The EMBO Journal (2005) 24, 3259–3265 | © 2005 European Molecular Biology Organization | All Rights Reserved 0261-4189/05
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THE EMBO JOURNAL

G-protein gamma subunit 1 is required for sugar reception in *Drosophila*

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Though G-proteins have been implicated in the primary step of taste signal transduction, no direct demonstration has been done in insects. We show here that a G-protein gamma subunit, *Gγ1*, is required for the signal transduction of sugar taste reception in *Drosophila*. The *Gγ1* gene is expressed mainly in one of the gustatory receptor neurons. Behavioral responses of the flies to sucrose were reduced by the targeted suppression of neural functions of *Gγ1*-expressing cells using neural modulator genes such as the modified *Shaker* K^+ channel (EKO), the tetanus toxin light chain or the *shibire* (*shi^{ts1}*) gene. RNA interference targeting to the *Gγ1* gene reduced the amount of *Gγ1* mRNA and suppressed electrophysiological response of the sugar receptor neuron. We also demonstrated that responses to sugars were lowered in *Gγ1* null mutant, *$Gγ1^{N159}$*. These results are consistent with the hypothesis that *Gγ1* participates in the signal transduction of sugar taste reception.
The EMBO Journal (2005) **24,** 3259–3265. doi:10.1038/sj.emboj.7600796; Published online 25 August 2005
Subject Categories: signal transduction; neuroscience
Keywords: *Drosophila*; G-protein; signal transduction; taste

Introduction

Gustatory sense enables animals to distinguish among nutritious and toxic soluble substances. Although gustatory sense plays a key role in determining the taste of food, the signaling mechanisms governing this important sensory system are not fully understood. As a model system, *Drosophila* offers several advantages for exploring the mechanism of gustatory sense perception at different levels of an organism (Ishimoto and Tanimura, 2004). In *Drosophila*, as in other insects, taste substances are detected by bipolar gustatory receptor neurons (GRNs). A typical chemosensilla houses four kinds of GRNs. Each GRN works as a specialist characterized by its responsiveness to taste substances. Sugar cells (S cells) respond to mono-, di- and trisaccharides. W and L1 cells respond to water and low concentration of salt, respectively. L2 cells respond to deterrent stimulants such as high concentrations of salt and bitter compounds (Meunier *et al*, 2003).

Previous studies have indicated that sugar taste information is initially received by G-protein-coupled receptors, both in mammals and insects (Hoon *et al*, 1999; Clyne *et al*, 2000; Dunipace *et al*, 2001; Nelson *et al*, 2001, 2002; Scott *et al*, 2001; Li *et al*, 2002). A typical model of G-protein signaling involves G-protein-coupled receptors coupled to a membrane-associated heterotrimer composed of a GTP-hydrolyzing Gα subunit and a Gβγ dimeric partner. G-protein subunits that mediate intracellular taste signaling pathways have been identified only in mammals (Lindemann, 2001). Several mammalian molecules, including G-protein subunits, a phospholipase C (PLC), a phosphodiesterase (PDE), an inositol 1,4,5-triphosphate ($Ins(1,4,5)P_3$) receptor and a transient receptor potential-like (TRPL) channel, have been linked to the taste transduction pathway. One of the G-protein α subunits, α-gustducin (Mclaughlin *et al*, 1992), is known to be involved in the mammalian response to sweet and bitter compounds (Wong *et al*, 1996). Gγ13, a G-protein γ subunit, is involved in a signal transduction pathway for a bitter compound, denatonium (Huang *et al*, 1999). Phospholipase C-$β_2$ ($PLCβ_2$) is essential for sweet and bitter signal transduction pathways (Zhang *et al*, 2003). A TRPL channel, TRPM5, is involved in both sweet and bitter taste signaling pathways (Zhang *et al*, 2003). On the other hand, taste signal transduction pathways in insects remain to be determined. In the visual system, the signal transduction pathway is divergent between vertebrates and invertebrates (Hardie and Raghu, 2001). In the vertebrate phototransduction system, a G-protein α subunit, transducin, activates a PDE, resulting in hydrolysis of guanosine 3′,5′-cyclic monophosphate and closure of transduction channels. In *Drosophila,* rhodopsin activates a distinct G-protein isoform, dGqα (Talluri *et al*, 1995), which activates a PLC isoform encoded by the *norpA* gene. Thus, *Drosophila* phototransduction employs an $Ins(1,4,5)P_3$ pathway instead of a cGMP pathway for the signal transduction. The $Ins(1,4,5)P_3$ pathway likely plays a role in the signal transduction cascade eliciting sweet taste in the fleshfly, *Boettcherisca peregrine* (Koganezawa and Shimada, 2002). To understand the molecular mechanisms underlying taste reception, additional information on the signal transduction molecules is needed both in vertebrates and invertebrates. In this study, we determined that a G-protein γ subunit, *Gγ1*, is expressed in GRNs and demonstrated that *Gγ1* is involved in responses to sugars using the behavioral and electrophysiological analysis combined with molecular genetic techniques in *Drosophila*.

Results and discussion

G-protein subunits are expressed in gustatory sensory organs

In the *Drosophila* genome, 16 genes are predicted to encode G-protein subunits. These include sequences for 11

Author contributions: HI and TT designed the research; HI performed the research; HI and TT analyzed the data; KT and RU contributed new analytic tools, and HI and TT wrote the paper.
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Received: 18 April 2005; accepted: 3 August 2005; published online: 25 August 2005

α subunits, three β subunits and two γ subunits (FlyBase). We determined G-protein subunits expressed in a gustatory organ using RT–PCR. *Gα73B*, *Gβ13F*, *Gβ5* and *Gγ1* were detected by this analysis (Supplementary Figure S1). The role of these G-protein subunits in gustatory signal transduction has not been shown previously. In this study, we have characterized one of these four G-proteins, *Gγ1*. To study the role of G-proteins involved in signal transduction of taste, we chose the G-protein γ subunit, since *Drosophila* G-protein γ subunit includes the fewest variety of subtypes, only two: *Gγ1* and *Gγ30A*, among three G-protein subunits and only one subtype, *Gγ1*, is expressed in the gustatory organ labellum (Figure 1A). *Gγ1* (CG8261) is located on the second chromosome and is cytologically mapped to 44F3–5. There are five alternative transcriptional forms (RA–RE) of the *Gγ1* gene. To identify which transcripts are expressed in the labellum, we carried out RT–PCR and 3′ RACE using specific primers for these transcripts (Supplementary Figure S2). We found that the RC, RD and RE forms are present in labellum. We searched Gal4 enhancer-trap strains to study the role of *Gγ1* in the gustatory system (Brand and Perrimon, 1993). We found NP1535 strain in which a *P*{Gal4} element is inserted 73 bp upstream of the transcriptional start site of *Gγ1* (Figure 1B) (Ray and Ganguly, 1992, 1994). The UAS-green fluorescent protein (GFP) and NP1535 strains were crossed and their progeny enabled us to visualize *Gγ1* expression with GFP detection. GFP signals were observed in both the labellum and the tarsi (Figure 1C). Gustatory chemosensilla on the labellum are classified into three types: l-, s- and i-type, based on their shape and location (Ishimoto and Tanimura, 2004). Four GRNs are housed in s- and l-type chemosensilla as a cluster, while the i-type chemosensilla house two GRNs (Hiroi *et al*, 2004). We observed GFP signals in all GRNs from each cluster, though only one GRN exhibited a higher GFP signal at the high magnification (Figure 1C inset), suggesting that *Gγ1* mainly functions in one of the four GRN types.

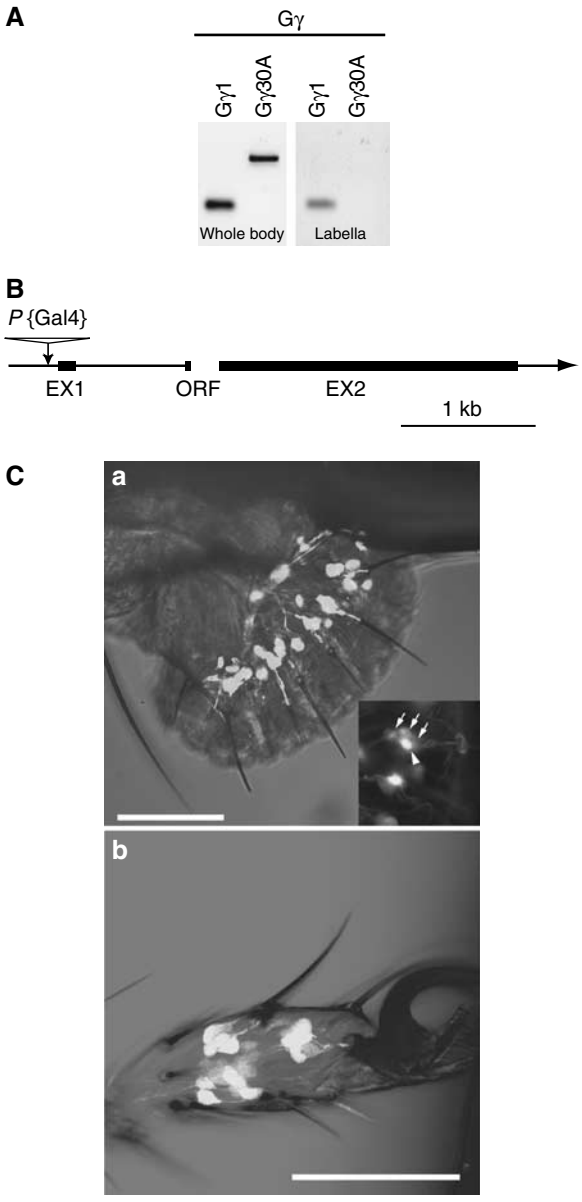

Figure 1 G-protein gamma subunit 1 is expressed in gustatory organ. (**A**) RT–PCR analysis shows an mRNA expression profile of two G-protein gamma subunits in the labellum and whole body. (**B**) A schematic diagram showing the gene structure of the *Gγ1* gene and the insertion site of *P*{Gal4} in the enhancer trap strain, NP1535. (**C**) GAL4 expression patterns in NP1535 visualized by GFP. GFP expression is in the sensory neurons of labellum (a) and tarsi (b). GFP signals were observed at the base of all gustatory sensilla in the labellum and tarsi. In a cluster of four GRNs, one GRN shows higher intensity of GFP signal. Arrows indicate GRNs showing lower intensity of GFP signals in a cluster of GRNs. An arrowhead indicates a cell with a higher intensity of GFP signal. Scale bars indicate 50 μm.

Neural suppression directed by NP1535 Gal4 driver reduces behavioral response to sucrose

We tested the possible role of *Gγ1* in gustatory signal transduction by measuring the proboscis extension reflex (PER) response to sucrose, since G-protein-coupled receptors are known to mediate the sweet signal transduction pathway both in vertebrates and invertebrates (Lindemann, 2001). As a control strain, we used ΔP NP1535#1–3, in which a *P*{Gal4} element was precisely removed by introducing a genomic transposase source. ΔP NP1535 flies showed an 83–91% PER response rate (Figure 2A), whereas the NP1535 homozygote flies exhibited significantly lowered PER rates ($P<0.001$). The NP1535/ΔP NP1535#1 heterozygote flies, like the ΔP NP1535 flies, showed normal PER. The *P*{Gal4} insertion induced a recessive phenotype in the behavioral response to sucrose. To compare the expression level of *Gγ1* mRNA between NP1535 and control strains, we performed quantitative PCR (QPCR) (Figure 4B). The results indicated that *Gγ1* expression level is actually reduced in NP1535 flies in which the amount of *Gγ1* mRNA is 9–14% of control strains (Figure 4B). To characterize *Gγ1*-expressing GRNs, we examined the PER response of flies expressing mutant type of generically modified *Shaker* K^+ channels (Osterwalder *et al*, 2001; White *et al*, 2001), EKO, driven by the Gal4 reporter of NP1535. In the NP1535/+; UAS-EKO/+ flies, EKO inhibits neural activity of the Gal4-expressing cells (Figure 2A). The NP1535/+; UAS-EKO/+ flies exhibited a significantly lowered PER rate of 36% ($P<0.001$), which is similar to the response of the NP1535 homozygote flies. Tetanus toxin (TNT) (Sweeney *et al*, 1995) inhibits docking of the synaptic vesicles to the membrane, thus blocking neurotransmitter release. The PER response of flies expressing TNT was also

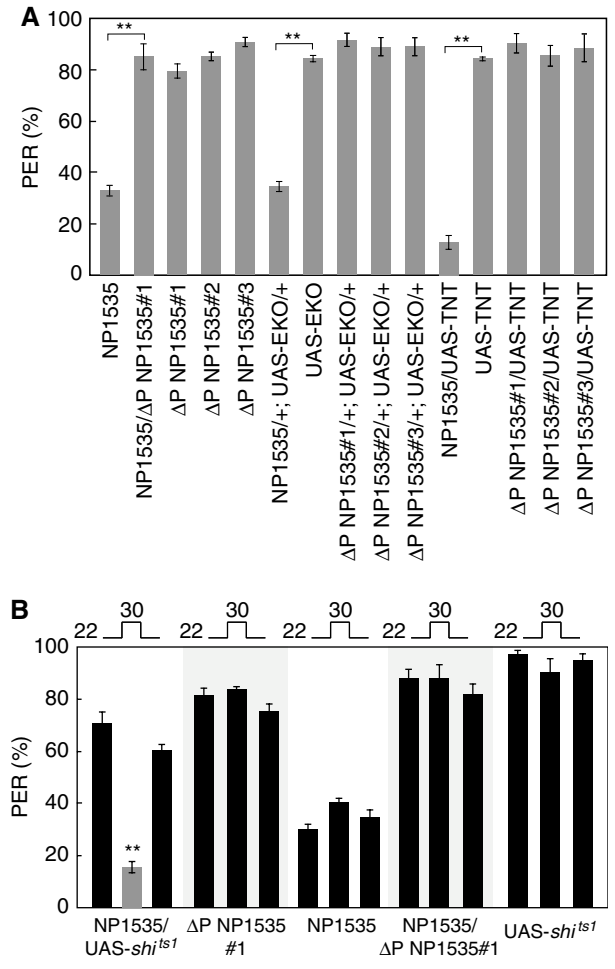

Figure 2 PER is suppressed by targeted expression of EKO or TNT. (**A**) PER responses of flies expressing either UAS-EKO or UAS-TNT induced by enhancer-trap line (NP1535) and control flies (NP1535, UAS-EKO, UAS-TNT, ΔP NP1535#1–3, NP1535/ΔP NP1535#1) to 100 mM sucrose. $^{**}P<0.001$. (**B**) PER responses of NP1535/UAS-shi^{ts1} flies and control flies (NP1535, UAS-shi^{ts1}, ΔP NP1535#1, NP1535/ΔP NP1535#1) to 100 mM sucrose at the permissive temperature (22°C) and restrictive temperature (30°C). Data were obtained from at least 150 flies of each strain. $^{**}P<0.001$.

measured (Figure 2A). In the NP1535/UAS-TNT flies, the PER responses were severely reduced, even in comparison to that of the NP1535/+; UAS-EKO/+ flies. These results suggest that the Gal4-expressing neurons in the NP1535 strain participate in the behavioral response to sucrose. However, there remains a possibility that the action of EKO or TNT might impair developmental processes in the nervous systems regulating the observed PER response.

To exclude this possibility, we used a temperature-sensitive allele of the dynamin mutant, shi^{ts1}, through which synaptic transmissions can be conditionally disrupted (Figure 2B). At a restrictive temperature (30°C), the neurotransmitter release is inhibited in the shi^{ts1}-expressing neurons. The NP1535/UAS-shi^{ts1} flies showed normal PER responses to 100 mM sucrose at the permissive temperature (22°C). When these flies were transferred to 30°C, the PER response was significantly reduced ($P<0.001$). This reduction was recovered when flies were returned to 22°C. The control flies, NP1535, ΔP NP1535#1, NP1535/ΔP NP1535#1 and UAS-shi^{ts1}, showed no significant differences of PER responses under both temperatures ($P>0.1$). These data suggest that the Gal4-expressing neurons in the NP1535 flies are required for the behavioral response to sucrose.

Nerve responses to sugars were reduced in NP1535/UAS-EKO and NP1535 flies

We showed that the reduced behavioral response to sucrose is due to suppression of the Gal4-expressing neurons in NP1535. Yet, we were not able to discern whether the cause of the behavioral defect is in the peripheral nervous system or in the central nervous system.

We then recorded the electrophysiological GRN response to determine whether the *Gγ1*-expressing GRNs are needed for the sugar reception. We found no significant reduction of nerve responses to water and salt in NP1535 and NP1535/+; UAS-EKO/+ flies (data not shown; $P>0.1$). In the ΔP NP1535 and UAS-EKO flies, we observed approximately 64 spikes/s from the labellum gustatory sensilla using 100 mM sucrose (Figure 3A), whereas the NP1535 flies showed a decreased firing rate (38 spikes/s). This result is consistent with the behavioral data obtained by the PER test (Figure 2). The NP1535/+; UAS-EKO/+ flies also demonstrated an attenuated nerve response (Figure 3A). These results support the view that *Gγ1* is functioning in S cells.

S cells respond to sugars with a glycopyranoside moiety, as well as fructose and trehalose (Rodrigues and Siddiqi, 1981; Tanimura and Shimada, 1981; Tanimura *et al*, 1982). We examined the neural responses of flies to four kinds of sugars: sucrose, glucose, fructose and trehalose, at a range of concentration from 10 to 300 mM (Figure 3B). In comparison to the control flies, the NP1535 flies showed markedly lower responses to all four kinds of sugars (10–100 mM of sucrose and fructose; $P<0.001$ at low sugar concentrations, 30 mM of glucose; $P<0.001$, 10 and 30 mM of trehalose; $P<0.001$). These results are in agreement with the nerve responses we recorded from the NP1535/+; UAS-EKO/+ flies in the present study. The lower *Gγ1* expression levels in the NP1535 flies likely cause the attenuation of sugar responses. We used RNA interference (RNAi) methodologies to verify the lower *Gγ1* expression response on another genetic background. Moreover, we examined fly nerve responses to water and salt to ask whether the *Gγ1* subunit specifically mediates the gustatory signal transduction utilized for sugar perception.

RNAi of Gγ1 suppressed nerve responses of S cells

We examined the nerve responses of flies expressing double-stranded RNA targeting to *Gγ1* mRNA (*Gγ1*.IR) in all neurons using the *elav*-Gal4 activator. For these studies, we used two independent UAS-*Gγ1*.IR strains in which a P{UAS-*Gγ1*.IR} is on the second (II) or the third (III) chromosome. The UAS-*Gγ1*.IR(II)/*elav*-Gal4 flies showed reduced nerve responses to 100 mM sucrose (Figure 4A). We also observed reduced nerve responses to 100 mM sucrose in the *elav*-Gal4/+; UAS-*Gγ1*.IR(III)/+ flies. The *Gγ1* response values using the RNAi methodology were similar to those we observed in NP1535/+; UAS-EKO/+ and NP1535 flies. We used 30 and 300 mM sodium chloride to examine the nerve responses of L1 and L2 cells, respectively. The L1 and L2 cells showed normal responses to sodium chloride. These results indicate that inhibition of the *Gγ1* mRNA reduced the S-cell nerve responses, but not those of the L1 (low salt) or L2 (high salt)

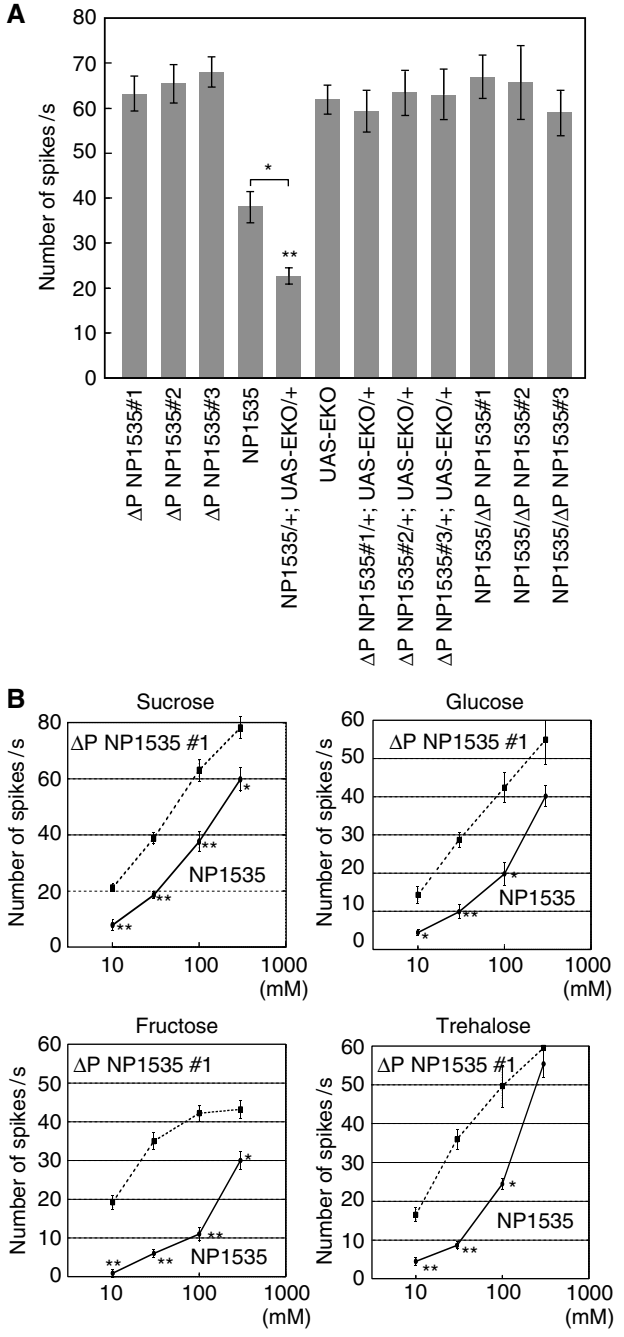

Figure 3 Nerve responses were reduced in NP1535 and NP1535/+; UAS-EKO/+ flies. (**A**) Nerve responses of NP1535/+; UAS-EKO/+ flies to 100 mM sucrose. Responses were recorded from l-type chemosensilla on the labellum. Significant differences were observed between control flies (ΔP NP1535#1–3, NP1535/ΔP NP1535#1–3, ΔP NP1535#1–3/+;UAS-EKO/+ and UAS-EKO) and NP1535/+; UAS-EKO/+ flies. Nerve responses were obtained from 10 flies. Error bars are s.e.m.; $^{**}P<0.001$; $^{*}P<0.05$. (**B**) Dose–response curves of nerve responses to sugars (sucrose, glucose, fructose and trehalose) of NP1535 and ΔP NP1535#1 flies. Each data point represents at least five recordings made from 12 flies in total. Error bars are s.e.m.; $^{**}P<0.001$; $^{*}P<0.05$.

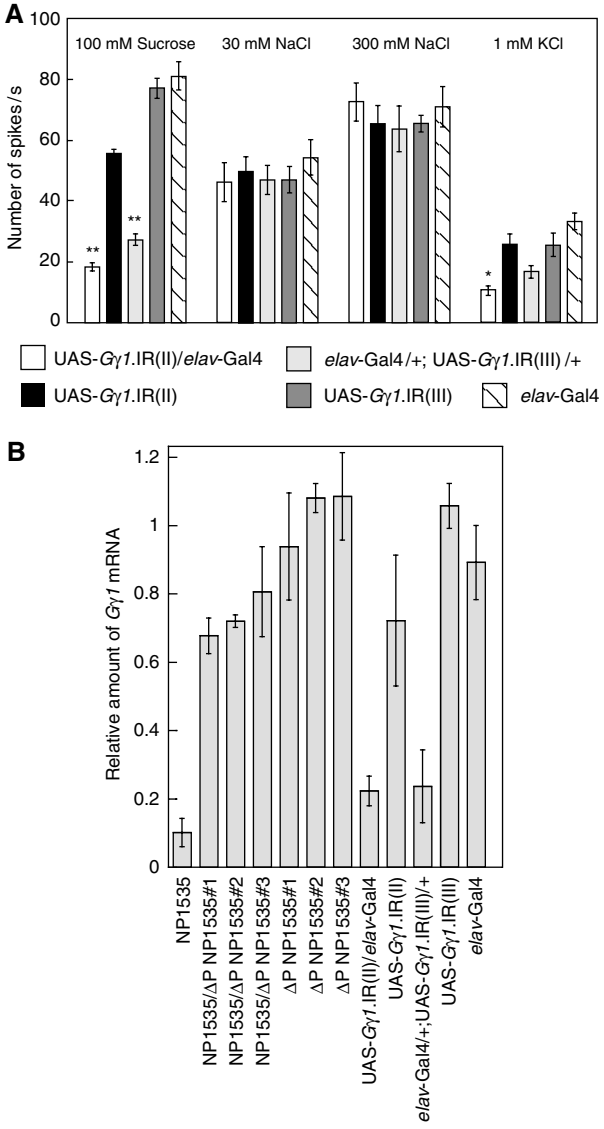

Figure 4 RNAi-mediated *Gγ1* silencing impairs nerve responses of GRNs to sugar. (**A**) Nerve responses were recorded from l-type chemosensilla on the labellum. Stimulating solutions were 100 mM sucrose for S cell, 30 mM NaCl for L1 cell, 300 mM NaCl for L2 cell and 1 mM KCl for W cell. We used UAS-*Gγ1*.IR flies with either the second or third chromosome linked. We obtained responses from at least 10 flies. Error bars are s.e.m.; $^{**}P<0.001$; $^{*}P<0.01$ (**B**) mRNA expression levels were measured by QPCR. Elongation factor 1 α48D (Ef1α48D:CG8280) was used to normalize the mRNA content among strains. Error bars are s.e.m.; $^{**}P<0.001$; $^{*}P<0.01$.

cells. We also examined nerve responses to water (1 mM KCl) using UAS-*Gγ1*.IR(II)/*elav*-Gal4 flies and *elav*-Gal4/+; UAS-*Gγ1*.IR(III)/+ flies. The *elav*-Gal4/+; UAS-*Gγ1*.IR(III)/+ and the background strain flies responded to water similarly ($P>0.1$). However, the water response exhibited by the UAS-*Gγ1*.IR(II)/*elav*-Gal4 flies was reduced by comparison. To demonstrate the function of *Gγ1* in water reception, water response of *Gγ1* null mutant should be tested. The RNAi assay for *Gγ1* revealed that *Gγ1* mediates signaling at some step for sugars. However, the observed nerve responses to sucrose were not totally inhibited in the *Gγ1*.IR flies, suggesting a possibility that the RNAi was not complete. We determined the amount of *Gγ1* mRNA in each strain using QPCR (Figure 4B). *Gγ1*.IR flies, UAS-*Gγ1*.IR(II)/*elav*-Gal4 and *elav*-Gal4/+; UAS-*Gγ1*.IR(III)/+, showed reduced expression of *Gγ1*, which was 20–29% of UAS-*Gγ1*.IR(II), UAS-*Gγ1*.IR(III) and *elav*-Gal4 strains. QPCR results suggest that *Gγ1* mRNA was not completely abolished, but substantially reduced by RNAi.

Cells homozygous for $G\gamma1^{N159}$ show reduced sugar responses

We examined nerve responses of GRNs bearing a nonsense mutation $G\gamma1^{N159}$ (Izumi *et al*, 2004), to determine whether the sugar response will be completely disappeared. Since $G\gamma1^{N159}$ homozygote mutants are embryonic lethal, we employed a directed mosaic system using a GAL4-responsive yeast site-specific recombinase, called FLP (*flippase*). In this system, a clonal analysis can be restricted to the tissue of interest (Duffy *et al*, 1998). We combined the *elav*-GAL4 driver with the UAS-*flp* responder for the directed recombination of neural precursors including progenitor cells of GRNs. Directed FLP expression then induces mitotic recombinations and generates cells containing the $G\gamma1^{N159}$ homozygotes. We found that GRNs bearing homozygous $G\gamma1^{N159}$ mutants exhibited normal responses to water, high (300 mM) and low (30 mM) concentrations of salt (Figure 5). These results suggest that *Gγ1* participates neither in water nor salt reception mechanisms. The reduction of the W-cell response of the UAS-*Gγ1*.IR(II)/*elav*-Gal4 flies was possibly caused by an effect of genetic background.

GRNs bearing the wild-type (WT) *Gγ1* gene responded to sucrose normally (Figure 5). GRNs bearing the homozygous $G\gamma1^{N159}$ mutation showed lower nerve responses to 10–300 mM sucrose. The neural activity in response to the low concentration of sucrose was significantly reduced in the $G\gamma1^{N159}$ homozygote-carrying GRNs ($P<0.001$), though they still responded to high sucrose concentrations. Therefore, the S cell was not completely suppressed in the *Gγ1* null mutant type. There are two G-protein γ subunits in the *Drosophila* genome. One of the Gγ subunits, *Gγ30A*, is involved in the phototransduction pathway (Schulz *et al*, 1999). RT–PCR failed to detect the *Gγ30A* transcript in the taste organ. Hence, it is unlikely that *Gγ30A* mediates a sugar reception pathway. A G-protein-independent signal transduction pathway might exist for the sugar signal transduction. In the fleshfly, *B. peregrine*, Murakami and Kijima (2000) demonstrated that a putative ion channel is directly gated by sucrose using an *in situ* patch clamp. The null mutant analysis of *Gγ1* supports the idea that a G-protein-independent pathway is involved in the sugar reception mechanism.

When we monitored the expression of the *Gγ1* gene using the Gal4/UAS system, one of the four GRNs showed the stronger gene expression. Our results proved that only the sugar responses were affected by interfering with the function of the GFP-positive cells. Therefore, it is reasonable to conclude that the cells most strongly labeled by GFP expression are the S cells. However, if *Gγ1* is expressed in other GRNs, then there might be an additional role of the gene. One possible function of *Gγ1* may be to facilitate the bitter taste signal transduction. The mouse G-protein γ subunit, *Gγ13*, is colocalized within α-*gustducin* circumvallate papillae, and is thought to be coupled to mediate sweet and bitter signals (Huang *et al*, 1999). Since a minority of GRNs are activated by bitter substances (Meunier *et al*, 2003), there remains a possibility that common G-proteins mediate both sweet and bitter signal transduction pathways in *Drosophila*. An additional possible function of *Gγ1* is a role in developmental processes, since *Gγ1* is required for an asymmetrical division of neuroblasts in *Drosophila* (Izumi *et al*, 2004). A previous study showed that the expression of *Gγ1* is developmentally regulated in a variety of tissues (Ray and Ganguly, 1992). The

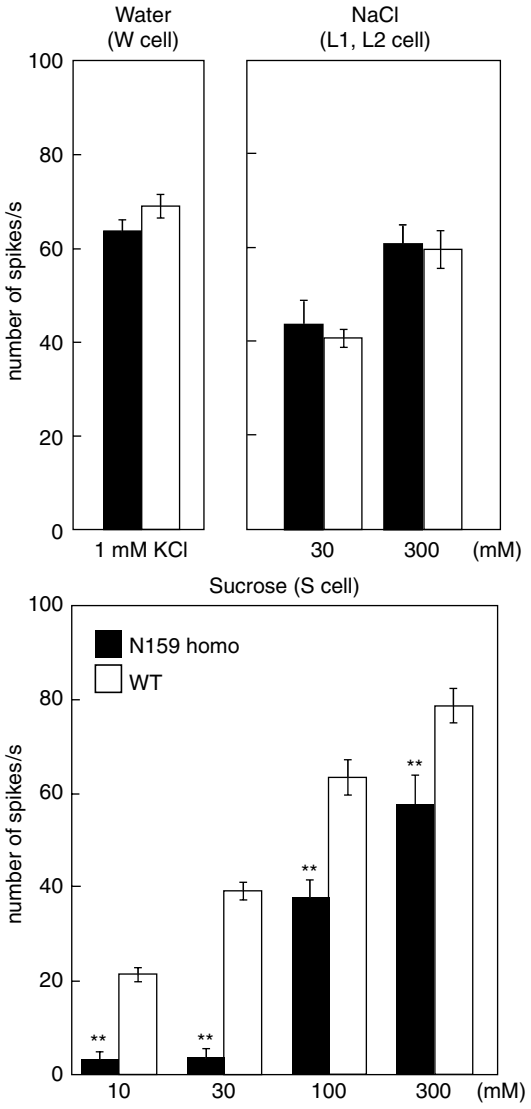

Figure 5 Nerve responses recorded from homozygotes of *Gγ1* nonsense mutant cells induced by mitotic recombination. Nerve responses were recorded from normal and somatic recombinant GRNs of l-type chemosensilla. We recorded electrophysiological responses from GRNs whose genotypes were WT or nonsense mutants of the *Gγ1* gene (N159 homo). Concentration of sucrose ranged from 10 to 300 mM. In all, 1 mM of KCl was used for stimuli to W (water) cells; 30 and 300 mM of NaCl were used for stimuli to L1 (low salt) and L2 (high salt) cells, respectively. Each recording was obtained from at least 32 chemosensilla of 14 flies. Error bars are s.e.m.; $^{**}P<0.001$.

Gγ1 gene produces at least five transcriptional products. We detected three types of *Gγ1* transcripts, RC, RD and RE, in taste organ (Supplementary Figure S2). One or more of these transcripts may function during the development of GRNs.

In this study, we identified *Gγ1* expression in the gustatory organs and demonstrated that the *Gγ1* subunit is involved in signal transduction pathways of sugar reception. This is the first report that implicates a specific G-protein gamma subunit in this pathway, which is required for sugar taste reception in *Drosophila*. This finding should lead to a better understanding of the molecular mechanism governing gustatory perception. Potential players include G-protein β subunits associating with Gγ1, downstream signaling molecules

and targeted effecters of the Gβ/Gγ1 complex. Our study also alludes to a potential G-protein-independent mechanism of sweet reception. Importantly, this has also been proposed through electrophysiological studies on knockout mice for the G-protein alpha subunit, α-*gustducin*. α-*gustducin* knockout mice showed a reduced, but not a completely abolished, response to sweet compounds (He *et al*, 2004). Thus, a G-protein-independent pathway for sweet taste reception is possibly used in both mammals and insects. Additional studies are needed to identify molecules mediating a G-protein-independent pathway.

Materials and methods

Fly strains

Strains of *Drosophila melanogaster* were maintained on a standard cornmeal-glucose agar medium at 25°C. A UAS-EKO (the modified *Shaker* K^+ channel) strain was obtained from W Benjamin in the Keshishian lab (Yale University, USA). A UAS-TNT strain was obtained from C O'Kane (Cambridge University, UK). UAS-GFP and *elav*-Gal4 strains were obtained from the Bloomington *Drosophila* Stock Center (Indiana, USA). UAS-*shibire*^{ts1} (Kitamoto, 2001) and *Gγ1*^{N159} (Izumi *et al*, 2004) flies were provided by T Kitamoto (University of Iowa, USA) and F Matsuzaki (Riken, Japan), respectively. The enhancer trap strain, NP1535 (Hayashi *et al*, 2002), was obtained from the *Drosophila* Genetic Resource Center in Kyoto Institute of Technology, Japan. For generating flippase-mediated somatic recombinant GRNs carrying the homozygous *Gγ1*^{N159} mutation, female flies carrying P elements (P{GawB}*elav*^{C155}, P{hs*flp*}1, *w**; P{FRT(*w*^{hs})}G13 P{tubP-*Gal80*}LL2/*CyO*, homozygous for hsp70-*flp*) were crossed to male flies of *y*^{1} *w**; P{FRT(*w*^{hs})}G13 P{UAS-mCD8::*GFP*.L}LL5, homozygous for {FRT}G13, UAS-mCD8::*GFP* (both strains were obtained from the Bloomington *Drosophila* Stock Center). Male flies carrying both hsp70-*flp* and UAS-mCD8::*GFP* were crossed to female flies carrying *Gγ1*^{N159}, {FRT}G13 heterozygous with a balancer, *CyO*. Flies carrying the homozygote form of the *Gγ1*^{N159} mosaic GRN clone were generated by inducing heat shock at 30°C three times for 2 h during the mid-pupal stage. Those flies were checked for disappearance of GFP signals under a dissecting microscope equipped with epifluorescence.

RT–PCR analysis

mRNA was prepared from 50 labella (dissected with a razor blade) using a QuickPrep™ Micro mRNA Purification kit (Amersham-Pharmacia Biotech, Uppsala, Sweden). cDNA synthesis and amplification were carried out sequentially using a SuperScript One-Step RT–PCR with Platinum Taq (Invitrogen, CA, USA). Primer sequences were designed to synthesize cDNA sized to 216 bp for *Gγ1* (forward: ATGGACGTAATGTCATCATC; reverse, TCCTTAGA GAACGGTGCAGG), and 840 bp for *Gγ30A* (forward, AGTCGCC CATCCTGCGAAGC; reverse, AGCCTAGATCGAACTCATAC).

Microscopy

The labellum and tarsus of the NP1535/UAS-GFP flies were fixed with phosphate-buffered saline (PBS) containing 4% formaldehyde for 15 min at room temperature. GFP fluorescence was observed with a confocal microscope (Zeiss LSM 510). The excitation wavelength was at 488 nm (argon laser), and the emission wavelength was at 515 nm. Z sections were collected at 1-μm intervals, and were processed to construct projections through an extended depth of focus. Images were processed minimally by using Photoshop (Adobe Systems, California, USA) to adjust the light levels, as well as background contrast and brightness.

Behavioral assays

The PER was examined principally as described (Kimura *et al*, 1986). Flies aged 3–6 days after eclosion were maintained on fresh medium for 1 day. Flies were starved for 20 h, but were allowed to take water. Before the assay with sugar solutions, the prothoractic tarsus of the fly was touched with a drop of water. If the water droplet induced the PER, the fly was allowed to intake sufficient water. This procedure was repeated between sugar stimulations to prevent water response. We tested PER reactions to sugar solution on 150 flies each, and repeated each experiment three times.

Electrophysiological recordings

Flies 3–6 days old were fed on a fresh medium for 1 day prior to experimentation. All electrophysiological recordings were obtained from labellar chemosensilla using the tip-recording method (Hodgson *et al*, 1955; Hiroi *et al*, 2002). Briefly, the proboscis was fixed at the base of labella using lanolin (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The *Drosophila* Ringer solution, filling the glass capillary tube, grounded the fly subject electrically. Labella chemosensilla were stimulated up to 2 s with a recording electrode with a 20-μm tip diameter. The electrolyte (1 mM KCl) does not elicit spikes from the S, L1, and L2 cells, but elicits spikes from the W cell.

RNA interference

cDNA fragment was amplified using PCR with primers for *Gγ1*. Primer sequences for RNAi construct were: forward, ATGGACG TAATGTCATCATC; reverse, TTAGAGAACGGTGCAGGACGA. The target sequence is the ORF, which is common to all transcriptional forms of *Gγ1* gene. The PCR product was cloned by TA cloning kit (Invitrogen, CA, USA) and sequenced. An inverted-repeat transgene for *Gγ1* in inducible Gal4 element was constructed and supplied by the Genetic Strains Research Center, Invertebrate Genetics Laboratory, National Institute of Genetics (Mishima, Japan).

Quantitative PCR

QPCR was performed on cDNA prepared from labella using Brilliant SYBR Green QPCR Master Mix reagents (Stratagene, California, USA) and the thermal cycler apparatus from Stratagene Mx3000P, according to the manufacturer's recommendations. Total cDNA was prepared following the RT–PCR method, except using poly dT_{24} primer. Primer sequences were designed to synthesize cDNA sized to 216 bp for *Gγ1* (forward, ATGGACGTAATGTCATCATC; reverse, TTAGAGAACGGTGCAGGACGA). Elongation factor 1, Ef1α48D (CG8280; 229 bp, forward, CCAACATGGGCAAGGAAAAG; reverse, ATCGATGGTGATACCACGCT), was used to normalize cellular mRNA contents of every preparation. Reactions were performed with 10 μl of enzyme mix, 10 pmol of forward primers, 10 pmol of reverse primers and 1 μl of diluted cDNAs in a final volume of 20 μl. PCR running was performed as follows: initial denaturation at 95°C for 5 s, 40 amplification cycles including annealing, elongation and real-time fluorescence measurement at 55°C for 15 s and denaturation at 95°C for 1 min. At the end of the 40 PCR cycles, the melting temperature was determined by continuously recording the fluorescence during progressive heating up to 95°C with a ramp rate of 0.1°C/s. Three duplicate reaction mixtures were averaged in each PCR run. We performed QPCR at least three times for each strain.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Acknowledgements

We thank W Benjamin, C O'Kane, T Kitamoto, F Mastuzaki and the *Drosophila* Genetic Resource Center in Kyoto Institute of Technology and the Bloomington Stock Center for providing fly strains. We thank P Lucas, N Meunier and F Marion-Poll for helpful suggestions and discussions. We also thank F Yokohari (Fukuoka University) for the use of confocal microscope, K Kimura and M Haruta for technical assistance. HI was supported by the Japan Society for the Promotion of Science. Financial support for this work was provided by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan to TT and HI.

References

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118:** 401–415

Clyne PJ, Warr CG, Carlson JR (2000) Candidate taste receptors in *Drosophila*. *Science* **287:** 1830–1834

Duffy JB, Harrison DA, Perrimon N (1998) Identifying loci required for follicular patterning using directed mosaics. *Development* **125:** 2263–2271

Dunipace L, Meister S, McNealy C, Amrein H (2001) Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. *Curr Biol* **11:** 822–835

FlyBase *Gene Ontology Report*, http://flybasenet/fbservlet/goreport?id=GO:0005834command=linkslink=GNF

Hardie RC, Raghu P (2001) Visual transduction in *Drosophila*. *Nature* **413:** 186–193

Hayashi S, Ito K, Sado Y, Taniguchi M, Akimoto A, Takeuchi H, Aigaki T, Matsuzaki F, Nakagoshi H, Tanimura T, Ueda R, Uemura T, Yoshihara M, Goto S (2002) GETDB, a database compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. *Genesis* **34:** 58–61

He W, Yasumatsu K, Varadarajan V, Yamada A, Lem J, Ninomiya Y, Margolskee RF, Damak S (2004) Umami taste responses are mediated by alpha-transducin and alpha-gustducin. *J Neurosci* **24:** 7674–7680

Hiroi M, Marion-Poll F, Tanimura T (2002) Differentiated response to sugars among labellar chemosensilla in *Drosophila*. *Zool Sci* **19:** 1009–1018

Hiroi M, Meunier N, Marion-Poll F, Tanimura T (2004) Two antagonistic gustatory receptor neurons responding to sweet–salty and bitter taste in *Drosophila*. *J Neurobiol* **61:** 333–342

Hodgson ES, Lettvin JY, Roeder KD (1955) Physiology of a primary chemoreceptor unit. *Science* **122:** 417–418

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJP, Zuker CS (1999) Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* **96:** 541–551

Huang LQ, Shanker YG, Dubauskaite J, Zheng JZ, Yan WT, Rosenzweig S, Spielman AI, Max M, Margolskee RF (1999) G gamma 13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium. *Nat Neurosci* **2:** 1055–1062

Ishimoto H, Tanimura T (2004) Molecular neurophysiology of taste in *Drosophila*. *Cell Mol Life Sci* **61:** 10–18

Izumi Y, Ohta N, Itoh-Furuya A, Fuse N, Matsuzaki F (2004) Differential functions of G protein and Baz-aPKC signaling pathways in *Drosophila* neuroblast asymmetric division. *J Cell Biol* **164:** 729–738

Kimura K, Shimozawa T, Tanimura T (1986) Isolation of *Drosophila* mutants with abnormal proboscis extension reflex. *J Exp Zool* **239:** 393–399

Kitamoto T (2001) Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J Neurobiol* **47:** 81–92

Koganezawa M, Shimada I (2002) Inositol 1, 4, 5-trisphosphate transduction cascade in taste reception of the fleshfly, *Boettcherisca peregrina*. *J Neurobiol* **51:** 66–83

Li XD, Staszewski L, Xu H, Durick K, Zoller M, Adler E (2002) Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* **99:** 4692–4696

Lindemann B (2001) Receptors and transduction in taste. *Nature* **413:** 219–225

Mclaughlin SK, Mckinnon PJ, Margolskee RF (1992) Gustducin is a taste-cell-specific G-protein closely related to the transducins. *Nature* **357:** 563–569

Meunier N, Marion-Poll F, Rospars JP, Tanimura T (2003) Peripheral coding of bitter taste in *Drosophila*. *J Neurobiol* **56:** 139–152

Murakami M, Kijima H (2000) Transduction ion channels directly gated by sugars on the insect taste cell. *J Gen Physiol* **115:** 455–466

Nelson G, Chandrashekar J, Hoon MA, Feng LX, Zhao G, Ryba NJP, Zuker CS (2002) An amino-acid taste receptor. *Nature* **416:** 199–202

Nelson G, Hoon MA, Chandrashekar J, Zhang YF, Ryba NJP, Zuker CS (2001) Mammalian sweet taste receptors. *Cell* **106:** 381–390

Osterwalder T, Yoon KS, White BH, Keshishian H (2001) A conditional tissue-specific transgene expression system using inducible GAL4. *Proc Natl Acad Sci USA* **98:** 12596–12601

Ray K, Ganguly R (1992) The *Drosophila* G-protein gamma-subunit gene (d-G-gamma-1) produces 3 developmentally regulated transcripts and is predominantly expressed in the central-nervous-system. *J Biol Chem* **267:** 6086–6092

Ray K, Ganguly R (1994) Organization and expression of the *Drosophila melanogaster* G-protein gamma-subunit gene. *FASEB J* **8:** A1399

Rodrigues V, Siddiqi O (1981) A gustatory mutant of *Drosophila* defective in pyranose receptors. *Mol Gen Genet* **181:** 406–408

Schulz S, Huber A, Schwab K, Paulsen R (1999) A novel G gamma isolated from *Drosophila* constitutes a visual G protein gamma subunit of the fly compound eye. *J Biol Chem* **274:** 37605–37610

Scott K, Brady Jr R, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R (2001) A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104:** 661–673

Sweeney ST, Broadie K, Keane J, Niemann H, O'Kane CJ (1995) Targeted expression of tetanus toxin light-chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. *Neuron* **14:** 341–351

Talluri S, Bhatt A, Smith DP (1995) Identification of a *Drosophila* G-protein alpha-subunit (dG(Q)alpha-3) expressed in chemosensory cells and central neurons. *Proc Natl Acad Sci USA* **92:** 11475–11479

Tanimura T, Isono K, Takamura T, Shimada I (1982) Genetic dimorphism in the taste sensitivity to trehalose in *Drosophila melanogaster*. *J Comp Physiol* **147:** 433–437

Tanimura T, Shimada I (1981) Multiple receptor proteins for sweet taste in *Drosophila* discriminated by papain treatment. *J Comp Physiol* **141:** 265–269

White BH, Osterwalder TP, Yoon KS, Joiner WJ, Whim MD, Kaczmarek LK, Keshishian H (2001) Targeted attenuation of electrical activity in *Drosophila* using a genetically modified K^+ channel. *Neuron* **31:** 699–711

Wong GT, Gannon KS, Margolskee RF (1996) Transduction of bitter and sweet taste by gustducin. *Nature* **381:** 796–800

Zhang YF, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu DQ, Zuker CS, Ryba NJP (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* **112:** 293–301