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Odorant Receptor Specificities and Receptor Combinatorials: Implications for Olfactory Coding

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The perception and discrimination of thousands of different odorants by the vertebrate olfactory system results from the activation of specific olfactory neurons within the olfactory epithelium of the nose (reviewed in Buck, 2000; Firestein, 2001). Activity from these cells is then interpreted by the brain to discern the molecular identity of a given odorant stimulus. How is this process of molecular recognition accomplished? The first step in olfactory discrimination resides at the level of the interaction of odorous ligands with odorant receptors. How many different odorants can bind to an individual receptor, and how many different receptors are employed to identify a given ligand as a discrete odor percept? In one model, discrimination may occur by the use of relatively few receptors, each capable of interacting with numerous odorants. The discrimination of one odorant from another would then be achieved by combining or blending the inputs from these relatively non-selective, low affinity receptors. Alternatively, the olfactory system may employ a large number of receptors, each capable of binding to a more restricted subset of odorants.

The OR family of odorant receptors

A large multigene family thought to encode odorant receptors was initially identified in the rat (Buck and Axel, 1991; reviewed in Mombaerts, 2004). The predicted structure of these receptors exhibits a seven transmembrane domain topology characteristic of the 'A family' or rhodopsin class of G protein-coupled receptors. The size of the OR gene family in mammals appears to be extremely large and is estimated to contain between 900 and 1400 individual genes (Zhang *et al.*, 2004). In the fish, where the number of perceived odorants is more limited, the size of the OR repertoire appears to be much smaller and may contain as few as 100 genes (e.g. Dugas and Ngai, 2001). These observations suggest that the initial step in olfactory discrimination is accomplished by the integration of signals from a large number of specific receptors, each capable of binding to a limited number of odorants.

An understanding of how information is encoded by odorant receptors has been facilitated by recent studies elucidating the molecular receptive field properties of individual odorant receptors. For the OR class of receptors, progress has been hampered by the difficulty in achieving receptor expression at the plasma membrane of heterologous cell systems. Nonetheless, a number of approaches have succeeded in characterizing the ligand response properties of a small collection of these receptor molecules (Zhao *et al.*, 1997; Krautwurst *et al.*, 1998; Malnic *et al.*, 1999; Touhara *et al.*, 1999). Perhaps the best functionally characterized OR family member is the I7 receptor from the rat (Zhao *et al.*, 1997; Araneda *et al.*, 2000). By using an adenoviral vector to overexpress the I7 receptor in olfactory neurons in situ, Firestein and colleagues demonstrated that this receptor is tuned to respond to n-aliphatic aldehydes. Interestingly, whereas the presence of the aldehyde moiety is essential for activation, the receptor is broadly tuned for the length (or hydrophobicity) of the n-aliphatic backbone, showing a preference for an 8-carbon structure. These results suggest that OR receptors may be specific for certain classes of molecules (e.g. aldehydes), but broadly tuned for certain molecular features (e.g. carbon chain length or hydrophobicity), and support the notion that a combination of receptors—each tuned to discriminate distinct chemical features—is required to identify the molecular identity of an odorant stimulus.

Receptors of the vomeronasal organ and related receptors

In terrestrial vertebrates, the vomeronasal organ (VNO) functions to receive non-volatile cues of a pheromonal as well as non-pheromonal nature (Halpern, 1987). Subsequent to the initial discovery of the OR family of odorant receptors, two unrelated G protein-coupled receptor gene families were identified in the mammalian VNO, the V1R receptors and the V2R receptors (Mombaerts, 2004). The V1R receptors, which belong to the 'A family' or rhodopsin-class of G protein-coupled receptors are expressed within the subpopulation of $G\alpha_i$-expressing VNO sensory neurons. Recent genome-wide surveys have revealed the presence of ~150 V1R genes in the mouse genome (Zhang *et al.*, 2004). The V2R receptors belong to the 'C family' of G protein-coupled receptors, which includes the metabotropic glutamate receptors (mGluR), extracellular calcium sensing receptors (CaSR) and GABA-B receptors. Members of this receptor family are characterized by their long N-terminal extracellular domain, which contains the primary determinants for ligand binding (reviewed in Pin *et al.*, 2003). There are probably ~100 mammalian V2R genes and these receptors are localized to the subclass of $G\alpha_o$-expressing neurons, in a pattern complementary to V1R/$G\alpha_i$ expression. Whereas one V1R receptor has been shown to be activated by pheromonal compounds (Boschat *et al.*, 2002), ligands for the V2R receptors have yet to be identified.

In the fish, receptors homologous to the V2R receptor family have been shown to be expressed in the olfactory epithelium (Cao *et al.*, 1998; Naito *et al.*, 1998; Speca *et al.*, 1999). As the fish do not possess a VNO (the VNO is a specialization of terrestrial vertebrates), we will refer to these 'V2R-like' receptors more generally as 'C family olfactory receptors'. The fish C family olfactory receptors are expressed by the subpopulation of microvillous sensory neurons in the fish's single olfactory organ, distinct from the ciliated sensory neurons, which express members of the OR family of odorant receptors (Cao *et al.*, 1998; Speca *et al.*, 1999). Significantly, one C family receptor from the goldfish, receptor 5.24, has been shown to interact with amino acid ligands (Speca *et al.*, 1999), which are used as olfactory feeding cues in fish (Hara, 1994). This receptor was shown to bind to arginine and lysine with high affinity, exhibiting lower affinities for other amino acids (Speca *et al.*, 1999). Thus, the receptor is preferentially tuned to respond to basic amino acids, although the tuning is

rather broad. As for the case of the rat I7 receptor (Araneda *et al.*, 2000; Zhao *et al.*, 1997), these observations suggest that olfactory discrimination is afforded by the combinatorial activity from an array of broadly tuned odorant receptors. Future studies focusing on the ligand specificities of other C family olfactory receptors will allow a more comprehensive understanding of the combinatorials used to receive and process sensory information through this receptor system.

Receptor structure and function: determinants of odorant specificity

The identification of the activating ligands for specific odorant receptors provides the opportunity to understand the principles governing the molecular receptive field properties of these protein sensors. For example, it would be of great interest to elucidate what features of the receptor molecule are responsible for determining ligand specificity. Are certain regions of the ligand binding pocket tuned to interact with certain chemical moieties? What aspects of the ligand–receptor interaction provide for the broad tuning profiles for certain ligands within a class of compounds? In C family G protein-coupled receptors, ligand binding is thought to occur primarily, if not exclusively within the extracellular N terminal domain (NTD) (Pin *et al.*, 2003). The homology of the goldfish C family odorant receptor to the mGluR receptors, about which extensive information is known regarding receptor structure and ligand-receptor interactions (reviewed in Pin *et al.*, 2003), therefore provides an excellent opportunity to formulate a structural model of an odorant receptor binding pocket. We are currently focusing on generating such molecular models and testing them through site-directed mutagenesis and direct functional validation. Our results indicate that the general principles governing ligand binding in other amino acid receptors indeed apply to the goldfish olfactory receptor. We have further identified key residues in the binding pocket that determine the receptor's selectivity for specific amino acids. Together these studies lay the foundation for a broader understanding of the molecular determinants of ligand selectivity in this class of chemosensory receptor.

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