Published in final edited form as:
Ann N Y Acad Sci. 2009 July ; 1170: 153–156. doi:10.1111/j.1749-6632.2009.03888.x.

Trafficking of mammalian chemosensory receptors by RTPs

Hiroaki Matsunami, **Joel D. Mainland**, and **Sandeepa Dey**
Department of Molecular Genetics and Microbiology Duke University Medical Center Durham, NC 27710

Abstract

Although mammalian odorant receptors (ORs) were identified over 15 years ago, we still do not understand how odorant molecules interact with ORs at a molecular level. Previous studies of mammalian ORs have tested small numbers of ORs against large numbers of odorants. Some fundamental properties of the olfactory system, however, require investigation of a wide panel of diverse ORs with a large number of chemically diverse odorants. Previously, we identified OR accessory proteins, RTP1 and RTP2. They are expressed specifically in olfactory neurons, are associated with OR proteins and facilitate the OR trafficking to the plasma membrane when coexpressed in mammalian cell lines. Using this approach, high-throughput screening using a large repertoire of mammalian ORs is now possible. The activation profiles can be used to develop a predictive model relating physicochemical odorant properties, receptor sequences, and their interactions, enabling us to predict a tested receptor's response to a novel odorant and a novel receptor's response to a tested odorant. This will provide a basis for understanding how structurally diverse odorant molecules activate the mammalian OR repertoire. Similarly, two families of vomeronasal receptors, V1Rs and V2Rs, are also notoriously difficult to functionally express in heterologous cells. However, coexpression of the RTP family members with V1Rs or V2Rs does not seem to facilitate trafficking of the receptor proteins. This suggests that the vomeronasal organ has a unique biosynthetic pathway for membrane proteins.

Since mammalian odorant receptors (ORs) were identified over 15 years ago, many chemosensory (olfactory and taste) receptors have been identified. These include ORs and TAARs expressed by the olfactory sensory neurons in the olfactory epithelium, V1Rs and V2R expressed in the vomeronasal organ, T1Rs and T2Rs expressed in the taste buds [1–9]. These are seven transmembrane, G-protein coupled receptors. Insects use a completely different set of chemoreceptors, the Ors and the Grs, to detect chemicals [10–14]. These proteins likely function primarily as ligand-gated channels [15,16].

The vast majority of chemosensory receptors must form a complex with a partner protein to functionally respond to stimuli. Taste receptors T1R1, T1R2 and T1R3 show little response to taste stimuli when expressed alone in a heterologous system, however coexpression of T1R2 and T1R3 results in the formation of a sweet taste receptor and coexpression of T1R1 and T1R3 results in the formation of an umami (l-amino acid) taste receptor [17–19]. Similarly, candidate sour taste receptors PKD2L1 and PKD1L3 need to be coexpressed in the same cells to be efficiently trafficked to the cell surface in HEK293T cells [20]. In the case of V2Rs, a non-classical MHC class 1b molecules and ß2-microglobulin may form a complex with some V2Rs [21]. Finally, in insect olfaction, all Ors require Or83b as a partner for proper trafficking and function [22].

Following this pattern, mammalian ORs are typically retained in the endoplasmic reticulum (ER) in heterologous cells when expressed by themselves [23–26]. Expression cloning strategy was used to screen OR partner molecules. Using a SAGE (serial analysis of gene expression) library from single olfactory and vomeronasal neurons, candidate genes encoding membrane associated proteins were isolated and tested to determine if they enhanced surface expression

of an OR, MOR203-1, in HEK293T cell line. With this stragety, receptor-transporting proteins, RTP1 and RTP2, were identified. They are specifically expressed in the olfactory neurons, and promote functional cell-surface expression of various ORs in heterologous cells. A third molecule REEP1 was found to be expressed at lower levels in the olfactory epithelium and was also less effective in increasing functional heterologous expression of ORs. Increased odorant induced responses and co-immunoprecipitation of RTP1 with odorant receptors suggests that it may act as a trafficking chaperone or a co-receptor [27]. However the mechanism underlying the effect of RTPs on increased surface expression of ORs remains elusive.

Subsequently a shorter form of RTP1, RTP1S encoded from the second methionine residue in the same reading frame of the originally described RTP1, was shown to be predominantly expressed in the olfactory epithelium and had a more robust effect on promotion of OR trafficking and ligand induced response of ORs [28]. In addition to RTP1S, $G_{\alpha olf}$ (G_{α} subunit coexpressed in the olfactory epithelium) and Ric8B have been shown to act in synergy in increasing functional heterologous expression and response [28–30].

Using this system, our group has shown that immunological tags added to the N-terminal end of ORs, often to used to facilitate OR trafficking and to determine OR localization [31], do not broadly affect ligand specificities. However, increasing the amount of receptors at the cell surface can affect relative ligand selectivity such that a weak agonist can behave like a full agonist [28].

It is not known whether RTP1 and RTP2 are required for OR trafficking and function *in vivo*. Generation and analysis of RTP1 and RTP2 knockout animals will be critically important to assess the *in vivo* function of RTP1 and RTP2 not only in OR protein trafficking, but also in odor detection. Additionally, these mutant animals may also be used to assess axon pathfinding and targeting in the olfactory bulb. Lastly, it will be interesting to see if the development of the olfactory system is affected by the absence of these partner proteins.

Many fundamental questions in odor coding are unanswered. How do odorants bind and activate ORs? How many ORs are activated by a particular odor molecule? Are OR tuning profiles broad and overlapping similar to cones in color vision, or do they tile olfactory space as in the auditory system. Are some ORs specifically tuned to a particular compound, or do all the ORs have similar tuning specificities? What kind of physical or chemical odorant properties are important for OR selectivity? Which residues or structural elements of ORs are important for OR selectivity? Is there a direct correlation between activation of a given OR and odor perception? Do odorants that elicit attraction or repulsion activate distinct sets of ORs? Previous studies of mammalian ORs have tested small numbers of ORs. Some fundamental properties of the olfactory system, however, require investigation of a wide panel of diverse ORs with a large number of chemically diverse odorants. To date, approximately 40 out of ~1000 mouse ORs, 2 out of ~1200 rat ORs and 7 out of ~380 human ORs have been shown to respond to at least one ligand [32]. The discovery of the RTP partner proteins paves the way for systematic high-throughput screening of mammalian ORs. Such a screen will produce a powerful tool to answer a number of the important questions mentioned above. The activation profiles can be used to develop a predictive model relating physicochemical odorant properties, receptor sequences, and their interactions. This will provide a basis for understanding how structurally diverse odorant molecules activate the mammalian OR repertoire.

In addition to the main olfactory system, many vertebrates possess an accessory olfactory system consisting of the vomeronasal organ and a dedicated accessory olfactory bulb. This system appears to be important for detection of some pheromones, chemicals that influence innate behavior or physiology in the same species. Two families of putative pheromone

receptors, that are expressed in the vomeronasal organ, V1Rs and V2Rs, appears to be notoriously difficult to functionally express in heterologous cells with some exceptions [21, 33]. Like ORs, the vast majority of V1Rs and V2Rs seem to be retained in the ER when expressed in heterologous cells. Coexpression of the RTP family members with V1Rs or V2Rs does not seem to facilitate trafficking of the receptor proteins in heterologous cells, suggesting that the vomeronasal neurons may use different molecular machinery from that of ORs and other known chemosensory receptors for their trafficking. Recent progress in genome sequences from a wide variety of animal species, some with functional vomeronasal organs and some without, should facilitate screens to identify uncharacterized molecules potentially functioning in vomeronasal receptor trafficking. Developing a heterologous system to functionally express the vomeronasal receptors will help establish ligands for these receptors and allow us to relate particular ligand-receptor pairs or sets of such pairs to behavior; a high-throughput screening assay for vomeronasal receptors would also allow us to ask a number of important questions about coding of pheromone molecules and would allow us to delineate the distinct roles of the two olfactory systems.

References

1. Liberles SD, Buck LB. A second class of chemosensory receptors in the olfactory epithelium. Nature 2006;442:645–650. [PubMed: 16878137]
2. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 1991;65:175–187. [PubMed: 1840504]
3. Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell 1995;83:195–206. [PubMed: 7585937]
4. Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 1997;90:763–773. [PubMed: 9288755]
5. Matsunami H, Buck LB. A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell 1997;90:775–784. [PubMed: 9288756]
6. Ryba NJ, Tirindelli R. A new multigene family of putative pheromone receptors. Neuron 1997;19:371–379. [PubMed: 9292726]
7. Adler E, et al. A novel family of mammalian taste receptors. Cell 2000;100:693–702. [PubMed: 10761934]
8. Matsunami H, Montmayeur JP, Buck LB. A family of candidate taste receptors in human and mouse. Nature 2000;404:601–604. [PubMed: 10766242]
9. Hoon MA, et al. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 1999;96:541–551. [PubMed: 10052456]
10. Vosshall LB, et al. A spatial map of olfactory receptor expression in the Drosophila antenna. Cell 1999;96:725–736. [PubMed: 10089887]
11. Clyne PJ, et al. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron 1999;22:327–338. [PubMed: 10069338]
12. Clyne PJ, Warr CG, Carlson JR. Candidate taste receptors in Drosophila. Science 2000;287:1830–1834. [PubMed: 10710312]
13. Scott K, et al. A chemosensory gene family encoding candidate gustatory and olfactory receptors in Drosophila. Cell 2001;104:661–673. [PubMed: 11257221]
14. Dunipace L, et al. Spatially restricted expression of candidate taste receptors in the Drosophila gustatory system. Curr Biol 2001;11:822–835. [PubMed: 11516643]
15. Sato K, et al. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 2008;452:1002–1006. [PubMed: 18408712]
16. Wicher D, et al. Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 2008;452:1007–1011. [PubMed: 18408711]
17. Nelson G, et al. An amino-acid taste receptor. Nature 2002;416:199–202. [PubMed: 11894099]
18. Nelson G, et al. Mammalian sweet taste receptors. Cell 2001;106:381–390. [PubMed: 11509186]

19. Li X, et al. Human receptors for sweet and umami taste. Proceedings of the National Academy of Sciences of the United States of America 2002;99:4692–4696. [PubMed: 11917125]
20. Ishimaru Y, et al. Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proceedings of the National Academy of Sciences of the United States of America 2006;103:12569–12574. [PubMed: 16891422]
21. Loconto J, et al. Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. Cell 2003;112:607–618. [PubMed: 12628182]
22. Larsson MC, et al. Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 2004;43:703–714. [PubMed: 15339651]
23. Lu M, Echeverri F, Moyer BD. Endoplasmic reticulum retention, degradation, and aggregation of olfactory G-protein coupled receptors. Traffic (Copenhagen, Denmark) 2003;4:416–433.
24. Lu M, et al. Endoplasmic reticulum degradation impedes olfactory G-protein coupled receptor functional expression. BMC Cell Biol 2004;5:34. [PubMed: 15369603]
25. Gimelbrant AA, et al. Truncation releases olfactory receptors from the endoplasmic reticulum of heterologous cells. Journal of neurochemistry 1999;72:2301–2311. [PubMed: 10349839]
26. McClintock TS, et al. Functional expression of olfactory-adrenergic receptor chimeras and intracellular retention of heterologously expressed olfactory receptors. Brain research 1997;48:270–278. [PubMed: 9332724]
27. Saito H, et al. RTP family members induce functional expression of mammalian odorant receptors. Cell 2004;119:679–691. [PubMed: 15550249]
28. Zhuang H, Matsunami H. Synergism of accessory factors in functional expression of Mammalian odorant receptors. The Journal of biological chemistry 2007;282:15284–15293. [PubMed: 17387175]
29. Yoshikawa K, Touhara K. Myr-Ric-8A Enhances G{alpha}15-Mediated Ca2+ Response of Vertebrate Olfactory Receptors. Chemical senses. 2008
30. Von Dannecker LE, Mercadante AF, Malnic B. Ric-8B promotes functional expression of odorant receptors. Proceedings of the National Academy of Sciences of the United States of America 2006;103:9310–9314. [PubMed: 16754875]
31. Krautwurst D, Yau KW, Reed RR. Identification of ligands for olfactory receptors by functional expression of a receptor library. Cell 1998;95:917–926. [PubMed: 9875846]
32. Mombaerts P. Genes and ligands for odorant, vomeronasal and taste receptors. Nature reviews 2004;5:263–278.
33. Shirokova E, et al. The human vomeronasal type-1 receptor family--detection of volatiles and cAMP signaling in HeLa/Olf cells. FASEB J 2008;22:1416–1425. [PubMed: 18096815]