

Functional Expression of Mammalian Odorant Receptors

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The olfactory sensory neurons (OSNs) in the olfactory epithelium of the nose detect and discriminate a large number of volatile environmental chemicals. This ability is essential for animals to find prey, food, mates, predators and toxic compounds. In OSNs, odorant receptor (OR) proteins, members of the G-protein coupled receptor (GPCR) superfamily, are concentrated at the membrane of the cilia at the tip of the dendrite. OR proteins are also present at axon terminals, where they may have roles in target recognition of developing olfactory axons (Mombaerts *et al.*, 1996; Wang *et al.*, 1998; Barnea *et al.*, 2004; Feinstein *et al.*, 2004). The great diversity of existing odorant molecules demands a large number of different ORs to allow differential discrimination. Accordingly, rodents have >1000 different ORs and humans have ~350 (Buck and Axel, 1991; Axel, 1995; Mombaerts, 1999; Firestein, 2001; Young *et al.*, 2002; Zhang and Firestein, 2002). Interestingly, expression of an OR is restricted to that of only one allele per olfactory neuron (Malnic *et al.*, 1999; Serizawa *et al.*, 2003; Lewcock and Reed, 2004).

Although mammalian ORs were identified over 13 years ago, the chemical selectivity of individual ORs is largely unknown, mainly because it has been difficult to express ORs on the surface of heterologous cells to assay their ligand-binding specificity. It is thought that OR proteins are retained in the endoplasmic reticulum (ER) (McClintock *et al.*, 1997; Lu *et al.*, 2003). Despite these difficulties, various methods to match ORs with odorants have been proposed and tested (reviewed in Mombaerts, 2004).

One method to aid in heterologous cell surface targeting is to add the N-terminal amino acids of rhodopsin or a foreign signal peptide to the N-terminus of the OR (Krautwurst *et al.*, 1998; Hatt *et al.*, 1999). ODR-4, which is required for proper localization of chemosensory receptors in *Caenorhabditis elegans*, has a small effect on facilitating cell-surface expression of one rat OR, but not another OR (Gimelbrant *et al.*, 2001). Thus, for most ORs, this and other modifications do not seem to promote cell-surface expression.

Accordingly, methods using homologous cells were developed by various groups, in which OSNs or a cell line derived from OSNs were used (Murrell and Hunter, 1999; Touhara *et al.*, 1999; Zhao *et al.*, 1998; Gimelbrant *et al.*, 2001). Zhao *et al.* (1998) used adenovirus as a vector to express ORs in OSNs in the nose. Screening of putative ligands and subsequent activation of foreign ORs was measured either by electroolfactogram or calcium imaging of single cells. They found that a rat receptor, I7, was activated by a few chemicals including octanal. In a different homologous assay system, a gene targeting protocol was used to create mice in which OSNs that express a specific OR are labeled by green fluorescent protein (GFP). Dissociated GFP-labeled OSNs were loaded with calcium indicator so that calcium imaging could be used to measure the activation of OSNs upon ligand exposure (Bozza *et al.*, 2002). Two groups used a combination of calcium imaging in dissociated OSNs and single-cell RT-PCR to identify the OR gene expressed by the odorant-activated OSNs (Malnic *et al.*, 1999; Touhara *et al.*, 1999; Kajiya *et al.*, 2001), as each OSN expresses only one OR gene. Using this method, Malnic *et al.* (1999) identified 13 different ORs that responded to various aliphatic alcohols and acids. However, it is difficult to verify OR

ligand specificity. Touhara's group used heterologous expression systems to verify activation for some of the ORs they cloned. Finally, a new method using a combination of functional imaging of the olfactory bulb, fluorescent dye injection and single-cell RT PCR was recently proposed (Mizrahi *et al.*, 2004). Success is limited to a handful of ORs as it is extremely laborious to test each individual ORs using these techniques compared to methods that involve heterologous expression systems.

Further progress in understanding olfactory coding in mammals is likely to require a development of new assay system(s) that enable functional expression of a large number of ORs in heterologous cells in order to identify cognate ligands. OSNs may have unique molecular machinery to promote proper targeting of OR proteins to the cell surface (McClintock and Sammeta, 2003), explaining why OR expression in heterologous systems has been difficult. To overcome this, identification of accessory protein(s) that are required for cell-surface expression of ORs would be critical. There is precedence for this protein targeting problem. Many GPCRs are known to require accessory proteins for correct targeting to the cell surface membrane (for a review, see Brady and Limbird, 2002). Examples of this include NinaA for *Drosophila* rhodopsin (Baker *et al.*, 1994), RanBP2 for cone opsin (Ferreira *et al.*, 1996), ODR-4 for *Caenorhabditis elegans* olfactory receptors (Dwyer *et al.*, 1998), RAMPs for the calcitonin receptor-like receptor (CRLR) (McLatchie *et al.*, 1998) and finally the M10 family of MHC class I proteins and β 2 microglobulin for V2Rs, the putative mammalian pheromone receptors (Loconto *et al.*, 2003). Importantly, with the exception of NinaA and RanBP2, none of these accessory proteins share any sequence homology to each other; their only common feature is their association with the membrane.

The putative accessory proteins required for OR trafficking are likely to be expressed by OSNs, interact with OR proteins, and enhance responses to odorants when co-expressed with ORs in heterologous cells. How might putative accessory proteins aid OR trafficking? They might function as chaperones promoting correct folding of ORs in the ER. Alternatively, they could facilitate the transport of specific vesicles/cargos that includes ORs. Lastly, they might form a stable complex with ORs, potentially masking ER retention signals of ORs, and thereby functioning as co-receptors to the ORs. Establishment of functional OR expression system using heterologous cells, together with the annotation of virtually all the ORs in the mouse and human genomes, will provide a platform to investigate OR-odorant interaction in a comprehensive manner.

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