although the regulatory role of GDP versus GTP binding is unknown. Using a constitutively active form of GPA1 (which does not require GTP for activation), Pandey et al. have elegantly shown that the interaction of the GTG proteins with GTP-GPA1 inhibits their GTPase activity. The regulation of a GPCR-type protein by GTP-GPA1 is unprecedented. If GTG1 and GTG2 act like regular GPCRs, one would expect that ABA perception leads to GTP-GPA1 formation, which subsequently inhibits GTG1 and GTG2 GTPase activity. This finding is, however, difficult to reconcile with the *gpa1* and *gtg1 gtg2* single or double mutant phenotypes. Indeed, GPA1 and GTGs play different roles in stomatal regulation. GPA1 is required for inhibition of stomatal reopening by ABA, whereas GTG1 and GTG2 are required for ABA to induce stomatal closure. The occurrence of epistatic interactions in a *gpa1 gtg1 gtg2* triple mutant would provide evidence that GPA1 and the GTGs indeed act in the same signaling pathway, as implied by the biochemical analyses. In the current study, GTP-GPA1 is proposed to function as a rheostat downregulating ABA binding to GTGs. Binding of ABA to GTG1 and GTG2 did not affect their GTPase activity; however, the analyses were performed with purified proteins and only approximately 1% of GTG1 and GTG2 were functional in binding the phytohormone. Expression and functional analysis of GTG1 and GTG2 in a more physiological environment, such as membranes of an organism or cells devoid of an ABA signal pathway, could uncover their mode of regulation.

The identification of GTG1 and GTG2 reveals fascinating insights into a new class of integral membrane-localized G proteins of eukaryotes. The prediction that they represent ABA receptors will undoubtedly motivate further study. Future work establishing a robust link to central components of the ABA signaling pathway could bolster the claim that GTGs are ABA receptors. Such components would, for example, include the homologous protein phosphatases ABI1 and ABI2, which are key regulators of early steps in the ABA signal transduction cascade.

REFERENCES

Bhasin, M., and Raghava, G.P. (2005). Nucleic Acids Res. *33*, W143–W147.

Christmann, A., Moes, D., Himmelbach, A., Yang, Y., Tang, Y., and Grill, E. (2006). Plant Biol. *8*, 314–325.

Eng, E.T., Jalilian, A.R., Spasov, K.A., and Unger, V.M. (2008). J. Mol. Biol. *375*, 1086–1097.

Hamilton, D.W., Hills, A., Kohler, B., and Blatt, M.R. (2000). Proc. Natl. Acad. Sci. USA *97*, 4967–4972.

Levchenko, V., Konrad, K.R., Dietrich, P., Roelfsema, M.R., and Hedrich, R. (2005). Proc. Natl. Acad. Sci. USA *102*, 4203–4208.

Maeda, Y., Ide, T., Koike, M., Uchiyama, Y., and Kinoshita, T. (2008). Nat. Cell Biol. *10*, 1135–1145.

Pandey, S., Chen, J.G., Jones, A.M., and Assmann, S.M. (2006). Plant Physiol. *141*, 243–256.

Pandey, S., Nelson, D.C., and Assmann, S.M. (2009). Cell, this issue.

Razem, F.A., El-Kereamy, A., Abrams, S.R., and Hill, R.D. (2008). Nature *456*, 824.

Vahisalu, T., Kollist, H., Wang, Y.F., Nishimura, N., Chan, W.Y., Valerio, G., Lamminmaki, A., Brosche, M., Moldau, H., Desikan, R., et al. (2008). Nature *452*, 487–491.

A New Family of Odorant Receptors in *Drosophila*

Maria L. Spletter[1,2] and Liqun Luo[1,2,*]
[1]Howard Hughes Medical Institute
[2]Department of Biology
Stanford University, Stanford, CA 94305, USA
*Correspondence: lluo@stanford.edu

DOI 10.1016/j.cell.2008.12.031

In the fruit fly *Drosophila*, not all olfactory sensory neurons express a seven transmembrane odorant receptor, suggesting that other types of odorant receptors might exist. Benton et al. (2009) now present evidence that a family of proteins related to ionotropic glutamate receptors is a previously unrecognized class of odorant receptors.

Odor detection is accomplished by odorant receptors, originally identified in rodents as a large family of seven transmembrane G protein-coupled receptors (GPCRs). Odorant receptors have subsequently been found in fish and nematodes, and eventually in the fruit fly *Drosophila* (Bargmann, 2006). Surprisingly, *Drosophila* seven transmembrane odorant receptors (ORs) were recently found to have inverted membrane topology compared to typical GPCRs, with their N terminus facing the cytoplasm rather than the extracellular space (Benton et al., 2006). Additionally, *Drosophila* ORs require Or83b, another seven transmembrane protein highly conserved in insects, as an obligate coreceptor (Lars-

son et al., 2004). Indeed, evidence suggests that OR/Or83b complexes form ligand-gated ion channels (Sato et al., 2008; Wicher et al., 2008), a striking difference to GPCRs in worms and vertebrates that rely on second messengers to activate ion channels. In this issue, Benton et al. (2009) report the identification of a new class of odorant receptors that are related to ionotropic glutamate receptors, thus expanding the known repertoire of odorant receptors beyond the classical family of seven transmembrane receptors.

In the *Drosophila* olfactory system, olfactory sensory neurons (OSNs, also known as olfactory receptor neurons or ORNs) located in the antennae and maxillary palps send axons to the antennal lobe in the central brain. OSN dendrites are present in sensory organs called sensilla, where they are exposed to the environment, and the different types of sensilla (basiconic, coeloconic, and trichoid) respond to different types of odorants (Figure 1). Just as in the mammalian olfactory system, most *Drosphila* OSNs express a single OR, and OSN axons expressing the same OR converge on the same glomerulus in the antennal lobe (Vosshall and Stocker, 2007). Nearly every OR has been genetically mapped for its expression in specific sensilla and for its corresponding OSN axon projection to a specific glomerulus (Couto et al., 2005). With one exception, all ORs are expressed in basiconic and trichoid OSNs. Or35a is expressed in coeloconic OSNs which project to only one of eight glomeruli predicted to be targets of coeloconic OSNs. Exhaustive study of ORs and gustatory receptors (two gustatory receptors are coexpressed in CO_2-sensing OSNs, see Figure 1) still leaves most coeloconic OSNs unaccounted for with regard to the identity of the odorant receptors they express (Couto et al., 2005). This indicated that an unidentified class of odorant receptors could be present in most coeloconic OSNs. Benton et al. (2009) now present convincing evidence that a family of proteins related to ionotropic glutamate receptors fill this role.

Benton et al. identified this family of receptors, which they named ionotropic receptors (IRs), in a bioinformatic screen for genes expressed in the fly antennae.

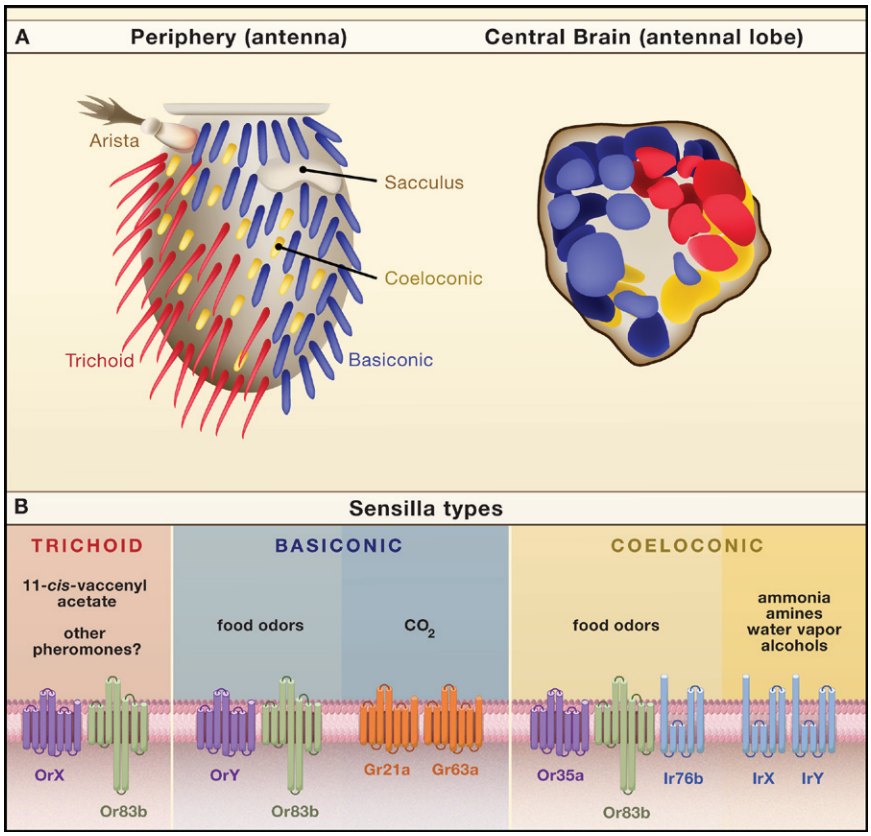

Figure 1. Odorant Receptors in *Drosophila*

(A) A schematic of an antenna and the antennal lobe of the fruit fly *Drosophila*. Basiconic, trichoid, and coeloconic sensilla containing olfactory sensory neurons (OSNs) are present in overlapping domains on the antenna (left). OSNs in these sensilla send axonal projections to distinct regions in the antennal lobe in the central brain, with axons expressing the same odorant receptor converging on a single glomerulus (right). Ionotropic receptors (IRs) are expressed in coeloconic sensilla, as well as in neurons of the arista and sacculus.

(B) Summary of odorant sensitivities and odorant receptor expression in different types of sensilla. In *Drosophila*, classical seven transmembrane odorant receptors (ORs) have a reversed membrane topology compared to typical GPCRs. Or83b, another seven transmembrane protein highly conserved in insects, is an obligate coreceptor for other ORs. Although most basiconic OSNs express ORs, one class of basiconic OSNs express two gustatory receptors (GRs) involved in the detection of CO_2. Benton et al. (2009) show that IRs act as odorant receptors in coeloconic sensilla.

Subsequent BLAST searches revealed a family of 61 genes in *Drosophila*. Despite having a similar modular organization to the ionotropic glutamate receptors extensively studied for their role in synaptic transmission, such as AMPA and NMDA receptors, the IRs show wide divergence at the sequence level and in particular show considerable variations in residues known to be important for glutamate binding. Thus, these IRs are unlikely to be glutamate receptors, but may instead bind other ligands.

Multiple lines of evidence indicate that IRs are indeed the missing coeloconic odorant receptors. First, mRNAs of 15 IRs are specifically expressed in neurons of the adult antenna. Second, an antibody that specifically recognized one IR protein stained the dendrites of OSNs, consistent with a role in odor detection. Third, the IR-positive OSNs are distinct from Or83b-expressing OSNs, as assayed by double in situ analysis (with the exception of coeloconic OSNs coexpressing Or35a/Or83b/Ir76b). Fourth, in mutants that disrupt coeloconic sensilla development, IR expression is lost. Fifth, IR expression maps into four clusters of neurons corresponding to the four coeloconic sensilla types previously characterized physiologically (Yao et al., 2005). Sixth, just as OSNs expressing a common OR project their axons to a single glomerulus, axons labeled by Ir76a-GAL4 converge to a single glomerulus

previously identified as having coeloconic input. Finally, misexpression of IRs is sufficient to bestow new odor sensitivity to coeloconic OSNs, indicating that IRs determine odor specificity.

There appear to be important organizational differences between OSNs that express IRs and those that express ORs. Multiple IRs can be coexpressed per neuron, which is strikingly different than OR expression, which generally follows a one neuron-one receptor rule. This may reflect a functional requirement for more than one IR to form a functional channel. NMDA receptors, for instance, are a tetramer composed of different subunits. Although Ir76a axons target a single glomerulus, this may not be a general rule, as Ir76a is one of the few IRs expressed in a single class of coeloconic OSNs. The odor responses of IR-expressing OSNs may be defined by expression of a particular set of IRs rather than a single IR gene, so a given IR may label axons projecting to multiple glomeruli.

These important results by Benton et al. lend themselves to several lines of future study. Biologically, the identification of IRs should allow comprehensive mapping of odor specificity and glomerular projection of specific IR-expressing OSNs. It is already known that a division of labor exists between the trichoid sensilla that putatively sense pheromones and the basiconic sensilla that sense food odorants, and this division is even propagated to higher olfactory centers (Jefferis et al., 2007). Future studies will determine whether coeloconic OSNs have particular odor specialties and how this information is represented in the brain. Mechanistically, it is important to extend the sufficiency test beyond ectopic expression of IRs in other coeloconic OSNs. For instance, reconstituting the odor response in ectopic cells such as *Xenopus* oocytes would allow the dissection of biochemical and biophysical properties of the IR receptor/channel complex.

The identification of IRs as odorant receptors also raises interesting evolutionary questions. Ionotropic glutamate receptors at synapses and IRs in odor detection both function by sensing molecules in the extracellular environment, an ancient faculty dating back to prokaryotes (Bargmann, 2006). Various families of "chemosensors" may have been coopted numerous times through evolution for purposes as diverse as olfaction, immunity, and neurotransmission. A second consideration is the difference between olfaction in insects and other animals, namely that insect odorant receptors could function independently from second messengers. Whether this distinction allows some evolutionary advantage, such as a more rapid signaling, is yet to be determined. One thing is certain: insights from *Drosophila* olfaction continue to yield surprises while deepening our understanding of general biological principles.

REFERENCES

Bargmann, C.I. (2006). Nature *444*, 295–301.

Benton, R., Sachse, S., Michnick, S.W., and Vosshall, L.B. (2006). PLoS Biol. *4*, e20.

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Cell, this issue.

Couto, A., Alenius, M., and Dickson, B.J. (2005). Curr. Biol. *15*, 1535–1547.

Jefferis, G.S., Potter, C.J., Chan, A.M., Marin, E.C., Rohlfing, T., Maurer, C.R., Jr., and Luo, L. (2007). Cell *128*, 1187–1203.

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., and Vosshall, L.B. (2004). Neuron *43*, 703–714.

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., and Touhara, K. (2008). Nature *452*, 1002–1006.

Vosshall, L.B., and Stocker, R.F. (2007). Annu. Rev. Neurosci. *30*, 505–533.

Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). Nature *452*, 1007–1011.

Yao, C.A., Ignell, R., and Carlson, J.R. (2005). J. Neurosci. *25*, 8359–8367.