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An expression system for Gustatory receptors—and why it failed

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A recent paper by the Dahankuar laboratory suggested that single *Drosophila* sugar receptors proteins accurately mediate sugar detection when ectopically expressed in olfactory neurons of the antenna. These findings contradict numerous previously published electrophysiological and behavioral investigations, which all point towards heteromultimeric sugar taste receptors. Here, I provide some explanation why this "pseudo-heterologous" expression system may have led to this misleading conclusion.

Freeman, Wisotsky and Dahanukar recently described an ectopic expression system for insect gustatory receptors (Grs).[1] Such a system, if faithfully reporting receptor-ligand interaction observed in taste neurons, would provide an invaluable tool for matching the large number of insect taste receptors, which, depending on the species, range from about a dozen to more than 100, with specific ligands.[2] Using olfactory neurons as the cellular vehicle, Freeman and colleagues find that single sugar Gr proteins alone are capable of mediating responses to various sugars, a contention at odds with published data from several other laboratories, which have provided several lines of evidence that taste receptors function as heteromeric complexes of 2 or more Gr subunits. The prowess—and ultimately usefulness—of any ectopic or heterologous expression system demands that the components whose functions it analyzes are not expressed in this system, and it would seem appropriate that extra precaution is used when new findings contradict published work. Unfortunately, the Dahanukar group failed to do so.

The strategy employed by Dahanukar and co-workers used the *GAL4/UAS* system to drive expression of insect sugar *Gr* genes in the ab1C olfactory sensory neurons (OSNs). Ab1C neurons are unusual compared to most other OSNs in that they appear to be devoid of odorant receptors (Ors) or ionotropic chemoreceptors (Irs). Instead, they express a narrowly tuned carbon dioxide receptor formed by 2 Gr proteins, Gr21a and Gr63a.[3,4] By expressing single sugar *Gr* genes, the authors recorded firing patterns of ab1 sensilla injected with various sugar solutions.[1] For example, 4 receptors (Gr5a, Gr64f, Gr64e or Gr64b) conferred neural responses to trehalose, glucose and melezitose, and 2 receptors (Gr64b and Gr61a) to glucose. Likewise, Gr64a, Gr64c or Gr64d alone were sufficient to induce firing in ab1C olfactory neuron when stimulated with maltose, fructose or glycerol. All these findings are inconsistent with both electrophysiological recordings of taste sensilla and behavioral analysis of flies with mutations in various sugar *Gr* genes.[5-7]

Recent expression analyses of all but one sugar *Gr* gene have revealed complex expression overlap among them, generating at least 8 different subtypes of GRNs present in the labellum and the last 2 segments of the foretarsi.[8] Activation of sweet GRNs is initiated through binding of a sugar to a specific sugar receptor, which are thought to be composed of 2 or more Gr proteins. Specifically, numerous behavioral analyses have shown that mutations in different sugar *Gr* genes reduce or abolish proboscis extension responses to the same sugars. For example, flies lacking *Gr5a* (*ΔGr5a*) or the entire *Gr64* locus (*ΔGr64*) exhibit severe reduction or complete loss of behavioral responses to the sugar trehalose, implying that a functional trehalose receptor requires the Gr5a subunit as well as one or more subunits encoded by the *Gr64* genes. *ΔGr64*

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mutant flies, with only 2 functional sugar *Gr* genes, *Gr5a* and *Gr61a*, provide the best example that the claims by Dahanukar and colleagues are not reflective of true sugar receptor action. Over-expression in the ab1C neuron of either *Gr5a* or *Gr61a* elicits responses from 4 different sugars, including glucose and trehalose, yet *ΔGr64* flies lack PER responses to both these sugars.[5] But the most conclusive support for multimeric sugar receptors was derived from electrophysiological studies by Montell and colleagues.[9] In this paper, the authors showed that sweet taste neuron responses to trehalose are completely lost in *ΔGr64* or *ΔGr5a* mutant flies, while providing a single member of the *Gr64* family (*Gr64f*) to *ΔGr64* flies completely restored trehalose induced firing in sweet neurons. This clearly indicates that *Gr5a* and *Gr64f* together constitute a functional trehalose receptor, and that either of them alone fails to do so. Lastly, and most conclusively, we have recently generated a sugar-blind fly lacking all 8 sugar *Gr* genes, and we find that transgene mediate expression of single *Gr* genes fails to induce any cellular responses in sweet taste neurons to any sugars, while certain pairs of *Gr* genes restores responses to select sugars.[10]

Freeman et al. did perform select co-expression analyses, combining *Gr5a* with each of the other sugar *Gr* genes, but claim to find no evidence for heterodimeric complexes. Yet, the interpretation of their data seems odd and counterintuitive. First, they find that the combination of Gr5a and Gr64f does not increase the firing rate as opposed to single Gr expression alone. However, if both of these receptors respond to glucose on their own, one would expect that co-expression should have an additive effect on neuron firing rate. Second, the authors state that "co-expression of Gr64a generally depressed responses to Gr5a-dependnet sugars, which is consistent with their non-overlapping function in taste neurons." Why would "non-overlapping function of these receptors lead to depression of the function of one by the other? The simplest interpretation of this observation, in my view, is that Gr5a and Gr64a interact with each other to form a receptor that depletes the pool of receptors responding to sugars mediated by "Gr5a alone". And lastly, the authors failed to notice that one of the more relevant sugar receptors (Gr64f), as established by both behavioral and electrophysiological studies,[6,8,9] was by far the least effective one in the ab1C expression system (Figure 2A in ref 1), for which no explanation was provided.

Freeman et al.'s observations, however, are consistent by co-operation of endogenously expressed Gr proteins in ab1C neurons with the ectopically expressed sugar Gr protein. The obvious candidates for such a cooperation are the 2 other Gr proteins expressed in ab1C neurons (Gr21a and Gr63a). In none of the experiments were both these Gr proteins eliminated through mutation in their respective genes. Thus, an ectopically expressed sugar Gr may combine with Gr21a or Gr63a to form a novel receptor complex not occurring in wild type flies, but acquires sugar binding properties that are less specific than those generated from naturally formed sugar Gr complexes. A second possible explanation might be endogenous expression of a sugar *Gr* gene in ab1C neurons themselves, which the authors did not contemplate at all. Indeed, we recently found that 4 of the 8 sugar *Gr* genes are expressed in numerous olfactory sensory neurons,[8] providing yet another example of previously reported cases of *Gr* genes expressed in atypical fashion in a variety of sensory and central neurons.[11-13] In any case and whatever the specific reasons for the observed, sugar-induced responses, the ectopic expression system of ab1C olfactory neurons does not accurately describe the properties of insect taste receptors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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