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COMMENTARY

Using GEFs to Deorphanize Odorant Receptors

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Odorant perception is initiated when odorants activate unique combinations of odorant receptors (ORs) expressed in the cilia of olfactory sensory neurons in the nose (Buck and Axel 1991; Malnic et al. 1999). One of the greatest challenges in the olfactory science is to correlate ORs with their ligands (to deorphanize the ORs). The determination of OR–ligand pairs should reveal how the OR family is used to generate diverse odorant perceptions (Keller et al. 2007, Menashi et al. 2007). However, these studies are complicated by the poor functional expression of the ORs in heterologous cells because they are retained in the endoplasmic reticulum and cannot reach the plasmatic membrane (McClintock and Sammeta 2003). Consequently, a very small number of mammalian ORs have been linked to odorants they recognize to date (Malnic 2007).

Methods that enhance OR trafficking to the cell membrane have been successfully used to obtain functional expression of ORs in heterologous cells. The fusion of the 20 N-terminal amino acids of rhodopsin (rho tag) to the N-terminal region of ORs facilitates cell surface expression of the receptors (Krautwurst et al. 1998). Also, coexpression with the receptor transporting proteins (RTPs, Saito et al. 2004) enhances surface expression of ORs in HEK293 cells.

It has been previously demonstrated that Ric-8B promotes functional OR signaling through Gαolf in HEK293T cells (Von Dannecker et al. 2006). Ric-8B is an olfactory-specific putative guanine nucleotide exchange factor (GEF), which is normally expressed in olfactory sensory neurons and interacts with Gαolf (Von Dannecker et al. 2005). GEFs catalyze the exchange of guanosine 5′-diphosphate for guanosine 5′-triphosphate to generate an active form of the Gα protein and, therefore, are considered to work as positive regulators of G-protein-coupled receptor signaling. Therefore, in this case, functional expression of ORs is not mediated by an increase in the amount of receptors on the cell surface but instead results from the amplification of the OR signaling through the G protein. It was also shown that the use of a combination of Ric-8B and RTP1S (a short form of RTP1) results in an improved heterologous expression of ORs (Von Dannecker et al. 2006; Zhuang and Matsunami 2007).

In this issue of *Chemical Senses*, Yoshikawa and Touhara show that another GEF, Ric-8A, can be used to improve functional expression of ORs through exogenously transfected Gα15 in HEK293 cells. Ric-8A is a well-characterized GEF (Tall et al. 2003) and is similar in amino acid sequence to Ric-8B. Coexpression of 3 different ORs (MOR31-2, MOR23, and MOR204-34, which recognize respectively valeric acid, methyl isoeugenol, and lyral) with Gα15 and myr-Ric-8A (a myristoylated form of Ric-8A which is localized to the cell membrane) resulted in specific ligand induced Ca^{2+} responses in HEK293 cells. Coexpression of these same ORs with Gα15 and RTP1 produced similar results. When the same experiments were repeated, but now with myr-Ric-8A, Gα15 and RTP1 being coexpressed in the same cells, the Ca^{2+} responses were significantly increased. Therefore, the combination of Ric-8A and RTP1 also further enhances functional heterologous expression of ORs. Parallel experiments were also performed using the same 3 ORs and Ric-8B, and it was observed that the combination of Ric-8B and RTP1 produced increased Gαs-mediated cyclic adenosine 3′,5′monophosphate (cAMP) accumulation, when compared with Ric-8B or RTP1 alone, as previously demonstrated for other ORs (S6, MOR203-1, OREG, and olfr62, Von Dannecker et al. 2006; Zhuang and Matsunami 2007). It is important to note that in the present experiments, only the rho-tagged versions of the ORs were used. It would be interesting to determine whether, in these conditions, untagged ORs show the same ligand properties because the presence of the rho tag could alter the affinities of the ORs (Von Dannecker et al. 2006; Zhuang and Matsunami 2007). It is possible that for the majority of the ORs, no rho tag is required for functional expression when the Ric-8 proteins are used in combination with RTP1.

Yoshikawa and Touhara then used the 2 assays (the Ca^{2+} imaging approach using Ric-8A, Gα15, and RTP1 or the cAMP assay using Ric-8B and RTP1) to deorphanize the mouse OR MOR139-3. This OR was originally cloned using the single-cell reverse transcriptase polymerase chain reaction method (Malnic et al. 1999; Touhara et al. 1999) from a mouse olfactory sensory neuron that had responded to *m*-cresol but had never been successfully expressed in heterologous cells. The *m*-cresol induced Ca^{2+} responses in cells expressing MOR139-3, myr-Ric-8A, Gα15, and RTP1, but no responses were observed in the absence of myr-Ric-8A, indicating that this GEF was required for the functional expression of MOR139-3. The responses of MOR139-3 to a panel of 19 aliphatic and aromatic odorants were also tested, and consistent results were obtained using the two different assays.

The results of Yoshikawa and Touhara show that another GEF, besides Ric-8B, can be used to functionally express ORs in heterologous cells. They also show that Ric-8A can be used in combination with RTP1 to produce robust OR functional expression, as was shown for Ric-8B. The use of these methods in the future should facilitate and expedite the deorphanization of mammalian ORs.

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