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COMMENTARY

The structure of Orco and its impact on our understanding of olfaction

Frank Zufall[1] and Ana I. Domingos[2,3]

The olfactory system is able to discriminate between an almost unlimited number of odorant molecules. It does so by means of specific receptors expressed on the surface of olfactory sensory neurons. Mammalian odorant receptors comprise a large family of G protein–coupled receptors (GPCRs), numbering ∼1,100 expressed odorant receptors (ORs) in the mouse genome, which couple to cyclic nucleotide–gated cation channels via a second messenger pathway. However, insect ORs comprise a more modest family, around 60 in *Drosophila melanogaster*, which form heteromultimers containing a divergent OR subunit conferring odor specificity and the highly conserved and essential co-receptor Orco. In 2008, two independent studies revealed that the OR/Orco complex formed nonselective cation channels. Now, a landmark study by Butterwick et al. has solved the cryo-EM structure of an Orco homomer at 3.5-Å resolution, providing structural insight into this large and unique family of ion channels.

During his doctoral thesis in the late 1980s in Munich, Germany, one of us (Zufall) was given the task to investigate the chemoelectrical signal transduction mechanism of insect olfactory sensory neurons. Although he was able to make some progress on this question (Zufall and Hatt, 1991), had he known that it would take the field nearly 30 yr to get to the core of this problem, he probably would have looked for another project. The other one of us (Domingos) moved from Lisbon, Portugal, to New York City in early 2000 to perform her doctoral thesis in the group of Leslie Vosshall at Rockefeller University. It was there where she made an important contribution to understand the function of a *D. melanogaster* odorant receptor gene, then known as *Or83b* (Larsson et al., 2004) but now called *Orco*, short for "olfactory receptor co-receptor" (Vosshall and Hansson, 2011). Here we have teamed up to consider the functional consequences of the recent cryo-EM structure of the insect olfactory receptor Orco (Butterwick et al., 2018). At the latest Annual Meeting of the European Chemoreception Organization (ECRO) in Würzburg, Germany, our colleague Bill Hansson called this work by the group of Vanessa Ruta nothing short of a "quantum leap." What is it that makes the work of Butterwick et al. (2018) so remarkable? To address this question, we will first discuss some of the background that will be necessary to appreciate the functional role of Orco and its structure.

A central problem in olfaction has always been how the olfactory system recognizes and discriminates an almost unlimited number of chemical cues in the environment, and how the binding of odorant molecules to specific receptors is then transformed into an electrical signal within specialized nerve cells, the olfactory sensory neurons, in a process that we call chemosensory transduction (Zufall and Munger, 2016). Ever since the discovery that olfaction in the mammalian olfactory epithelium is mediated by a large family of GPCRs, known as ORs, from the rhodopsin-related superfamily (Buck and Axel, 1991), together with the finding that these receptors couple through a G protein cascade to a cAMP-mediated second messenger pathway that opens cyclic nucleotide–gated cation channels (Pace et al., 1985; Nakamura and Gold, 1987; Zufall et al., 1994), our view of olfactory signal transduction has been somewhat GPCR centric. Subsequent discoveries of additional GPCR families in the mammalian nose—the vomeronasal receptor type 1 and type 2 families and the formyl peptide receptors expressed in the vomeronasal organ, as well as the trace amine–associated receptors expressed in the main olfactory epithelium—all supported the view that chemosensory transduction is a process that depends on GPCRs coupled through a second messenger pathway to a dedicated ion channel, albeit with specific adaptations in the identity of the G proteins, enzymes, second messengers, and channels for each receptor family and chemosensory cell type (Zufall and Munger, 2016). This GPCR-centric view was further strengthened by parallel studies revealing the receptors and second messenger pathways for mammalian taste (Chandrashekar et al., 2006). Furthermore, in an effort to understand invertebrate olfaction, the genome of the nematode worm *Caenorhabditis elegans* was found to encode more than 1,500 predicted GPCRs in nematode-specific families (Troemel et al., 1995; Bargmann, 2006). But it is important to

[1]Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany ; [2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK; [3]Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Correspondence to Frank Zufall: frank.zufall@uks.eu.

note that there is now also abundant evidence for noncanonical, non-GPCR chemosensation mechanisms in both *C. elegans* and the mammalian nose. For example, several cGMP-dependent detection and signaling mechanisms have evolved that use either receptor guanylate cyclases (membrane receptor–enzyme complexes) or soluble guanylate cyclase homologues (Bargmann, 2006; Leinders-Zufall et al., 2007; Bleymehl et al., 2016).

Against the background of these groundbreaking developments, it is easy to understand that initial efforts to discover insect odorant receptor families were focused on GPCRs. Surprisingly, however, and as we know today, the story of insect ORs became a very different one. After intensive efforts, a family of over 60 ORs was identified in olfactory sensory neurons of *D. melanogaster* (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). This relatively small number raised the immediate question of how the vast number of odorants can be discriminated by those relatively few receptors. Subsequently, it became clear that insect ORs are very different from mammalian GPCRs (Kaupp, 2010). In fact, insect ORs are not GPCRs; they adopt an inverse heptahelical topology and are expressed as heteromultimers composed of a divergent OR subunit that confers odor specificity and the highly conserved co-receptor Orco. Orco appears to be functionally orthologous across all insects (Jones et al., 2005) and its genetic disruption in the fruit fly (Larsson et al., 2004), in mosquitoes (DeGennaro et al., 2013), and most recently in ants (Trible et al., 2017; Yan et al., 2017) confirmed its essential role for insect olfaction at the molecular, cellular, and behavioral levels. In the absence of Orco, ORs cannot assemble, traffic, or function (Larsson et al., 2004; Benton et al., 2006). Together, these studies, as well as others that cannot be referenced here because of space limitations, established that Orco occupies a central and critical position in understanding and targeting insect olfaction. A recent review by Suh et al. (2014) stated that "the characterization of the ubiquitous, highly conserved, and insect-specific Orco odorant receptor co-receptor has opened the door to the design and development of novel insect control methods to target agricultural pests, disease vectors, and even nuisance insects."

How does the OR/Orco complex function in detail? In 2008, two papers published back to back (Sato et al., 2008; Wicher et al., 2008) set out to address this question and to define the signal transduction mechanism of insect olfactory sensory neurons. Both papers reached an important conclusion, namely, that insect olfactory receptors are heteromeric ligand-gated ion channels that constitute a new class of nonselective cation channels. Sato et al. (2008) stated that their results "support the hypothesis that the complex between OR and Or83b itself confers channel activity." Wicher et al. (2008) stated that "application of odorants to mammalian cells co-expressing Or22a and Or83b results in nonselective cation currents activated by means of an ionotropic and a metabotropic pathway." And further: "Expression of Or83b alone leads to functional ion channels not directly responding to odorants, but being directly activated by intracellular cAMP and cGMP" (Wicher et al., 2008). In contrast, Sato et al. (2008) failed to observe a G protein–dependent cyclic nucleotide activation of Or83b alone. Although these data provided compelling evidence that a heteromeric OR/Orco complex functions as a nonselective cation channel directly gated by odor or pheromone ligands, they also raised several new questions and disagreements that were intensely debated in the field (Nakagawa and Vosshall, 2009; Kaupp, 2010; Stengl and Funk, 2013; Suh et al., 2014). Indeed, one study found that the Orco family can form functional ion channels in the absence of an odor-binding OR and can be gated by a chemical agonist known as VUAA1, but cannot be activated by membrane-permeant cAMP or cGMP analogues (Jones et al., 2011). As a consequence, there is currently no single agreed-upon model for insect odor transduction.

In hindsight, and on the basis of the evidence summarized above, it seems logical that someone would set out to advance the field by providing a detailed molecular structure of Orco. The work by Butterwick et al. (2018) now provides the first cryo-EM structure for any olfactory receptor, and offers important new insight to understand the mechanism of insect peripheral olfaction. The authors were able to solve the single-particle cryo-EM structure of an Orco homomer from the parasitic fig wasp *Apocrypta bakeri*, at a resolution of 3.5 Å. Using whole-cell and single-channel recordings in excised patches, the authors showed that heterologous expression of *A. bakeri* Orco results in the formation of Ca^{2+}-permeable cation channels activated by the agonist VUAA1. The channel recordings had a single-channel conductance of ∼10 pS, as determined from the slope of current–voltage plots. The authors also presented new insight into the architecture of the Orco homotetramer—the channel pore, ion selectivity, and the extracellular gate—and the way in which Orco subunits may interact with ORs. Together, these data provided conclusive "structural and functional confirmation that insect olfactory receptors form a novel class of heteromeric ligand-gated ion channels" (Butterwick et al., 2018). Below are a few of the key findings from this work:

Overall structure of the Orco homotetramer

The work of Butterwick et al. (2018) reveals that each Orco subunit comprises seven membrane-spanning helical segments (S1–S7) with an amino terminus that lies intracellularly and a carboxy terminus that lies extracellularly. Viewed from the extracellular surface, Orco forms a tetrameric pinwheel with four subunits encircling a central pore. The authors identify an intracellular "anchor domain," which forms interactions between subunits and thus seems to anchor the four loosely packed subunits within the lipid membrane. Within the extracellular leaflet, the S1–S6 helices form a crevice that is likely to serve as a binding site for VUAA1 and other small molecules that gate the channel, fully consistent with several previous mutation studies by others.

The ion conduction pathway

A single helix from each subunit, S7b, lines the ion conduction pathway of Orco, in stark contrast to classical tetrameric cation channels, whose central pores normally comprise multiple helices. The pore is narrowest near the extracellular end, where it is too small for hydrated ions to pass; thus, this structure of Orco appears to represent a closed state. Overall, the pore of Orco resembles acid-sensing ion channels and ATP-gated P2X channels.

Gating and ion selectivity

Butterwick et al. (2018) measured ion selectivity in the expressed channel under bi-ionic conditions by analyzing shifts in the re-

versal potential of ionic currents. Na^+, K^+, and Cs^{2+} pass through the pore as readily as each other, and more readily than Ca^{2+} or Mg^{2+}, suggesting that ions with smaller hydrated radii permeate more easily. A series of point mutations revealed how hydrophobic residues at the extracellular constriction (the hydrophobic gate) contribute to ion selectivity by the channel. A particularly interesting finding is that the Ca^{2+} permeability of heteromeric OR/Orco complexes depends also on the identity of the OR, indicating that both subunits contribute to the conduction pathway in the heteromeric receptor. Additional experiments provided insight into gating mechanisms of the ion pathway by altering the sensitivity to VUAA1 or to a cognate odorant ligand.

Interaction of Orco with ORs

By aligning OR sequences from four distantly related insect species separated by nearly 400 million years of evolution, the authors gained insight into the pattern of relative sequence conservation. By mapping these sites to key interaction domains in Orco, potential interactions between ORs and Orco could be revealed. On the basis of this analysis, the authors proposed that the Orco homotetramer can be used as a structural template for OR/Orco heteromers—more specifically, that Orco subunits can be replaced with one or more ORs and the complex will still adopt a similar overall architecture.

It should be possible in the near future to provide a structure of OR/Orco heteromeric complexes, which have been characterized functionally by Butterwick et al. (2018). In turn, using such structural advances as a guide will enable the design of new experiments aimed at a better understanding of the in vivo function of OR/Orco complexes in various insect species. As in vertebrate olfactory sensory neurons (Zufall and Leinders-Zufall, 2000), we also expect a variety of mechanisms for Ca^{2+}-dependent feedback regulation and other modulatory pathways in insect olfaction. The pace of discovery in insect olfactory systems is likely to speed up dramatically in the near future.

What are the major implications of this study? For one, as stated by the authors, the architecture of Orco now provides a framework for understanding the enormous diversity of odor receptors and their ability to discriminate odorants across insect lineages. Given that a single Orco must assemble with up to hundreds of distinct ORs within a given species, the Orco structure ultimately may help to understand both conservation and variability of the insect OR family at a molecular level. Butterwick et al. (2018) propose that the OR/Orco receptor–channel complexes are likely to be the largest family of ion channels in nature, with "many hundreds of thousands of different variants distributed across the hundreds of thousands of insect species." Therefore, the work by Butterwick et al. (2018) certainly will have a major impact on subsequent investigations directed at understanding odor coding mechanisms in insects and the rest of the animal kingdom and on the ion channel field in general. A second major implication of this work is its promise to further aid the design and development of novel insect control methods—investigations that are already well underway (for examples, see DeGennaro et al., 2013; Suh et al., 2014).

Finally, one would hope that it should be possible to transfer the knowledge obtained from the Orco structure to nonionotropic olfactory receptors. For instance, in the mammalian vomeronasal organ, type 2 vomeronasal receptors (V2rs) of family ABD are coexpressed with other widely expressed V2rs of family C, as well as with nonclassical MHC molecules known as H2-Mv (Chamero et al., 2012). The implication is that these diverse molecules should form a large number of heteromultimeric receptor complexes that should vastly enhance the combinatorial and discriminative power of the resulting receptor proteins and the vomeronasal sensory neurons that express them. A similar situation exists for some of the mammalian taste receptors (Chandrashekar et al., 2006). Advances in heterologous expression methodology, together with combined structural analyses, are now badly needed for those and all other chemoreceptor families.

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