Review series

The cell biology of smell

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The olfactory system detects and discriminates myriad chemical structures across a wide range of concentrations. To meet this task, the system utilizes a large family of G protein–coupled receptors—the odorant receptors—which are the chemical sensors underlying the perception of smell. Interestingly, the odorant receptors are also involved in a number of developmental decisions, including the regulation of their own expression and the patterning of the olfactory sensory neurons' synaptic connections in the brain. This review will focus on the diverse roles of the odorant receptor in the function and development of the olfactory system.

Introduction

A major challenge of sensory neurobiology—and neuroscience in general—is to understand the pathways that bridge an external stimulus to its representation in the brain as a percept, and how such pathways ultimately drive behaviors. The first step of this process involves the primary sensory neurons, which detect physical stimuli through a variety of highly specialized receptors. The perception of a smell begins with the activation of receptors expressed by the olfactory sensory neurons (OSNs), which reside in the main olfactory epithelium lining the nasal cavity (Fig. 1 A). Each OSN extends a single dendrite to the lumenal surface of the epithelium, from which immotile cilia extend to catch inhaled odorants from the air. These olfactory sensory cilia are enriched in the odorant receptors and other signaling components that mediate the initial transduction events in the cell (Firestein, 2001). At the other end of the neuron, a single, unbranched axon projects to the olfactory bulb, a specialization of the forebrain that serves as the first relay station in this neural pathway. The axons of OSNs in the periphery together comprise the olfactory nerve. Once the axons reach the olfactory bulb, they make synapses with the dendrites of projection neurons, within discrete structures known as glomeruli. In the mouse, there are 5–10 million OSNs in the olfactory epithelium and ~1,800 glomeruli in each olfactory bulb, which translates to an $\sim 10^3$-fold convergence of primary sensory axons onto each olfactory glomerulus (Firestein, 2001). This convergence lies at the heart of the coding strategy for olfactory sensory information. In parallel to the main olfactory epithelium, the vomeronasal organ—an anatomical specialization of the nose in terrestrial vertebrates that is separate from the main olfactory epithelium—senses nonvolatile chemical stimuli, including pheromones (Dulac and Torello, 2003; Mombaerts, 2004). The present review focuses on the main olfactory epithelium and the multiple roles that the "OR" family of odorant receptors play, not only as detectors of volatile chemicals in the environment, but also as regulators of key developmental decisions made by differentiating OSNs.

The nose, its receptors, and connections to the brain

A large multigene family of olfactory-specific G protein–coupled receptors (GPCRs) was initially identified in the rat (Buck and Axel, 1991) and belongs to what is now referred to as the OR family of odorant receptors (Mombaerts, 2004). The predicted structure of these receptors exhibits a seven-transmembrane domain topology, and their sequences place them in the rhodopsin class of GPCRs. The size of the OR gene family in mammals is extremely large and ranges from ~700 genes in humans (about half of which are functional) to over 1,200 genes in rodents (about two-thirds of which are functional; Mombaerts, 2004; Nei et al., 2008). In the fish, the size of the OR repertoire appears to be much smaller, containing only 40–140 intact genes depending on the species (Alioto and Ngai, 2005; Niimura and Nei, 2005). The ORs exhibit extensive sequence diversity within their transmembrane domains—the presumed sites of ligand binding in this class of GPCR. Thus, the OR family has evolved to detect a wide range of chemical structures present in the animal's environment. In addition to the ORs, members of the much smaller trace amine–associated receptor (TAAR) family are expressed in OSNs of the main olfactory epithelium and are thought to mediate the reception of amine cues (Liberles and Buck, 2006). Finally, the neurons of the vomeronasal epithelium express receptors from three unrelated GPCR families, the V1R,

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Abbreviations used in this paper: CNG, cyclic nucleotide gated; OSN, olfactory sensory neuron.

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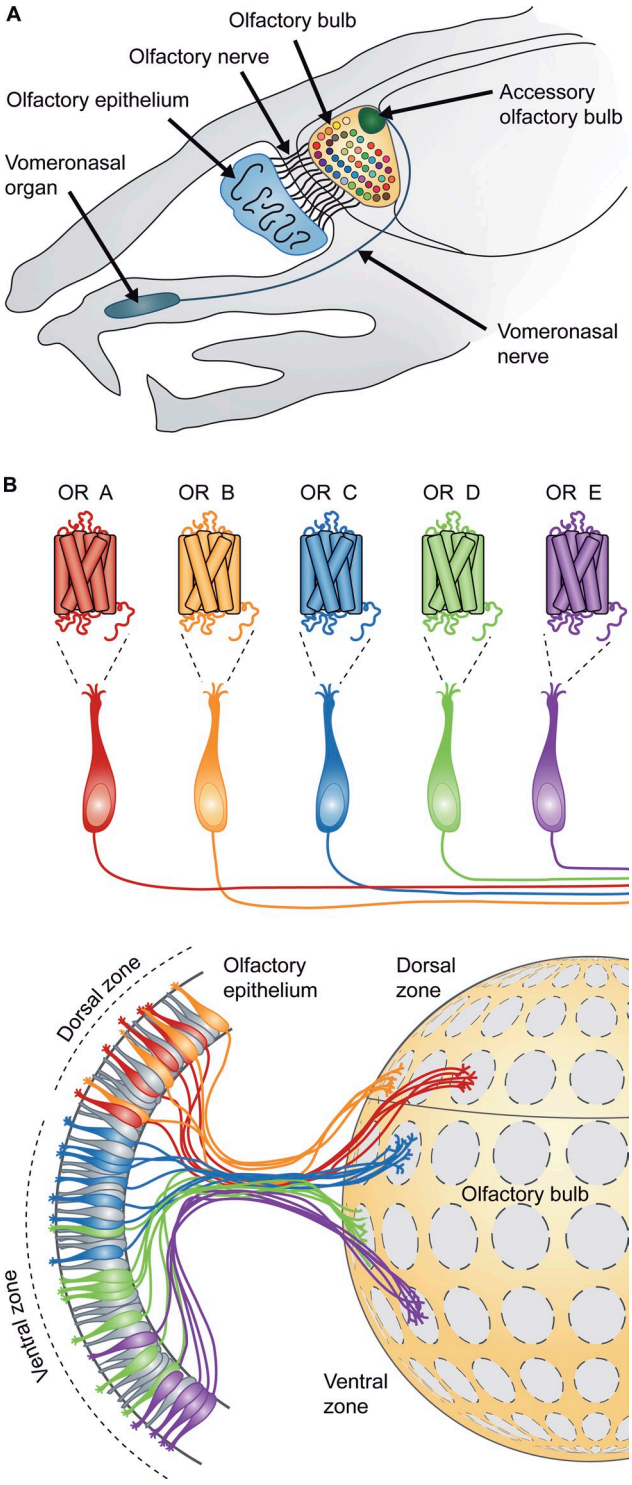

Figure 1. **Anatomy of the rodent peripheral olfactory system.** (A) Schematic representation of a parasagittal section through adult mouse head. Axons of the OSNs in the main olfactory epithelium comprise the olfactory nerve and innervate the olfactory bulb. Vomeronasal sensory neurons project their axons via a separate tract, the vomeronasal nerve, to innervate the accessory olfactory bulb. (B) Each OSN of the main olfactory epithelium expresses only one odorant receptor gene (OR A, OR B, OR C, etc.) out of a repertoire of over 1,000 genes. Neurons expressing a given OR are organized into broad zones along the dorsal–ventral axis of the olfactory epithelium (OE) and converge to a common glomerulus at corresponding dorsal–ventral zones in the olfactory bulb (OB). Each glomerulus thus receives innervation from sensory neurons expressing a single odorant receptor, providing the anatomical basis of the olfactory sensory map.

V2R, and formyl peptide-like receptors (Mombaerts, 2004; Yang et al., 2005; Rivière et al., 2009).

Upon binding its cognate odor ligand, the activated OR (and presumably also TAAR) couples through Gαolf, a Gαs isoform enriched in OSNs (Belluscio et al., 1998). Activated Gαolf in turn activates type III adenylyl cyclase (Wong et al., 2000), which catalyzes the production of cAMP. The increase in intracellular cAMP "gates" or opens a cyclic nucleotide-gated (CNG) channel, leading to an influx of sodium and calcium ions and depolarization of the neuron (Brunet et al., 1996). This initial depolarization is further amplified by the subsequent activation of calcium-activated chloride channels and, owing to the low concentration of extracellular Cl^- in the mucus bathing the olfactory cilia, the efflux of Cl^- from the cell (Stephan et al., 2009; Fig. 2 A). The odor-induced depolarization in the olfactory cilia spreads throughout the neuron, resulting in the opening of voltage-sensitive ion channels in the sensory neuron's axon hillock, the firing of action potentials, and the release of neurotransmitter at the synaptic terminal in the olfactory bulb.

To determine the identity of an odorant stimulus, the nervous system must discern which of the ∼1,000 ORs has been activated. Two organizing principles of the peripheral olfactory system underlie the encoding of this information. First, each OSN in the olfactory epithelium expresses just one allele of a single OR (Chess et al., 1994; Serizawa et al., 2003; Lewcock and Reed, 2004; Fig. 1 B)—a phenomenon referred to as the "one receptor, one neuron" rule. In this way, the spectrum of odorous compounds to which an individual OSN can respond (its "receptive field") is a direct function of the ligand-tuning properties of its singularly expressed OR. How, then, is activity from a particular class of OSNs (here defined by the OR that it expresses) distinguished from activity of all the other OSN classes, and by extension, from activity of other ORs? This second level of processing relies upon the projection of axons from OSNs expressing the same OR to common glomeruli in the olfactory bulb (Fig. 1 B). Interestingly, the projection of OSNs to the olfactory bulb comprises a discontinuous map; neurons expressing a given OR, although distributed broadly in the peripheral sensory epithelium (Ressler et al., 1993; Vassar et al., 1993), converge to discrete glomeruli in the olfactory bulb in a spatially invariant pattern (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Mori et al., 1999). This sensory map displays a mirror symmetry, such that OR-specific neurons typically innervate one glomerulus in the olfactory bulb's medial hemisphere and another glomerulus in the lateral hemisphere (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Nagao et al., 2000). The dendrites of the projection neurons of the olfactory bulb—the mitral and tufted cells—in turn innervate the glomeruli and carry this information via their axons to the olfactory cortex. The spatial patterns of activity elicited in the olfactory bulb appear to be represented not as a corresponding spatial map in the olfactory cortex, but rather in a sparse and distributed manner at this level (Poo and Isaacson, 2009; Stettler and Axel, 2009).

The odorant receptor: chemical sensor par excellence

Since their discovery in 1991, the ORs have been widely assumed to comprise a family of odorant receptors based on their

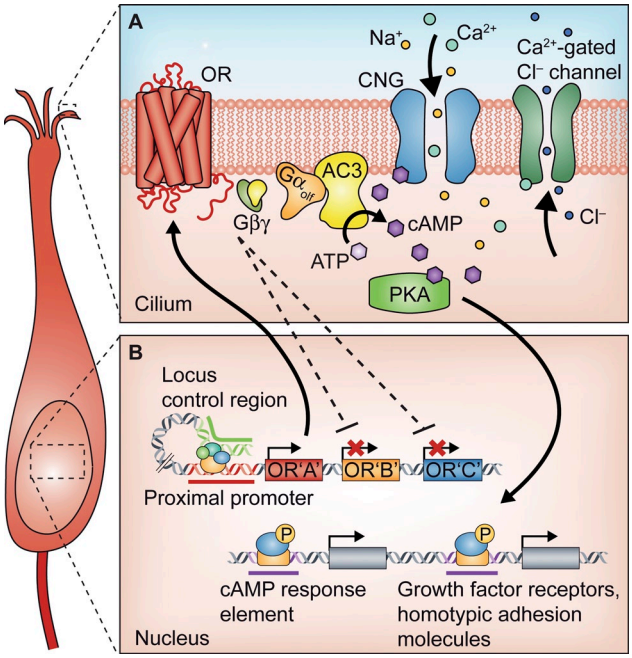

Figure 2. **Signal transduction in the OSN.** (A) Representation of the receptors, enzymes, and ion channels—present in the olfactory cilia—that transduce activity of the odorant receptor (OR) into changes in membrane potential and gene expression. Binding of an odorant to its cognate OR results in the activation of heterotrimeric G protein (Gαolf plus Gβγ). Activated Gαolf in turn activates type III adenylyl cyclase (AC3), leading to the production of cyclic AMP (cAMP) from ATP. cAMP gates or opens the cyclic nucleotide-gated (CNG) ion channel, leading to the influx of Na^+ and Ca^{2+}, depolarizing the cell. This initial depolarization is amplified through the activation of a Ca^{2+}-dependent Cl^- channel. In addition, cAMP activates protein kinase A (PKA), which can regulate other intracellular events, including transcription of cAMP-regulated genes. (B) Events in the nucleus of OSNs important for establishing and maintaining sensory neuron identity. Selection of a particular OR gene by the cell is thought to occur via interaction of a cis-regulatory locus control region with the proximal promoter of a single OR gene within a cluster of OR genes. This choice is stabilized—and the expression from all other OR genes in the genome is silenced—by an OR-dependent feedback loop, which ensures the expression of a single OR per sensory neuron. The mechanism underlying OR-mediated, OR gene silencing is at present not understood. OR-mediated activity also leads to transcriptional regulation of cAMP response element binding protein (CREB)–dependent gene expression via CREB's phosphorylation by PKA.

numbers, sequence diversity, and patterns of expression in OSNs. Although work over the last two decades has unequivocally supported this view, finding the ligands for these receptors (a process often referred to as "de-orphaning") has been surprisingly difficult. A major impediment to identifying ligands for these chemosensory receptors has been the difficulty in obtaining cell surface expression of cloned receptors in heterologous cells (Touhara, 2007). One approach—obviating the problems found in heterologous cell expression—has been to study the properties of virally transduced or even endogenous ORs in OSNs in vivo, thereby allowing them to be tested in their native cellular environment (Zhao et al., 1998; Malnic et al., 1999; Touhara et al., 1999) and providing a rich source of information on OR ligand tuning properties. Indeed, the first OR to be functionally characterized was de-orphaned by virally transduced expression in vivo (Zhao et al., 1998). However, such approaches are cumbersome and preclude the ability to screen in a high-throughput fashion a large array of receptors against a similarly large panel of candidate odorants—arguably a necessity if we are to understand how this family of ~1,000 receptors is used to discriminate among the vast range of chemicals in odor space. One approach to achieve surface expression of ORs in cultured cells utilizes receptor chimeras in which an N-terminal peptide from rhodopsin is fused to the OR N terminus, facilitating cell surface expression and characterization of some (but not all) ORs tested in heterologous cells (Krautwurst et al., 1998). A more effective and generally applicable solution was found by Matsunami and colleagues, who found that coexpression of ORs with the olfactory-specific chaperones RTP1 and REEP allows a large number of ORs to be functionally expressed in heterologous cells (Saito et al., 2004, 2009; Matsunami et al., 2009). Together, these various strategies have yielded insights into the specificities of individual ORs, with several principles emerging. For example, while showing a preference for compounds with certain molecular features (e.g., *n*-aliphatic aldehydes over alcohols), an individual OR can be broadly tuned for other characteristics, such as carbon chain length (Zhao et al., 1998; Araneda et al., 2000; Kajiya et al., 2001; Abaffy et al., 2006; Repicky and Luetje, 2009; Saito et al., 2009). These observations provide the molecular underpinnings of a combinatorial code in which the identity of an individual odorant is encoded by the particular subset of receptors that it activates (Fig. 3). Variations in the ligand's structural features (e.g., in carbon chain length, or the nature of a functional group) would elicit activity in a different subset of receptors and give rise to the perception of a different smell (Repicky and Luetje, 2009; Saito et al., 2009). Given the combinatorial nature of the code and the large number of different ORs found in a given species, the number of different perceived odors is vast.

The ability to characterize the functional properties of ORs has allowed interesting insights into the mechanisms of human olfaction. For example, it has long been known that some humans can detect the steroid androstenone (a compound found in human sweat), whereas others cannot. The androstenone "detectors" can be further divided, with one group describing the odor as musky and even pleasant and the other finding it distinctly unpleasant, akin to the smell of sweaty socks. What underlies these differences in human perception? A solution was found through an elegant combination of human genetics and functional characterization of specific OR genes (Keller et al., 2007). Two single-nucleotide polymorphisms (SNPs) associated with androstenone nondetectors were identified within the coding sequences of an OR gene. Functional expression in heterologous cells showed that the OR is activated by androstenone, whereas the double-SNP variant is not. The detection and perception of androstenone can be predicted based on this polymorphic OR: individuals homozygous for the wild-type allele tend to perceive androstenone as unpleasant, whereas those possessing one or no functional alleles perceive androstenone as less unpleasant or undetectable (Keller et al., 2007). These results highlight the role that a single OR receptor can play in human olfactory perception.

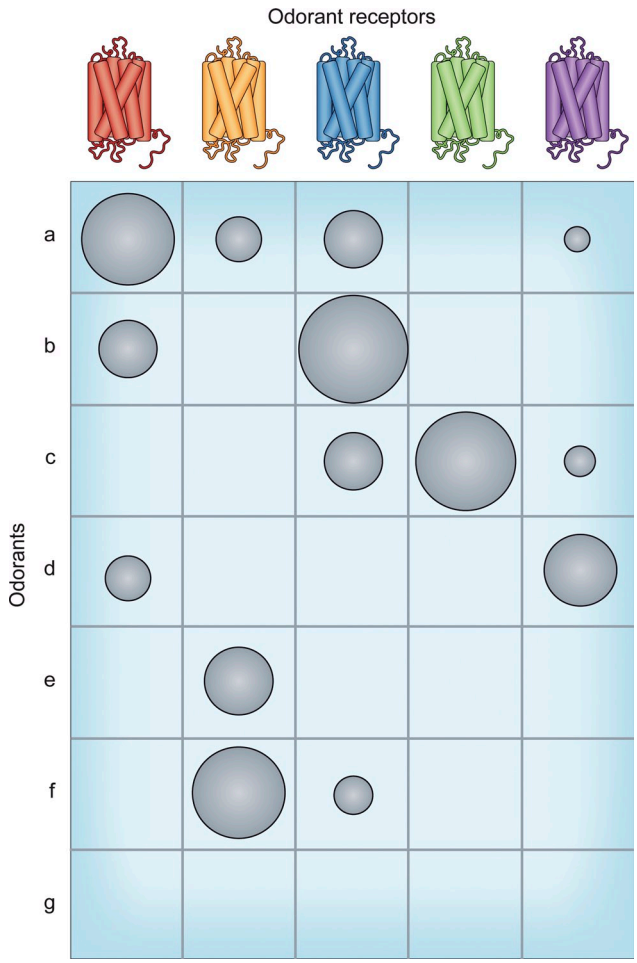

Figure 3. **Combinatorial coding of olfactory information.** Graphic representation of the olfactory receptor combinatorial code. In this hypothetical example, the responses of five odorant receptors to seven odorants (a–g) are shown, with the magnitudes of responses proportional to the sizes of the circles. Reflecting functional studies on individual odorant receptors, some receptors are more narrowly tuned than others, and individual odorants can activate different subsets (and numbers) of receptors. The pattern of receptor activation elicited by a particular compound is thought to represent that compound's chemical identity.

Odorant receptor gene expression: the problem of choosing one and forsaking all others

During development, each differentiating OSN must select and express a single receptor at the exclusion of all other receptors in the genome. How is this singularity in OR gene expression initially established and subsequently maintained over the lifetime of the cell? Several models have been advanced to explain how a single OR gene is expressed in each OSN. One idea posits somatic DNA rearrangements as a way of irreversibly selecting a single OR gene for expression while simultaneously repressing other genes in the repertoire. For example, DNA recombination events may be required to place the coding sequence adjacent to a promoter sequence or into a transcriptionally active locus, similar to the selection of a single antigen receptor gene via V(D)J recombination by cells in the immune system or antigenic variation of surface coat proteins in trypanosomes (Borst, 2002). If this were the case, mice cloned from an OSN in which the OR gene choice had already been made would show a grossly perturbed pattern of OR gene expression because all cells of the animal would have inherited the structural rearrangement that led to expression of that one OR allele. This idea was ruled out by the observation that mice cloned from a single, mature OSN in fact express the full complement of ORs with no apparent perturbations (Eggan et al., 2004; Li et al., 2004). Thus, the mechanism by which an OSN chooses an OR gene to express does not involve irreversible structural changes in genomic DNA.

How, then, is an OR gene selected for expression? Given that OR genes are found in clusters on multiple chromosomes (Mombaerts, 2004; Niimura and Nei, 2005), perhaps local sequence elements regulate the expression of genes within each cluster. In one model, a locus control region (LCR) governs OR gene expression as part of a hierarchy of cis-acting regulatory elements. In support of this model, an ∼2-kb sequence residing 75 kb upstream of a cluster of OR genes on mouse chromosome 14—termed the "H" region because of its homology to a similar sequence in the human genome—was shown to function as an enhancer of OR gene expression in transgenic mice made with yeast artificial chromosome (YAC) constructs with inserts sufficiently large to contain the H region together with the proximal OR gene sequences themselves (Serizawa et al., 2003). The H region has the hallmarks of an enhancer: moving it closer to an OR gene increases the number of cells expressing that gene, and deletion of the H region from the YAC transgene results in highly reduced expression of transgenic ORs (Serizawa et al., 2003). Cis-acting enhancers have also been identified in and around OR gene clusters in the zebrafish genome (Nishizumi et al., 2007); although these elements show no obvious sequence similarity to the mammalian H region, their presence supports the notion that each OR gene cluster is regulated by one or more LCR. Interestingly, only a single OR is expressed per cell from an H region–containing YAC transgene carrying a cluster of OR genes (Serizawa et al., 2003). Similar to the mechanism used to assure the expression of a single red or green opsin gene in the cone photoreceptor cell (Cook and Desplan, 2001), LCRs associated with OR clusters could account for the initial selection of a single OR gene by the OSN via a stable intrachromosomal interaction between the H region and the chosen OR's proximal promoter (Serizawa et al., 2003; Fig. 2 B). In an intriguing variation of this model, it was suggested that the H region might function as a master regulator of all OR genes in the genome, serving as both a cis- and trans-chromosomal regulatory element (Lomvardas et al., 2006). Indeed, experiments using chromosome conformation capture demonstrated physical associations between the H region and OR genes on other chromosomes (Lomvardas et al., 2006). This was a tantalizing observation, as an exclusive association of a single OR gene's proximal promoter with one allele's H region could help explain singularity of OR expression. However, subsequent studies showed that genetic ablation of the H region resulted in the loss of expression of only the most proximal OR genes in the neighboring OR gene cluster on chromosome 14, with normal expression of more distant genes in the cluster, as well as genes on other chromosomes (Fuss et al., 2007; Nishizumi et al., 2007). Thus, it is

likely that the H region—and similar sequences found within other OR gene clusters—act in cis to regulate the expression of OR genes in their immediate chromosomal vicinity. In a hierarchical mechanism, the selection of a single OR by a cell could come about through the initial restriction to a single LCR, which in turn stochastically makes a stable interaction with a single OR gene within its associated cluster.

The odorant receptor: enforcer of the "one receptor, one neuron" rule

Once an OR has been chosen by an OSN, it is stably expressed for the lifetime of the cell, at the exclusion of all other OR genes in the genome. What is the mechanism behind this exquisite mode of gene regulation? An important clue came from the observation that individual sensory neurons can in rare instances sequentially express multiple OR genes, with such "gene switching" events occurring more frequently when the initial OR gene expressed by the cell is a pseudogene (Serizawa et al., 2003; Lewcock and Reed, 2004; Shykind et al., 2004). These observations support a model in which a functional OR protein, once selected and expressed, silences the expression of other OR genes in the genome (Serizawa et al., 2004; Shykind, 2005). In a negative feedback mechanism, OR-dependent gene silencing prevents gene switching and ensures the stable expression of a single OR in each OSN (Fig. 2 B). There are two obvious consequences to this arrangement. First, if an OR pseudogene is initially selected for expression, the generation of a functional OSN is ensured by switching to another OR gene—an important quality control/quality assurance mechanism considering that a significant fraction of the OR gene repertoire comprises pseudogenes (25–30% in rodents and ∼50% in humans; Mombaerts, 2004; Niimura and Nei, 2005). Second, once a functional OR is selected for expression, gene silencing safeguards the functional identity of the OSN over the lifetime of the cell.

What are the intracellular signaling mechanisms mediating OR-dependent gene silencing? Several lines of evidence suggest that this feedback mechanism does not depend on OR activity (Imai et al., 2006). First, expression of multiple ORs per cell is repressed by transgenic expression of a mutant OR in which a conserved receptor activation motif is mutated, rendering the receptor unresponsive to odorant stimulation (Imai et al., 2006). Second, a constitutively active Gαs mutant (which is expected to bypass or substitute for an active receptor) expressed in lieu of an intact OR fails to suppress the expression of other OR genes (Imai et al., 2006). These observations leave us with a bit of a puzzle: the OR is required for silencing, but this process does not appear to operate through the expected G protein signaling pathway. Perhaps other, noncanonical, G protein–independent mechanisms are at play. It is also possible that the inactivated mutant receptor used in the above-mentioned study (Imai et al., 2006) retains a level of intrinsic activity—reflecting the receptor's equilibrium between inactive and active states in the absence of bound agonist (Rosenbaum et al., 2009)—to repress expression from other OR gene loci. Accordingly, OR-mediated gene silencing may well depend on intrinsic OR activity, which could be transduced not through Gαolf/cAMP-mediated signaling, but via Gβγ signaling, the other arm of the

Glossary

Axon growth cone. The leading, motile structure of a neuron's growing axon; the growth cone senses gradients of axon guidance cues via transmembrane receptors expressed on the plasma membrane. These interactions can cause local changes in actin polymerization and depolymerization, resulting in directional changes in the growth cone trajectory.

Axon guidance cue. A secreted, cell surface, or extracellular matrix protein that influences the growth of extending axons toward (attractive) or away from (repulsive) the source of the cue. These cues, which include proteins such as the semaphorins, netrins, ephrins, and slits, function by interacting with their cognate receptors on the axon growth cone.

Glomerulus. Functional unit of the olfactory bulb that segregates and organizes the synaptic inputs from the OSNs with their partner output neurons (the mitral cells), which in turn carry information from the olfactory bulb to higher cortical centers.

GPCR. G protein–coupled receptor; representing a large superfamily of receptors with a characteristic seven-transmembrane topology and whose intracellular signals are transduced by heterotrimeric G proteins; includes receptors for hormones, neurotransmitters, visual stimuli, and chemosensory (olfaction and taste) stimuli.

LCR. Locus control region; a class of transcriptional regulatory element that can influence the transcription of genes within a large stretch of genomic DNA. Regulation of transcription by LCRs is thought to involve a physical interaction of the LCR and the target gene's proximal promoter sequence—a regulatory DNA sequence found immediately adjacent to the protein-coding portion of a gene.

Odor. Scent or smell; natural sources of odors are often complex mixtures of many compounds, some of which (but not necessarily all) can contribute to the perception of the particular smell.

Odorant. A compound that elicits the perception of smell.

Odorant receptors. Receptors expressed by OSNs and belonging to the GPCR superfamily, responsible primarily for receiving "chemosensory" or chemical cues from the environment. There are multiple families of odorant receptors, which include the OR (the largest family), TAAR, V1R, V2R, and formyl peptide-like receptors.

Olfaction. The sense of smell.

Olfactory bulb. A specialized structure of the forebrain, which receives direct input from the OSNs in the nose.

Olfactory cortex. Collectively refers to the five brain regions that receive direct input from the olfactory bulb: the piriform cortex, anterior olfactory nucleus, olfactory tubercle, entorhinal cortex, and amygdala. The regions of the olfactory cortex are responsible for the perception of smell and for generating odor-evoked behaviors.

Olfactory epithelium. The sensory epithelium that resides in the nose and contains the primary sensory neurons (the OSNs) that are responsible for detecting chemical stimuli from the environment.

Olfactory sensory neuron. The primary sensory neuron of the olfactory system, responsible for receiving and transducing chemical information from the environment. The olfactory sensory neuron is where the olfactory system meets the outside chemical world.

Projection neuron. The mitral or tufted cell in the case of the olfactory bulb; these neurons receive information from the OSNs in the olfactory epithelium, and relay or "project" this information to the next level in the olfactory cortex.

heterotrimeric G protein signaling cascade. Future studies will hopefully soon identify the intracellular signaling pathways responsible for OR-dependent gene silencing, which is critical for maintaining the stable expression of a single OR gene and therefore the functional identity of the OSN.

Wiring up the olfactory sensory map

Patterning of connections by odorant receptor–independent mechanisms. In other sensory systems, neuronal activity—here defined as activity leading to changes in membrane potential of the sensory neuron—plays a role in the refinement of synaptic connections during development and the modification of such connections underlying experience-dependent plasticity (Fox and Wong, 2005). What role does such activity play in the establishment of the precise targeting of OSNs in the olfactory bulb? Early studies using gene knockouts of the olfactory-specific CNG channel—the ion channel responsible for transducing OR-driven increases in cAMP accumulation into membrane depolarization (Fig. 2 A)—showed that targeting and convergence of OSN axons to the appropriate regions of the olfactory bulb can occur in the absence of stimulus-evoked activity (Brunet et al., 1996; Zheng et al., 2000).

The projection of axons from OSNs expressing specific ORs to spatially invariant glomeruli in the olfactory bulb suggests that the targeting of sensory axons in the olfactory bulb depends at least in part on spatially restricted guidance cues in the target tissue and along the axonal trajectory. How is this feat of connectivity achieved during development? In one model, a hierarchy of cues guides axons of neurons expressing the same odorant receptor first to the general vicinity of their targets, and then to their singular target glomerulus (Lin and Ngai, 1999; St John et al., 2002). Regarding the initial steps of axon pathfinding, it is instructive to consider this problem in terms of the olfactory bulb's dorsal–ventral, medial–lateral, and anterior–posterior axes, given that the spatial identity of each glomerulus on the surface of this three-dimensional structure can be defined by its coordinates along these three principal axes. As we will discuss in the remaining sections, targeting of OSN axons to positions along the dorsal–ventral and medial–lateral axes occurs primarily via mechanisms independent of OR-mediated activity, whereas projection along the anterior–posterior axis and axon convergence both depend on OR activity.

Neurons expressing a specific odorant receptor are segregated within circumscribed zones in the epithelium along the dorsomedial–ventrolateral axis (corresponding more or less to the dorsal–ventral axis; Ressler et al., 1993; Vassar et al., 1993; Miyamichi et al., 2005). While axons arising from each zone project to a corresponding dorsal–ventral zone in the olfactory bulb (Mori et al., 1999; Miyamichi et al., 2005) within these latter zones odorant receptor-specific axons converge to form discrete glomeruli. How is this initial level of dorsal–ventral segregation established? The repulsive axon guidance cue, Slit1, and its receptor, Robo2, play a role in segregating OSN axons along the dorsal–ventral axis of the olfactory bulb. Slit1 is expressed in the ventral olfactory bulb, and its receptor Robo2 is expressed in OSNs in a high dorsomedial to low ventrolateral gradient across the olfactory epithelium (Cho et al., 2007).

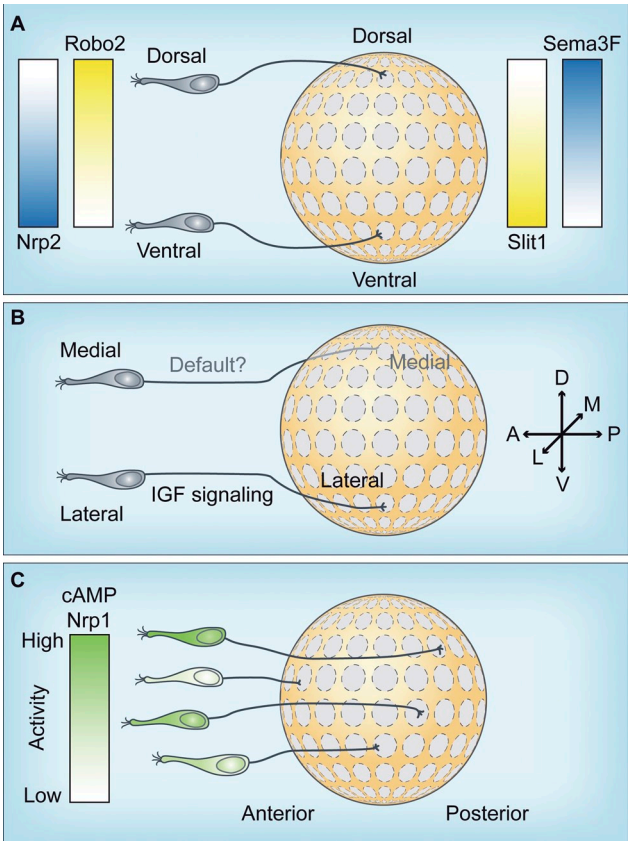

Figure 4. **Targeting of OSN axons to the olfactory bulb.** The projection of OSNs in the olfactory epithelium (OE) to their target glomeruli in the olfactory bulb (OB) can be considered along the bulb's three principal axes. (A) Zone-to-zone projection along the dorsal–ventral axis is shaped in part by complementary gradients of the chemorepellent molecules Slit1 and Semaphorin3F (Sema3F) and their receptors Robo2 and Neuropilin2 (Nrp2), respectively. (B) Innervation of the lateral olfactory bulb is dependent on IGF signaling, which may function to counteract a default tendency of all olfactory neurons to project medially. (C) Projection of olfactory sensory axons along the olfactory bulb's anterior–posterior axis depends not on the position of the cell in the OE, but rather on the level of intracellular cAMP, which in turn regulates the expression of the axon guidance receptor Neuropilin1 (Nrp1). By modulating the expression levels of axon guidance receptors such as Nrp1, the sensory axons are either more or less sensitive to guidance cues found in the OB or along the projection pathway.

Loss-of-function mutations either in Robo2 (the receptor) or Slit1 (the ligand) result in ectopic projections of OSN axons originating from the dorsomedial epithelium into the ventral olfactory bulb (Nguyen-Ba-Charvet et al., 2008). In addition, the chemorepellent Semaphorin3F is released by the axon terminals of dorsally projecting OSNs (which are the first to innervate the bulb) and prevents later-born ventrally derived axons from entering the dorsal olfactory bulb via its receptor, Neuropilin2, which is expressed in a high ventral to low dorsal gradient by OSNs in the olfactory epithelium (Takeuchi et al., 2010). Thus, complementary chemorepellent gradients of Slit1 and Semaphorin3F influence positioning of OSN projections along the dorsal–ventral axis (Fig. 4 A).

The projection of OSNs expressing a particular OR to the lateral versus medial olfactory bulb creates a mirror symmetry of innervation in this structure (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Nagao et al., 2000). What influences

the choice of an extending OSN axon to project laterally versus medially? Neurons located in medial or lateral positions of the olfactory epithelium innervate the medial or lateral olfactory bulb, respectively (Levai et al., 2003). Gene knockout studies in the mouse demonstrated that insulin-like growth factor (IGF) signaling plays an important role in the choice of OSNs to innervate the lateral versus medial olfactory bulb (Scolnick et al., 2008). Mice homozygous for a knockout of the IGF receptor, IGF1R, demonstrate a loss of innervation of the lateral olfactory bulb; axons normally destined for this bulb hemisphere rerouted to more medial locations, whereas the positions of medial glomeruli appear unperturbed (Scolnick et al., 2008). IGF can serve as a chemoattractant for OSN axon growth cones in culture, and this activity is dependent on PI3 kinase (Scolnick et al., 2008), a downstream target of IGF1R and a mediator of the growth cone's response to multiple axon guidance cues (Song and Poo, 2001). However, the absence of any clear-cut medial–lateral gradients of IGF1 and IGF2 (the two IGF ligands) either along the olfactory axon trajectory or in the olfactory bulb makes it difficult to explain how IGF signaling promotes innervation of the lateral olfactory bulb. One possibility is that IGF-binding proteins (Efstratiadis, 1998) bind to and mask the IGFs along the olfactory projection, thereby altering their spatial distribution of available IGF ligands. Another possibility is that growth toward the medial olfactory bulb is the default for all olfactory axons, regardless of their point of origin in the olfactory epithelium. Axons originating from the lateral olfactory epithelium would therefore require a counterbalancing lateral attraction in order to extend into the lateral hemisphere of the olfactory bulb; IGF ligands could serve as this attractive cue (Fig. 4 B). Whatever the case, it appears that IGF participates in concert with other guidance cues to direct OSN axons to the medial or lateral olfactory bulb.

Patterning of connections by odorant receptor–dependent mechanisms. In contrast to the mechanisms underlying the patterning of OSN axon projections along the dorsal–ventral and medial–lateral axes in the olfactory bulb, targeting along the anterior–posterior axis is influenced by the OR itself. This was first demonstrated through gene knock-in experiments in which the protein-coding region of one OR gene was replaced with that of another OR. In these "receptor swap" experiments, neurons expressing the modified allele projected their axons to an ectopic glomerulus close to—but distinct from—the target glomerulus of the substituted receptor (Mombaerts et al., 1996; Wang et al., 1998), indicating that the OR is just one determinant of the final position of the glomerulus. Two models can be entertained to explain these observations. In the first, the OR receives and transduces axon guidance cues at the growth cone, thereby serving a dual function as chemosensory receptor and axon guidance receptor. Consistent with this notion, OR protein has been observed in OSN axon terminals (Barnea et al., 2004), although their role as axon guidance receptors has yet to be demonstrated. This model also seems implausible in light of the demonstration that a receptor swap with the β2-adrenergic receptor coding sequence in place of the OR results in targeting of axons to glomeruli in the olfactory bulb (Feinstein et al., 2004). Alternatively, intrinsic activity of the receptor (i.e., activity in an unliganded state) could set the gain level or sensitivity of the cell to respond to what we might consider more traditional axon guidance cues. Indeed, cAMP (as well as cGMP) can modulate the growth cone's response to axon guidance cues (Song and Poo, 2001). In this scenario, OSNs project to different positions along the anterior–posterior axis of the bulb according to the particular OR's level of intrinsic activity (or in the case of the β2-adrenergic receptor swap, the β2-adrenergic receptor's intrinsic activity).

Support for this latter model has come from experiments in the mouse showing that perturbations in cAMP signaling cause anterior–posterior shifts in OSN axon targeting (Imai et al., 2006). A decrease in cAMP-mediated signaling caused by transgenic expression of either an inactive mutant OR or a dominant-negative protein kinase A (PKA) mutant results in an anterior shift in the target glomerulus (Imai et al., 2006). Conversely, transgenic expression of constitutively active Gαs or constitutively active PKA causes a posterior shift in innervation (Imai et al., 2006). Intriguingly, transcription of specific genes in OSNs correlates with the level of cAMP signaling (Imai et al., 2006). Notable among these is the gene encoding Neuropilin1, a receptor for the repulsive axon guidance cue Semaphorin3A (Imai et al., 2006); OSNs expressing high levels of Neuropilin1 project to the posterior olfactory bulb, whereas OSNs expressing low levels project anteriorly. Thus, a model emerges in which the position of a given OSN's target glomerulus along the olfactory bulb's anterior–posterior axis is in part determined by the OSN's sensitivity to axon guidance cues such as Semaphorin3A (Fig. 4 C). This sensitivity in turn is determined by the level of intrinsic activity manifested by the particular OR expressed by the OSN, which determines—via cAMP/PKA signaling—the level of expression of the Semaphorin3A receptor, Neuropilin1. In this way, OSN axons are sorted along the anterior–posterior axis of the olfactory bulb not based on their originating positions in the olfactory epithelium (as we discussed for dorsal–ventral and medial–lateral positioning), but rather based on the intrinsic activity of the OR expressed by each neuron. Interestingly, axons destined for the anterior versus posterior olfactory bulb are presorted in the olfactory nerve before they enter the olfactory bulb, and Semaphorin3A–Neuropilin1 signaling is involved in this presorting process (Imai et al., 2009).

The final step: convergence of like olfactory axons is influenced by OR-mediated neuronal activity

How do OSNs expressing the same OR that are distributed broadly in the olfactory epithelium converge upon a common glomerulus in each bulb hemisphere? OSN axons can converge to ectopic sites in mice missing their olfactory bulbs due to either surgical or genetic manipulations (Bulfone et al., 1998; St John et al., 2003; Chehrehasa et al., 2006; Ardiles et al., 2007), suggesting that convergence reflects a process intrinsic to the OSNs, independent of their cellular targets in the olfactory bulb. Insight into the mechanism underlying convergence was provided by a study showing that the homophilic cell adhesion molecules, Kirrel2 and Kirrel3, as well as the repulsive molecules EphrinA5 and EphA5 (a ligand–receptor pair), are

expressed in complementary patterns by OSNs, such that cells expressing high levels of Kirrel2 express low levels of Kirrel3, and vice versa; a similar pattern is observed for EphrinA5 and EphA5 (Serizawa et al., 2006). Moreover, expression of each of these (and other) molecules at high, low, or intermediate levels correlates with the particular OR they express (Serizawa et al., 2006; Kaneko-Goto et al., 2008). Additional investigations using the CNG channel knockout mouse further demonstrated that expression levels of Kirrel2–Kirrel3 and EphrinA5–EphA5 are dependent on neuronal activity, and CNG channel gain-of-function in OSNs results in the formation of ectopic glomeruli adjacent to the original targets (Serizawa et al., 2006). Together, these results suggest a mechanism in which homophilic Kirrel2–Kirrel2 or Kirrel3–Kirrel3 interactions play a role in promoting convergence of like axons, whereas repulsive EphrinA5–EphA5 signaling functions to segregate dissimilar axons. It seems unlikely, however, that interactions of a small number of cell adhesion or cell repulsion molecules can alone account for the sorting and segregation of >1,000 OR-specific axon types. Rather, such interactions may represent the final step in a hierarchy of mechanisms used to sort OSN axons as they innervate their target glomeruli. It is worth noting that for some OR-expressing neurons, the initial projection is made to a small number of adjacent glomeruli and is refined postnatally to a single glomerular target; this refinement depends on CNG channel–dependent neuronal activity (Zou et al., 2004). Postnatal refinement of glomerular innervation may reflect the final steps in a developmental process shaped by OR-mediated experience-dependent plasticity; it will be interesting to determine whether this process involves the activity-dependent expression of cell surface molecules such as Kirrel2/3 and Ephrin/Eph.

Conclusions

In addition to providing the basis for the recognition of diverse chemical stimuli in the olfactory sensory world, the OR plays critical roles in safeguarding the stability of odorant receptor gene choice and the wiring of OSN axons in the olfactory bulb. These latter duties of the OR ensure that the signals it initiates upon odorant activation are transmitted in a stable and coherent fashion to the next relay stations in the olfactory sensory pathway. A number of exciting challenges remain for the future. For example, deciphering and understanding the molecular principles underlying the "odor code"—i.e., how an animal's complete repertoire of ORs is used to detect the vast array of chemicals in "odor space"—is now possible with the latest advances in high-throughput cell-based screening and computational modeling of the OR proteins. Future research should also elucidate the molecular details of how the OR exerts its influence on the developmental decisions underlying OR gene regulation and OSN axon targeting and convergence. In terms of the regulation of OR gene expression, although we have some intriguing glimpses into the complexities behind the "one receptor, one neuron" rule, mechanistically this mode of gene expression remains a mystery. It will be fascinating to discover the genetic and epigenetic mechanisms that act to select a particular OR gene for expression and subsequently silence expression from all other OR gene loci. Finally, the targeting of OSNs from dispersed locations in the sensory epithelium to discrete and invariant glomeruli in the olfactory bulb represents a major feat of axon guidance and synaptic specificity. Future research in this domain should help to unravel the intertwined roles of classical axon guidance cues and their receptors (not to mention novel players that may well be involved), neuronal activity, and the ORs themselves in establishing and maintaining the olfactory sensory map in the olfactory bulb.

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