arrestin-mediated internalization of ORs may also facilitate desensitization [53,54].

Noncanonical signaling for odor recognition

Genetic studies of Gαolf, ACIII, and CNGA2 strongly suggest that cAMP is a major second messenger in odor recognition [39,41,43]. Mice that are deficient in the CNGA2 subunit of the cAMP-gated CNG channel retain some electro-olfactogram (EOG) responses to some ligands [55]. Such residual responses may be contributed from atypical OSNs that express TRPM5 [56•] or guanylyl cyclase-D (GC-D) [57], or from microvillar cells (Jourdan cells) [58•]. TRPM5 is a calcium channel that appears to mediate some pheromone responses and is expressed in a subset of OSNs that also express CNGA2 [56•]. These OSNs project their axons to the ventral part of the OB. However, it has not been tested whether the residual responses seen in the CNGA2 knockout mouse was because of the TRPM5 signaling. In contrast to TRPM5-expressing neurons, GC-D neurons respond to peptide hormones [59••], CO_2 [14••], and perhaps some pheromones [55], and they do not express the components necessary for canonical cAMP signaling [60,61]. GC-D neurons project their axons to the so-called 'necklace glomeruli' that form near the posterior limits of the OB [59••,60,62]. Odorant-responsive microvillar cells appear to utilize the PLC-IP_3 pathway. Because they do not extend their processes to the OB [58•], it is possible that they do not function as sensory neurons.

Monogenic OR expression and negative feedback

In addition to the odor detection, mammalian ORs are involved in establishing the olfactory system during the development. Functional expression of ORs ensures the one neuron–one receptor rule by negative-feedback regulation. In the mouse, each OSN expresses only one functional OR gene in a mono-allelic manner. Monogenic OR expression appears to be maintained by the combination of both positive and negative regulations (reviewed in [5]). As for the positive regulation, a *cis*-acting locus control region (LCR) is proposed to activate

only one OR gene promoter at any time by the physical interaction [9]. Based on the 3C and FISH analyses, it was proposed that the 'H region', a 2.1-kb DNA region in the MOR28 gene cluster, acts not only in *cis* but also in *trans* and that a single *trans*-acting H enhancer may allow the stochastic activation of only one OR gene in each OSN [63]. However, more recent knockout studies of the H region did not support the *trans*-acting model [64•,65•]. The H region, thus, is an LCR-type *cis*-acting enhancer. Because there are more than 40 different OR gene clusters, each of which appears to contain its own LCR(s), the LCR model alone cannot account for the monogenic OR expression. Once a functional OR gene is expressed, further trials to activate other OR gene promoters must be halted. However, if an OR pseudogene is chosen first, attempts to activate another OR gene should be continued. In negative feedback, the functional OR protein itself appears to have an inhibitory role on the activation of other OR genes. The deletion of, or a frame-shift mutation within, the OR coding region allows the coexpression of an additional OR gene [9,10,66,67]. These observations suggest that the OR gene product, most probably the protein and not the mRNA, mediates the negative-feedback regulation. Recently, it was reported that the forced expression of ORs using heterologous promoters could suppress the expression of endogenous ORs [68•]. As the forced expression was found to be inefficient, the OR coding sequence was proposed to have an inhibitory effects on the transcriptional regulation. However, it is yet to be demonstrated that the 'suppressive' effect was because of the DNA sequence, and not the potentially toxic effects of OR protein overexpression. It will be important to identify the essential nucleotide sequences responsible for the suppressive effect. For monogenic OR expression, the negative selection mechanism has been considered [69]. A recent study revealed that the negative selection is a fail-safe mechanism that ensures the one neuron–one receptor rule [70•]. In young animals, a small percentage of OSNs happen to express multiple ORs. These neurons are eliminated in an activity-dependent manner [70•].

The involvement of G proteins in negative-feedback regulation was tested using transgenic mice expressing a mutant type OR, in which the DRY motif sequence, essential for G-protein signaling, was changed to RDY [71••]. This mutation completely abolished odorant-evoked calcium responses in OSNs, indicating the loss of G-protein signaling. However, OSNs expressing the mutant OR maintained the one neuron–one receptor rule. These results indicate that the OR-mediated negative feedback does not require G-protein signaling. This notion was also supported by other investigators using a similar DRY motif mutant of an OR [68•]. 7-TMRs often utilize G protein independent signaling pathways, such as those involving β-arrestin (reviewed in [72]). It should be noted that the DRY motif is dispensable for the β-arrestin-mediated 7-TMR signaling [73].

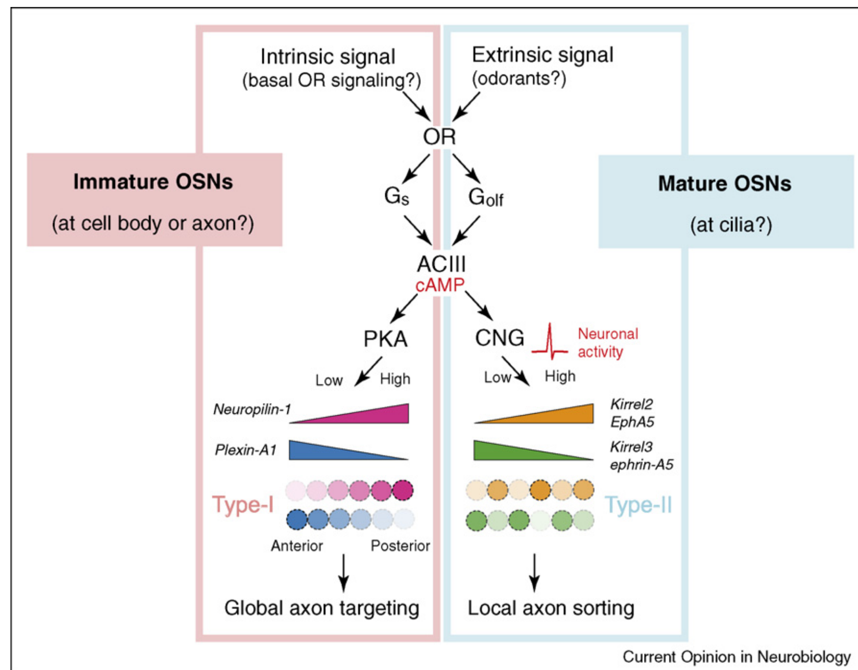
OR-instructed glomerular positioning

OSNs expressing a given OR converge their axons to a specific pair of glomeruli in stereotypical locations in the OB. The involvement of OR protein in OSN projection was elegantly demonstrated using genetically engineered mutant mice [11]. When the coding region of a particular OR gene was replaced with that of another OR gene, axons expressing the swapped OR gene targeted a novel position in the OB [11,74,75]. Thus, the OR protein was suggested to have an instructive role in projecting OSN axons. More recently, OR molecules were detected in axon termini by immunostaining with anti-OR antibodies [16,17]. On the basis of these observations, it has been suggested that the OR protein itself may recognize positional cues in the OB or mediate homophilic interaction of like axons. However, recent studies argue against these models. It has been reported that OR-derived cAMP signals direct axonal projection of OSNs through the transcriptional regulation of axon-guidance and axon-sorting molecules [71••,76••] (reviewed in [6••]).

To examine the role of G-protein signaling in the OR-specific convergence of OSN axons, the projection profile for OSNs expressing the DRY motif mutant of OR-I7 was analyzed [71••]. It was found that axons expressing the mutant OR-I7 remained in the anterior region of the OB and failed to converge to a specific site in the OB. Interestingly, coexpression of the constitutively active Gαs mutant restored the axonal convergence and glomerular formation defects. Partial rescue was also observed with the constitutively active mutants of PKA and CREB. Thus, Gαs-mediated and PKA-mediated transcriptional regulation appear to be involved in OSN projection. In this connection, it was reported that Gαs-coupled β2-adrenergic receptor could instruct OSN projection, whereas Gαi2-coupled vomeronasal receptor, V1rb2, cannot [23]. Furthermore, it was found that constitutively active Gαs or dominant-negative PKA, when expressed with wild-type OR-I7, causes a posterior or anterior shift of glomeruli, respectively [71••]. These findings suggest that the levels of OR-derived cAMP signals determine the anterior–posterior (A–P) positioning of glomeruli in the OB (Figure 2, left).

cAMP regulates the transcription of some axon-guidance molecules, for example, Neuropilin-1 in a positive manner and Plexin-A1 negatively. When protein levels are measured in axon termini of OSNs, Neuropilin-1 is found in an anterior-low and posterior-high gradient in the OB [71••,77,78], whereas Plexin-A1 is found as a counter gradient ([79]; Takeuchi *et al.*, unpublished). Increases or decreases of Neuropilin-1 cause posterior or anterior glomerular shifts, respectively (T.I. *et al.*, unpublished data). Furthermore, the A–P topography of the glomerular map is perturbed in mice deficient for Neuropilin-1 (Takeuchi *et al.*, unpublished) or its repulsive ligand, Sema3A [78,80].

Figure 2



Different utilization of cAMP signals for global versus local axon guidance. Type-I molecules are expressed at OSN axon termini, forming an anteroposterior gradient. In contrast, type-II molecules are expressed in a mosaic manner. Although both are regulated by cAMP signals, type-I appears to be regulated by intrinsic mechanisms (e.g. constitutive signaling through ORs), whereas type-II is affected by extrinsic sources (e.g. environmental odorants). The intrinsic and extrinsic signals are independently processed within OSNs through different signaling molecules (Gαs versus Gαolf and PKA versus CNG channel) at different maturational stages (immature versus mature OSNs).

Neuronal activity-dependent axon sorting

In addition to the global positioning of glomeruli in the OB, ORs also play a crucial role in sorting axons locally. The CNGA2 gene is X-linked and therefore, the female heterozygotes for the knockout of this gene are mosaic due to random X-inactivation. In these mice, glomeruli for some ORs are duplicated: one is CNGA2-positive and the other is CNGA2-negative [76••,81]. These observations indicate that neuronal activity affects the local sorting of OSN axons. To study how OR-instructed axonal fasciculation is regulated, Serizawa and colleagues screened the genes, whose expression profiles are correlated with the expressed OR species, [76••]. By comparing the OE expression profile of a wild-type mouse to that of the transgenic mouse in which the majority of OSNs express a particular OR, they identified genes such as those coding for homophilic adhesive molecules Kirrel2/Kirrel3 and repulsive molecules ephrin-A5/EphA5. In the CNGA2 knockout mouse, *Kirrel2* and *EphA5* were downregulated, while *Kirrel3* and *ephrin-A5* were upregulated, indicating that these genes are transcribed in an activity-dependent manner. Transgenic mosaic analysis demonstrated that the gain of function of these genes causes the duplication of glomeruli for some ORs [76••,82]. In the axon termini of OSNs, these molecules are expressed at differential levels in an OR-specific manner, exhibiting a mosaic pattern of glomeruli with different expression levels (Figure 2, right).

It has been reported that the dorsal–ventral (D–V) arrangement of glomeruli is roughly determined by the anatomical locations of OSNs in the OE [83]. This positional information appears to be represented by expression levels of guidance molecules, for example, Neuropilin 2, and Robo-2, forming a gradient or a counter gradient along the D–V axis [84,85•]. As described earlier, axon-guidance molecules, whose expression levels are uniquely determined by OR-derived cAMP levels, regulate the positioning of glomeruli along the A–P axis [71••]. After axons are guided to approximate destinations in the OB, the axon termini are sorted on the basis of the expressed OR species. This OR-specific sorting is mediated by the expression levels of multiple sets of adhesive/repulsive molecules that are regulated in an activity-dependent manner [76••].

Two distinct cAMP signals for OSN projection

Axon-guidance/axon-sorting molecules regulated by ORs can be categorized into two different types. One is type-I, which includes Neuropilin-1 and Plexin-A1, and is expressed at axon termini in a graded manner along the A–P axis in the OB [71••]. The other is type-II, which includes Kirrel2 and Kirrel3, and is expressed at axon

termini showing a mosaic pattern in the glomerular map [76••] (Figure 2). In the ACIII-deficient mouse, Neuropilin-1 and Kirrel2 are downregulated, whereas Plexin-A1 and Kirrel3 are upregulated, suggesting that both type-I and type-II genes are regulated by the same second messenger, cAMP ([86••]; Takeuchi *et al.*, unpublished). Similar results were obtained with the olfactory-specific Gαs/Gαolf-double knockout mouse where the OR-mediated cAMP production is blocked (T.I. *et al.*, unpublished). Indeed, in these mutant mice, both A–P positioning and OR-specific axonal segregation were perturbed ([86••,87••,88••]; T.I. *et al.*, unpublished). How is it, then, that the same second messenger, cAMP, is capable of regulating two types of genes in different ways?

cAMP signals for type-I and type-II genes appear to be derived from distinct sources. Naris occlusion affects the expression of type-II, but not of type-I. It is possible that the type-II genes are regulated by extrinsic signals like environmental odorants, whereas type-I genes are regulated by intrinsic signals such as endogenous ligands or by ligand-independent basal signaling by ORs (Figure 2). These differences may be, at least partly, because of the subcellular localization of ORs, namely, cilia versus axons.

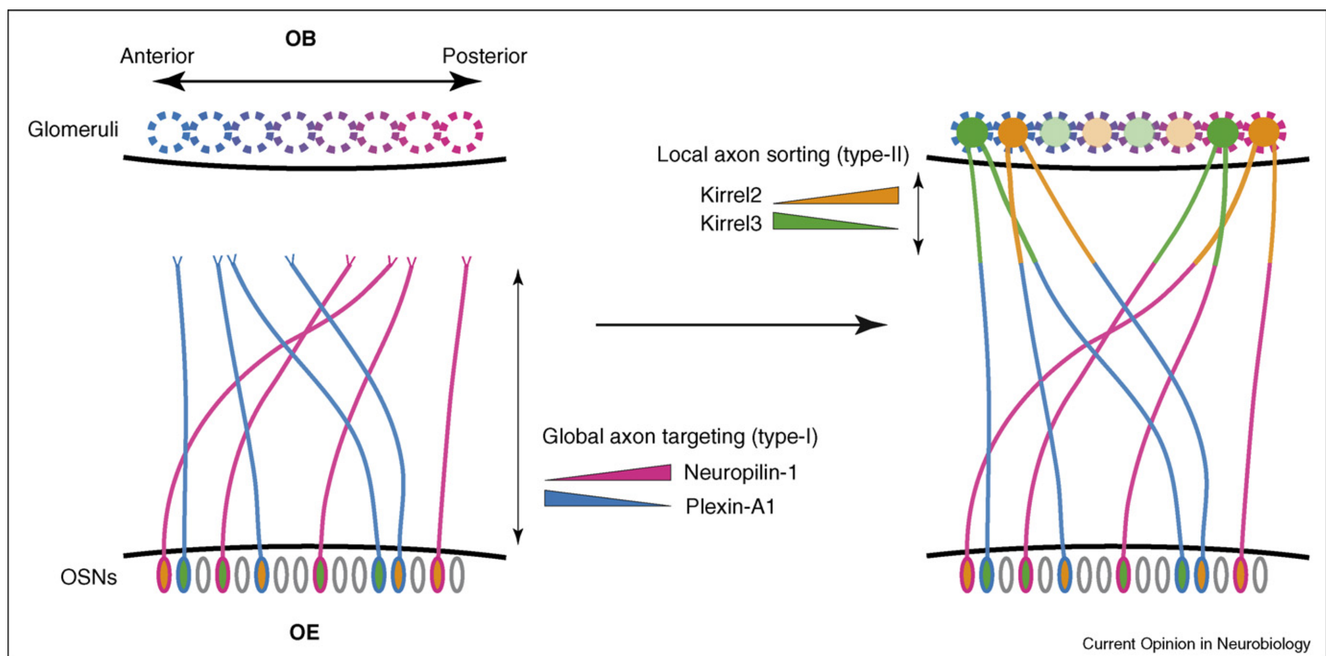
How are the cAMP signals independently processed within the OSNs for type-I and for type-II genes? It is possible that type-I and type-II genes are differentially regulated by cAMP signals at distinct stages of OSN maturation. Both Gαs and Gαolf are assumed to activate ACIII; however, expression profiles of these Gα proteins are quite different: Gαs is predominantly expressed in immature OSNs, whereas Gαolf is in mature OSNs [37,87••]. Expression of type-I genes is affected in the olfactory-specific Gαs knockout, whereas the type-II expression is affected in the Gαolf knockout (T.I. *et al.*, unpublished). These observations indicate that intrinsic cAMP signals regulate type-I genes in immature OSNs, whereas extrinsic cAMP signals regulate type-II genes in mature OSNs (Figure 2). Downstream signaling components also differ for the expression of type-I and type-II genes (Nakashima *et al.*, unpublished). Type-I genes, but not type-II genes, are affected by dominant-negative PKA. By contrast, CNGA2 deficiency affects type-II, but not type-I (Figure 2).

A recent study by Yoshihara and colleagues demonstrated that BIG2 is involved in the local sorting of OSN axons [89•]. BIG2 is expressed at axon termini of OSNs in an OR-specific manner and is affected by CNGA2 deficiency. Interestingly, however, BIG2 expression levels are not correlated with those of Kirrel2 and ephrin-A5. It is possible that BIG2 is regulated by yet another OR-dependent signaling mechanism to further diversify the neuronal identity code of sorting molecules.

Static versus dynamic signals of cAMP

Although the expression of both type-I and type-II molecules is cAMP-dependent, only type-II, but not type-I,

Figure 3



Step-wise model for OSN projection. Axonal projection of OSNs may occur in step-wise fashion. Axons are first guided to approximate anterior–posterior destinations with type-I molecules, and are further sorted locally with type-II molecules. This schema was modified from the 'hierarchical model' previously proposed by Key and St John [92].

is susceptible to odor exposure. Expression levels of type-I genes, determined by the intrinsic and static signals of cAMP, play an important role in establishing and maintaining the topography of the glomerular map. For example, expression level of Neuropilin-1 is an important determinant of A–P positioning (T.I. *et al.*, unpublished). Changes in the expression levels of type-I genes during the development would disrupt the A–P topography of the glomerular map. In contrast, temporal changes in the type-II gene expression, mainly caused by the environmental odorants, do not seem to perturb the glomerular map. Type-II molecules appear to mediate the local sorting of OSN axons via self/nonself recognition based on the relative differences of neuronal activities: activity-high and activity-low axons are segregated [76••]. In such a scenario, temporal changes in type-II gene expression should not disturb the self/nonself sorting of axons, as long as they occur in a concerted manner and are correlated with the OR species.

Step-wise regulation of OSN projection

Because type-I and type-II genes are seemingly independently regulated in immature and mature OSNs, respectively, the projection of OSN axons might occur in a step-wise fashion during the development (Figure 3). In OSN projection, axons are first guided to approximate destinations in the OB with type-I molecules and are later sorted locally with type-II molecules. It has been reported that, in the *Xenopus* olfactory system, axons expressing Neuropilin-1 and those expressing Plexin-A1 are sorted from each other within the axon bundles before they reach the OB [79]. A similar observation has also been made in mice [71••]. In contrast, the sorting Kirrel2-positive and Kirrel3-positive axons only occur after the arrival of OSN axons to the OB [76••]. Thus, axonal sorting probably occurs at two different stages of OSN projection in a step-wise manner: first in axon bundles with type-I and then in the glomerular layer with type-II molecules (Figure 3). The step-wise regulation of axon guidance appears to be a general feature of neural map formation, and is a powerful strategy for robust map formation (reviewed in [90,91•]).

Concluding remarks

In mammals, ORs are multifunctional signaling molecules, involved in odorant recognition, singular OR gene choice, and OSN projection at both global and local levels. For axonal projection, OR-derived cAMP signals regulate two distinct sets of axon-guidance/axon-sorting molecules in different ways at the transcriptional level. Recent studies indicate that in immature OSNs, intrinsic OR signals regulate type-I genes for global axon targeting, whereas in mature OSNs, extrinsic signals regulate type-II genes for local axonal sorting. In the mammalian nervous system, general second messengers, for example, cAMP, IP_3, and Ca^{2+}, often regulate the diverse neuronal functions through the transcriptional regulation. However, the precise molecular mechanism that converts the signaling specificity to the molecular code is poorly understood. The olfactory system will continue to provide insightful information as to how second messengers establish the neuronal identity that is represented by the combinatorial code of axon-guidance/axon-sorting molecules in axon termini.

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