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Molecular and structural bases for odorant recognition by olfactory receptors

Kazushige Touhara

Department of Integrated Biosciences, The University of Tokyo, Chiba 277-8562, JAPAN

Olfactory receptors (ORs), which comprise the largest G protein-coupled receptor family, are now known to play a pivotal role in recognizing a variety of odorants in the vertebrate olfactory system [1-5]. Until recently, however, functional evidence that the ORs indeed mediate odorant signal, had not been provided for many years since the discovery of the gene superfamily in 1991 [6]. My talk is divided into five topics: 1) functional cloning and expression of ORs, 2) a combinatorial receptor code for an odorant, 3) odorant-response assays in heterologous cells, 4) OR antagonism between odorants, and 5) binding site mapping of an OR. Here I present recent progresses in research for understanding how the olfactory system discriminates variety of odorants using an OR coding strategy.

Instead of screening cognate ligands for orphan ORs, we initially devoted a few years to develop a cloning strategy to functionally isolate OR genes. The observation that single mouse olfactory neurons express only one OR gene among hundreds of the OR family [1-3], suggested that an OR expressed in single olfactory neurons was responsible for the physiological function of the neurons. We thereby designed a functional cloning approach that included two-step experiment: recording odorant responses of isolated olfactory neurons by calcium imaging, and subsequent single cell RT-PCR using degenerate primers designed from the conserved sequences of the OR family [7,8]. The functionally-cloned

receptors were then reconstituted in olfactory neurons or in mammalian cell lines, providing evidence that the OR superfamily indeed mediated responses to odorants [7,8].

Using several functionally-cloned ORs, we showed that structurally-related ORs recognized overlapping sets of odorants with distinct affinities and specificities [8]. ORs discriminate subtle differences in chemical structures of odorants, but also tolerate other molecular features in the ligand-binding site. These results suggest that the identities of different odorants are specified by distinct combinations of ORs that possess unique molecular receptive ranges. Activation of a combinatorial receptor repertoire by an odorant determines the odor quality. We further demonstrated that an odorant was recognized by different sets of ORs at different odorant concentrations [8], which potentially explains our experience that an odorant seems to smell different at different concentrations.

Although it has been difficult to functionally express ORs in a heterologous system, we have established several odorant-response assays by virtue of a signaling pathway mediated by endognenous Gas or transfected Ga15 in mammalian cell lines and *Xenopus* oocytes [9]. Various functional assays for a heterologously-expressed OR are potentially useful for high-throughput ligand screening and functional analyses of hundreds of ORs. Functional cloning and expression of ORs followed by structure-function relationship studies will provide data to facilitate construction of ligand-activity matrices of ORs.

Despite increasing information on agonist-OR combinations, little is known about antagonism of ORs in the mammalian olfactory system. We found that odorants inhibit odorant-responses of OR(s), evidence of antagonism between odorants at the receptor level [10]. The antagonism was demonstrated in a heterologous OR-expression system and in single olfactory neurons that expressed a given OR, and was also visualized at the level of the olfactory

epithelium. Dual functions of odorants as an agonist and an antagonist to ORs indicate a new aspect in the receptor code determination for odorant mixtures. When antagonism occurs between components in an odorant mixture, the activated ORs by the mixture is not simply the sum of those for its components, leading to a novel perceptual quality that is not present in each component.

The recent atomic-level structure for bovine rhodopsin has provided an opportunity to elucidate a molecular basis for odorant recognition by ORs. A combination of computer modeling and ligand-docking analysis with site-directed mutagenesis experiments revealed the active site of an OR [11]. Structural basis for binding of agonists and antagonists to ORs will shed light on strategies for designing novel odorants with commercial potential. This line of research would no doubt activate pharmacological research for screening and designing agonists and antagonists for other G protein-coupled receptors, at which over 50% of the pharmaceuticals are targeted.

In conclusion, the functional cloning approach enabled us to examine not only odorant specificity of an OR but also receptor specificity and diversity of a particular odorant of interest. Odor discrimination is determined by differences in the receptive ranges of the ORs that together encode specific odorant molecules. We also provided molecular and cellular evidence for antagonism of OR activities between odorants. An odorant appears to act as an agonist and an antagonist to ORs. The observation not only provides an explanation for a peripheral mechanism for odorant mixture suppression, but also insight into strategies to modulate perceived odorant quality. Molecular and structural basis for odorant recognition by an OR has been investigated by combining dry and wet experiments. Emerging evidence on odorant-OR interaction by diverse approaches in molecular biology, pharmacology, and structural biology will eventually converge on fully understanding chemical sensing in the olfactory system. Considering importance

of sensation and perception of information from the external environment, elucidation of molecular mechanisms underlying odor recognition and discrimination by ORs is one of targeted researches in the post-genomic era with implication for a better quality life.

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