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Phosphorylation Switch of Orco Shapes the Sense of Smell in Insects

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Review of Guo et al.

Neuronal adaption exists in all sensory systems. With continuous exposure to one stimulus, sensory neurons often raise their sensitivity threshold and thereby reduce their subsequent response to stimuli of the same intensity. This adaptation allows sensory neurons to extend the dynamic range of their responsiveness. Although much research has focused on sensory transduction, little is known about mechanisms that underlie sensory adaption.

Sensory systems of insects have been established as models for studying sensory processing and plasticity. In *Drosophila*, odorants are detected by olfactory receptor neurons in sensilla. Most of the neurons express one odorant coreceptor (Orco) and one olfactory tuning receptor (OrX) that is either broadly or narrowly tuned to odorants. Although accumulating evidence indicates that olfactory adaptation occurs in *Drosophila* (Wilson, 2013), the mechanisms of adaptation, and how they operate over different timescales, remain to be understood. A recent article in *The Journal of Neuroscience* by Guo et al. (2017) reports that phosphorylation regulation at one specific amino acid residue (S289) in Orco shapes neuronal responses upon prolonged odorant preexposure.

Guo et al. (2017) first identified a conserved phosphorylation site of Orco that regulates olfactory activity by recording electrophysiological responses to the male-specific pheromone *cis*-vaccenyl acetate (cVA) in antennal trichoid neurons. cVA sensitivity was reduced in a phosphodeficient Orco mutant (S289A), and increased in a phosphomimetic Orco mutant (S289D), suggesting that the phosphorylation state of this specific residue plays an important role in modulating olfactory responses (Guo et al., 2017, their Figs. 1*F*, 2*A*). Immunohistochemistry experiments with an S289 phospho-specific antibody revealed that the observed variation in sensitivity was not a result of altered receptor trafficking in the dendritic cilia of olfactory neurons (Guo et al., 2017, their Figs. 3*B*, 4*E*), suggesting that the phosphorylation state of this residue affects efficacy of the olfactory receptor complex. Interestingly, the authors observed reduced levels of phosphorylated Orco after 30 min of odor exposure, and this trend was reversed after the odor was removed (Guo et al., 2017, their Fig. 4*F*). Together, the results suggest that dephosphorylation of Orco contributes to olfactory adaptation by reducing neuronal sensitivity after prolonged odor exposure.

If dephosphorylation of Orco after prolonged odor exposure is solely responsible for olfactory adaptation, there should be no desensitization of olfactory responses in phosphodeficient Orco mutants (S289A) because they cannot undergo dephosphorylation. But when Guo et al. (2017) preexposed S289A mutant flies to cVA for 1 h, the neurons retained the ability to be desensitized to cVA, albeit at a reduced level (Guo et al., 2017, their Fig. 2*D*). This implies that other factors contribute to response attenuation as well. Therefore, the authors asked whether additional phosphorylation sites were involved.

There are five putative PKC phosphorylation sites in Orco, including S289. Previous studies have generated a mutant phosphodeficient at all five residues in which olfactory sensitivity is decreased (Sargsyan et al., 2011; Getahun et al., 2013, 2016) (Fig. 1). Yet, the contribution of each phosphorylation residue in neuronal desensitization has not been tested. Guo et al. (2017) first created phosphodeficient Orco mutants for each of the three intracellular PKC residues and only observed changes in olfactory sensitivity in the S289A mutant. Nonetheless, whether the two extracellular PKC phosphorylation sites also contribute to modulating olfactory sensitivity remains an open question.

Interestingly, transient or sustained odor stimulation can increase or decrease the

Received Nov. 2, 2017; revised Dec. 18, 2017; accepted Dec. 21, 2017.

Y.-C.D.C. is a Howard Hughes Medical Institute International Student Research Fellow. We thank Dr. Anupama Dahanukar, Dr. Ryan Joseph, and Christi Scott for helpful comments and editorial assistance on the manuscript.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.3157-17.2017

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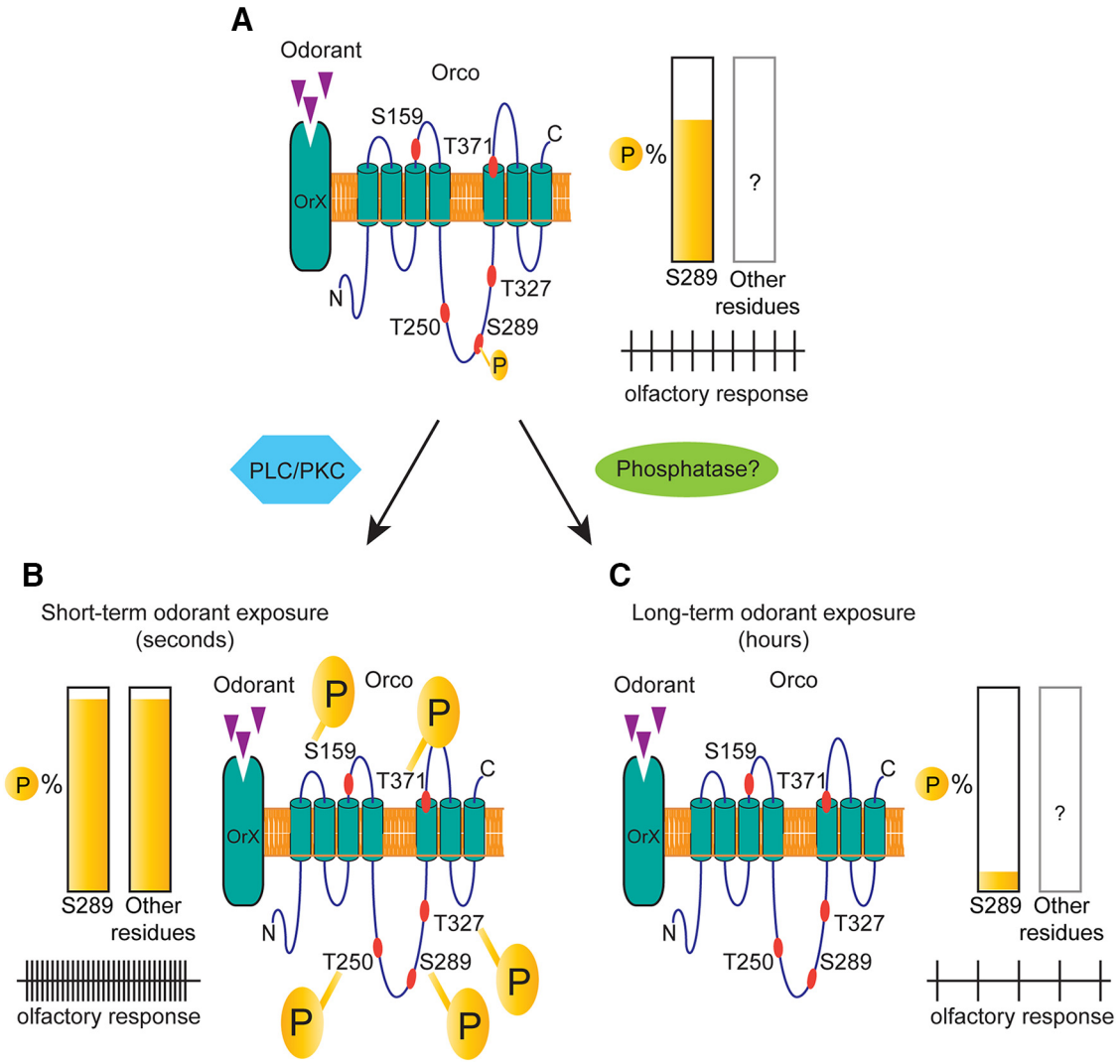

Figure 1. A proposed model for the modulation of olfactory responses by Orco phosphorylation over time. ***A***, Olfactory responses and phosphorylation status of Orco without preexposure to odor. An olfactory-receptor-neuron-specific tuning protein (OrX) and Orco form a receptor complex for detecting odorant molecules (purple triangles). Red represents five PKC phosphorylation residues. There is a basal level of phosphorylation at S289 of Orco. ***B***, Olfactory responses and phosphorylation status of Orco with short-term odor preexposure. When the receptor is preexposed to an odorant for a brief period (i.e., seconds), Orco undergoes PLC/PKC-dependent phosphorylation, increasing the number of phosphorylated residues, thereby potentiating the olfactory responses (higher spike frequency) to future odorant exposure. ***C***, In contrast, odorant preexposure over a longer time course (i.e., hours) leads to a reduction in the number of phosphorylated S289 residues, possibly through unidentified phosphatase-mediated mechanisms. The reduction in S289 phosphorylation desensitizes the olfactory neurons, thereby attenuating subsequent odorant-induced activity (lower spike frequency). ***A***, ***C***, Question marks indicate that the phosphorylation status for other residues has not been tested in these conditions.

Orco phosphorylation, leading to greater or less sensitivity, respectively (Fig. 1). The increased phosphorylation of Orco observed in response to brief and intermittent odor exposure (Sargsyan et al., 2011; Getahun et al., 2013, 2016) (Fig. 1*B*) might facilitate tracking concentration gradients of odor plumes during flight (Nagel and Wilson, 2011; Getahun et al., 2012), by making initially subthreshold concentrations perceptible. Conversely, the decreased phosphorylation of Orco S289 observed in Guo et al. (2017) occurs over a longer exposure period (>30 min) and leads to long-term olfactory adaptation (Fig. 1*C*). If both short- and long-term olfactory stimulation involves phosphorylation of the same Orco residues, it will be of interest to determine how and when in the course of odor exposure the effect switches from phosphorylation to dephosphorylation of Orco. It is possible, however, that short- and long-term olfactory stimulation has different effects on the overall pattern of phosphorylation of Orco, and this pattern determines whether sensitivity is increased or decreased. This type of regulation by the overall pattern of phosphorylation can be observed in long-term light adaptation in the visual system, where phosphorylation of different residues of the TrpL sensory receptor exerts different effects in response to different light conditions (Cerny et al., 2013). This hypothesis is consistent with the observed residual desensitization in Orco S289A and S289D mutants (Guo et al., 2017, their Fig. 2*D*), suggesting that as yet unidentified residues contribute to desensitization of olfactory responses. Future studies that examine how short- and long-term odor exposure affects phosphorylation at individual Orco sites and how phosphorylation of these sites affects olfactory responses will help to clarify this issue.

To distinguish whether Orco phosphorylation is regulated by binding of an odorant to the receptor complex or by subsequent depolarization of olfactory neurons, Guo et al. (2017) used optogenetics to depolarize olfactory sensory cells independently of odorant binding. Light activation mimicked odor exposure, leading to both a decrease in Orco phosphorylation and desensitization of neuronal

responses to cVA (Guo et al., 2017, their Fig. 6). This suggests that these effects are triggered by depolarization, rather than conformational changes in the receptor induced by odorant binding. Future investigations examining olfactory responses over different timescales by similar optogenetic activation in mutants of other Orco phosphorylation residues would uncover additional roles of individual phosphorylation residues in both short- and long-term olfactory adaptation.

In conclusion, Guo et al. (2017) demonstrated that phosphorylation of a specific residue (S289) in Orco reduces olfactory responses upon prolonged odor exposure. Orco is evolutionarily conserved across insect species. Modulation of olfactory responses by the phosphorylation status of Orco S289 could lead to discovery of new generations of insect repellents against insect vectors and agricultural pests. Recently, a screening system for small molecules that targets phosphorylation-dependent protein–protein interaction has been established (Watanabe and Osada, 2016). Future efforts in identifying small molecules that block phosphorylation-dependent interactions of Orco might potentiate insect repellency by reducing adaptation to repellents.

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