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Mutants in Phospholipid Signaling Attenuate the Behavioral Response of Adult *Drosophila* to Trehalose

Pinky Kain[1,4], Farhath Badsha[1], Syed Mubarak Hussain[1,4], Amit Nair[2,5], Gaiti Hasan[1] and Veronica Rodrigues[1,3]

[1]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India, [2]Inositide Laboratory, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK and [3]Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400005, India
[4]Present address: Institut für Neurobiologie, Westfälische Wilhelms Universität, Münster, Germany
[5]Present address: Oakland Innovation, 18 Cambridge Science Park, Milton Road, Cambridge CB4 0FH, UK

Correspondence to be sent to: Veronica Rodrigues, National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India. e-mail: veronica@ncbs.res.in

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Abstract

In *Drosophila melanogaster*, gustatory receptor genes (*Grs*) encode putative G-protein–coupled receptors (GPCRs) that are expressed in gustatory receptor neurons (GRNs). One of the *Gr* genes, *Gr5a*, encodes a receptor for trehalose that is expressed in a subset of GRNs. Although a role for the G protein, Gsα, has been shown in Gr5a-expressing taste neurons, there is the residual responses to trehalose in *Gsα* mutants which could suggest additional transduction mechanisms. Expression and genetic analysis of the heterotrimeric G-protein subunit, Gq, shown here suggest involvement of this Gα subunit in trehalose perception in *Drosophila*. A green fluorescent protein reporter of Gq expression is detected in gustatory neurons in the labellum, tarsal segments, and wing margins. Animals heterozygous for *dgq* mutations and RNA interference-mediated knockdown of *dgq* showed reduced responses to trehalose in the proboscis extension reflex assay and feeding behavior assay. These defects were rescued by targeted expression of the wild-type *dgqα* transgene in the GRNs. These data together with observations from other mutants in phospholipid signaling provide insights into the mechanisms of taste transduction in *Drosophila*.

Key words: *dgq*, *plc21C*, proboscis, *rdgA*, taste transduction, wing margins

Introduction

Compelling evidence suggests that sugar taste information is initially received by G-protein–coupled receptors (GPCRs), 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 receptors coupled to a membrane-associated heterotrimer composed of a GTP-hydrolyzing Gα subunit and a Gβγ dimeric partner. In mammals, there is evidence for the role of G-protein subunits, a phospholipase C (PLC), a phosphodiesterase, an inositol 1,4,5-triphosphate ($InsP_3$) receptor, and a transient receptor potential-like (TRPL) channel during taste transduction. The α subunit of a G-protein, α-gustducin, has been implicated in transduction of sweet and bitter compounds in mammals (McLaughlin et al. 1992; Wong et al. 1996). A distinct subunit, Gγ13, underlies response to the bitter compound, denatonium (Huang et al. 1999). Phospholipase C-β_2 (PLCβ_2) (Zhang et al. 2003) and a TRP channel, Transient receptor potential cation channel, subfamily M, member 5 are essential for sweet and bitter signal transduction pathways (Zhang et al. 2003).

In *Drosophila melanogaster*, gustatory receptor neurons (GRNs) express 60 gustatory receptor genes (*Grs*), which are similar to GPCRs (Clyne et al. 2000; Dunipace et al. 2001; Scott et al. 2001; Robertson et al. 2003). Among these, Gr5a is the most extensively studied gustatory receptor

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that is thought to be coupled to a heterotrimeric G-protein (Neves et al. 2002; Wong 2003). The natural ligand recognized by Gr5a is trehalose (-D-glucopyranosyl–D-glucopyranoside; Dahanukar et al. 2001; Ueno et al. 2001; Chyb et al. 2003). Recently, Dahanukar et al. (2007) have shown that mutants in *Gr5a* show defective behavior and electrophysiological responses toward trehalose, whereas animals lacking *Gr64a* show defective responses to a complementary subset of sugars, namely sucrose, maltose, and glucose (Dahanukar et al. 2007). Flies lacking both receptors are unable to respond to a large subset of sugars. Gr64f (a member of Gr64 cluster) is required in combination with other gustatory receptors for sugar detection in *Drosophila* (Jiao et al. 2007). Sugar receptors function as multimers and Gr64f is required broadly as a coreceptor for the detection of sugars. Although the roles of different receptors and their substrate specificities are beginning to be understood, studies on the mechanism of transduction have lagged behind.

A role for Gsα has been demonstrated because *DGsα*-null mutant heterozygotes as well as RNAi-mediated knockdown of this protein in taste neurons lead to a drop in sensitivity to sugars in electrophysiological assays (Ueno et al. 2006). Interestingly, Talluri et al. (1995) have documented the expression of *dgqα3* in chemosensory neurons of the proboscis. Because responses to trehalose are not completely abolished in *DGsα*-null, a role of other G-protein subunits in taste transduction remains possible.

In this paper, we demonstrate the role of dGq, in trehalose taste transduction in *Drosophila.* The $G_q\alpha$ pathway stimulates PLCβ to produce the intracellular messengers inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG). $G_q\alpha$ and NorpA (PLCβ) are expressed in the chemosensory neurons of proboscis (Talluri et al. 1995; Koganezawa and Shimada 2002). We used mutants in *dgq* and molecules that are believed to act downstream during signal transduction (*plcβ21c*, *rdgA*, and *trp*/*trpl*/*trpγ* channels) in behavioral assays to test whether this pathway operates during trehalose sensing in *Drosophila.* Results from these experiments coupled with those using RNA interference (RNAi)-mediated knockdown of $G_q\alpha$ lead us to suggest that sugar sensing by the gustatory neurons utilizes transduction via the $G_q\alpha$ pathway together with the *DGsα* pathway demonstrated previously.

Materials and methods

Fly stocks

The following stocks, dgq^{221c} and dgq^{1370} alleles of *dgq*, *Gq-GAL4*, RNAi construct for *dgq* (*UAS-Gq*^{1F1}), *UAS-dgqα3*, *plc21c* insertion allele (*plc21c*^{P319}) and the deficiency *Df(2L)p60A* which uncovers *plc21C*; *rdgA*^{1} and *rdgA*^{3}, have been described earlier (Weinkove et al. 1999; Hardie et al. 2002, 2003; Banerjee et al. 2006; Kain et al. 2008). *Gr5a-GAL4* on second and third chromosomes was kindly provided by Kristin Scott, Berkeley, USA. *trp*^{343}/*trp*^{343} (null allele of *trp*; Raghu et al. 2000), *Trp*^{365} (Yoon et al. 2000; Hong et al. 2002), and *trpl*^{302}/*trpl*^{302} (null allele of *trpl;* Scott et al. 1997) were generously gifted by Raghu Padinjat. *UAS-RNAi* lines *for plc21c* (*UAS-plc21c RNAi*^{557}; Kain et al. 2008), *rdgA* (*UAS-rdgA RNAi*^{100}; Kain et al. 2008), *trpl* (*UAS-trpl RNAi*^{571}), and *trpγ* (*UAS-trpγ RNAi*^{338}) are from Vienna Drosophila RNAi Center (http://stockcenter.vdrc.at/). The *UAS-itpr RNAi* stock is from National Institute of Genetics Japan and has been described previously (Agrawal et al. 2009). GAL80^{ts}, *UAS-TNTG*, *UAS-IMPTV*, and *UAS-2XEGFP* were obtained from the Bloomington stock centre. All stocks were grown on standard cornmeal medium at 25 °C.

Visualization of reporter activity in gustatory organs

Appendages–proboscis, legs, and wings from a *Gq-GAL4*/*UAS-2XEGFP* genotype were chopped and mounted in Vectashield mounting media (Vector laboratories, Inc.). The samples were analyzed for green fluorescent protein (GFP) expression using an Olympus FV1000 confocal microscope. The confocal sections were of 0.5 μm thickness.

Behavioral assays

Proboscis extension reflex behavioral assay

The protocol described in Rodrigues and Siddiqi (1978) was used. Newly eclosed flies were transferred to fresh vials with standard cornmeal agar medium and maintained at room temperature for 3–6 days. Flies were then starved for 18 h in vials with moist filter paper and immobilized by cooling on ice. They were mounted ventral side up on microscope slides with the help of nail polish. The preparations were kept in a moist chamber for 2–3 h to allow recovery. Stimuli were prepared in water and taken into a 1-mL plastic syringe such that the tarsus could be stimulated by touching a hanging drop on the needle of hypodermic syringe. Flies were first stimulated with water and allowed to drink until satiated. During stimulation with sugars, the flies were not allowed to drink; following each response, the fly was retested with water to control against the possibility that it was not fully water satiated. Each fly was tested 5 times; the interval between 2 consecutive sugar stimulations was at least 3 min to avoid the possibility of adaptation. Flies showing 3 or more extension of 5 trials were taken as responders. A set of 8–10 batches of 10 flies each was tested, and the percentage response of each batch was calculated. All experiments were double blinded. Graphs show the mean ± standard error of the mean (SEM) of the percentage response obtained from these batches. Statistical significance was calculated using the Student *t*-test. The stimulus used in these experiments is trehalose (Beckton Dickenson and Company, India Pvt. Ltd).

Feeding plate assay

The protocol is followed as been described in Arora et al. (1987). Freely moving flies are allowed to choose between

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2 agar based solutions; one containing trehalose and the other tasteless food dye (Carmosine red; Anand dyes and Co Ltd.). The solutions are placed in alternate wells of a microtiter plate. Flies, 2–4 day old, starved for 17–24 h at 25 °C in a moist vial were introduced into the plate by tapping them through a hole in the lid (without anesthesia). After feeding for 1 h, flies were immobilized by freezing plates at –20 °C and scored on the basis of color in the abdomen as bright red (+++), pink (++), intermediate (+), and white (–). Flies that respond to sugar will eat largely from the uncolored wells and their abdomens appear bloated but uncolored. The percentage of flies with uncolored abdomens was calculated by the following formula:

$$\%\ \text{uncolored} = \frac{(\text{No. of uncolored flies}) + (\text{No. of intermediate color flies}/2)}{(\text{Total number of flies in the assay})} \times 100$$

All experiments were double blinded. The results are represented as mean ± SEM is plotted. The significance of the differences in response between populations was measured by the Student *t*-test.

Results

$G_q\alpha$ is expressed in GRNs

We confirmed the localization of $G_q\alpha$ in taste neurons using a *Gq-GAL4* strain which bears a construct carrying 3.9 Kb of the $G_q\alpha$ upstream sequence fused in frame with GAL4. The GFP reporter driven by this strain has been shown to faithfully reflect endogenous $G_q\alpha$ expression (Kain et al. 2008). The diagram in Supplementary Figure S1A shows the distribution of GRNs (marked as green spots) on the taste organs that are found on the labellum, tarsal segments, and wing margins in adult *Drosophila*. The expression of $G_q\alpha$ in the taste neurons was monitored in a strain where *Gq-GAL4* drives GFP expression (Supplementary Figure S1B–F); expression was seen in the sense organs of the labellum (Supplementary Figure S1B), legs (Supplementary Figure S1C,D), and wing margins (Supplementary Figure S1E,F). There are between 2 and 4 chemosensory neurons innervating a single taste sensillum, of which only one is believed to be sugar sensitive (Falk et al. 1976; Nayak and Singh 1983). GFP expression could be detected in 3–4 cells within each taste organ (arrow in Supplementary Figure S1B), suggesting that multiple taste neurons express the $G_q\alpha$ subunit.

Sugar response is defective in heterozygous *dgq*-null mutants

Application of sugar stimuli to the taste organs on the legs or labellum stimulates extension of the proboscis and an attempt to feed. This response was used extensively by Dethier (1976) to characterize taste behavior in a variety of insects including *Drosophila*. We used this assay to measure the gustatory capabilities in mutants with lowered function of $G_q\alpha$. The P-excision allele, dgq^{221c}, carries a lesion in the 5′ end of *dgq* spanning the translation start site in exon 3 rendering it a null (Banerjee et al. 2006; Kain et al. 2008). Both dgq^{221c} and dgq^{1370} (induced by chemical mutagenesis) are lethal as homozygotes and as heterozygotes with *Df(2R)vg-C*, which uncovers the *dgq* locus (Kain et al. 2008). Because these alleles are homozygous lethal, we tested their ability to respond to trehalose sugars as heterozygotes (Figure 1A,B). The heterozygotes show a strong reduction in response to trehalose at all concentrations compared with canton-special (CS) background genotypes ($P < 0.005$ compare with CS). We verified this result using a previously characterized *dgq* RNAi line (Banerjee et al. 2006) to knock down levels in the *Gq-GAL4* expression domain (*Gq-GAL4*/*UAS-Gq*^{1F1}; Figure 1A,B). These animals also show a reduced response to trehalose at all concentrations tested ($P < 0.05$ for 0.01, 0.1, and 1 mM concentrations and $P < 0.005$ for 10, 100, and 1000 mM concentrations) compared with background genotypes. We confirmed that the parental strains did not harbor any background mutations that affect trehalose sensing, by measuring the proboscis extension reflex (PER) responses of homozygotes (Supplementary Figure S2).

We showed that cells expressing $G_q\alpha$ are responsible for eliciting a response to sugars by silencing these neurons through targeted expression of an active form of tetanus toxin (*UAS-TNTG*) (*Gq-GAL4*/*UAS-TNTG*; Figure 2). The gustatory responses to trehalose of these animals were measured in the feeding preference assay (Tanimura et al. 1982; Arora et al. 1987). The assay offers starved flies a choice between agar containing 10 mM trehalose and control agar that is tainted with a tasteless food color. The response is quantified by counting the number of flies with colored abdomens. We compared the responses of test flies with controls that expressed an imperfect form of tetanus toxin (TNT) (*IMPTV*); the responses of *Gq-GAL4*/*UAS-IMPTV* flies were comparable with other controls (*Gq-GAL4*/+ and CS). Flies expressing the active subunit of TNT (*Gq-GAL4*/*UAS-TNTG*) show a reduced response to trehalose as compared with control animals (Figure 2; $P < 0.0001$). Knockdown of $G_q\alpha$ in neurons using the RNAi construct driven by *Gq-GAL4* also leads to a reduced preference for trehalose as compared with control responses (*Gq-GAL4*/+, *UAS-Gq*^{1F1}, and CS; $P < 0.0001$; Figure 2).

Further evidence for the role of $G_q\alpha$ in trehalose-sensitive neurons was provided by RNAi-mediated knockdown of *dgq* in the Gr5a neurons using a specific GAL4 line (*Gr5a-GAL4*). The behavioral responses to trehalose in strains of transgenic flies carrying both *Gr5a-GAL4* and *UAS-Gq*^{1F1} constructs were significantly lower from control flies carrying either the *Gr5a-GAL4* or the *UAS-Gq*^{1F1} construct alone (Figure 1C; $P < 0.005$).

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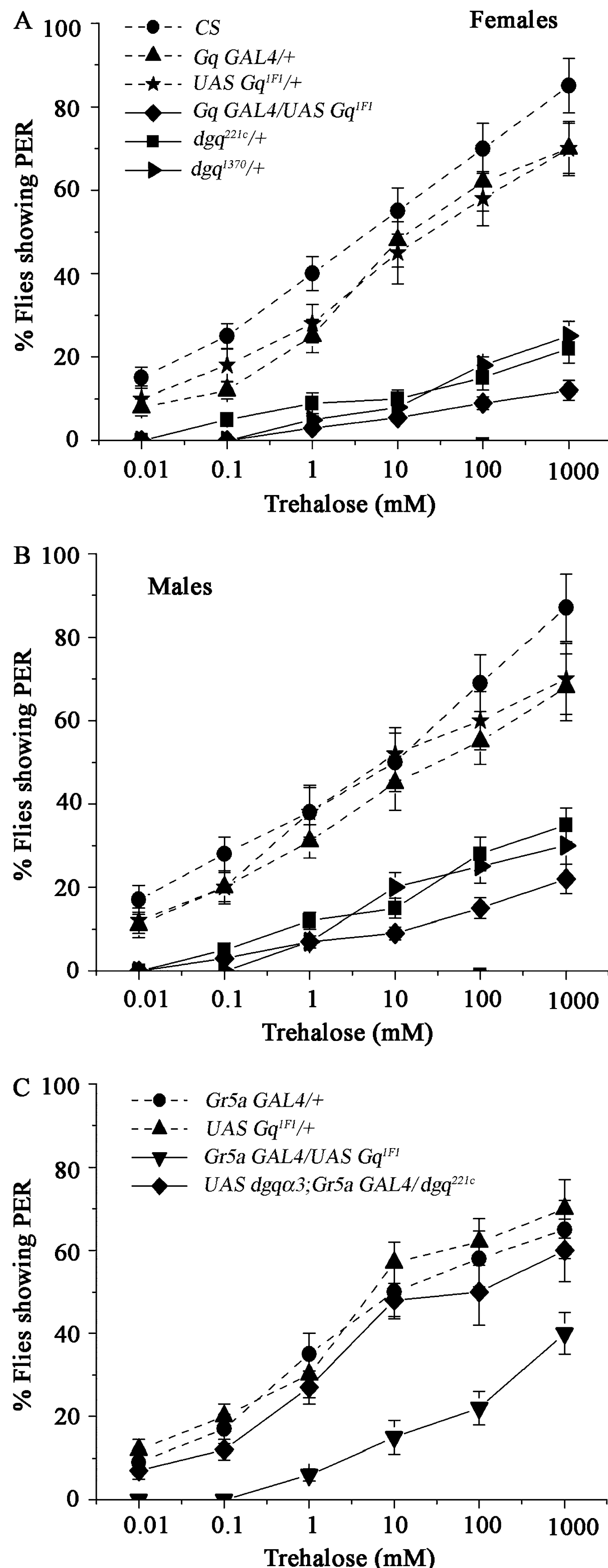

Figure 1 Taste sensitivity of *Drosophila* mutants for *dgq* to trehalose. The genotypes for PER shown in **A** (females) and **B** (males) are shown with different symbols in A (dash lines for control animals). The PER for dgq^{221c}/+ ($P < 0.005$ compare with wild-type CS), dgq^{1370}/+ ($P < 0.005$ compare with CS), and $Gq\text{-}GAL4/UAS\text{-}Gq^{1F1}$ (*dgq* RNAi; $P < 0.05$ for 0.01, 0.1, 1 mM concentrations and $P < 0.005$ for 10, 100, 1000 mM concentrations) are significantly lower than that of *CS* (WT), *Gq-GAL4*/+, and $UAS\ Gq^{1F1}$/+ flies. **(C)** The effect of *dgq* RNAi on Gr5a-positive neurons. The PER for trehalose in *dgq* RNAi-expressing flies ($Gr5a\text{-}GAL4/UAS\text{-}Gq^{1F1}$) is reduced compared with controls *Gr5a-GAL4*/+ and $UAS\text{-}Gq^{1F1}$/+ at all the concentrations of trehalose tested ($P < 0.005$). PER are rescued when *UAS dgqα3* is introduced in the heterozygous null mutant ($UAS\ dgq\alpha3;\ Gr5a\text{-}GAL4/dgq^{221c}$) background ($P > 0.001$ compare with control animals) at all the concentrations of trehalose. Each data point represents the mean ± SEM of 8–10 experimental sets.

Transgene expression of $G_q\alpha$ can rescue the gustatory defects of *dgq* mutants

To confirm that reduced trehalose responses in heterozygous *dgq*-null mutants is a consequence of reduced signaling through $G_q\alpha$, a wild-type copy of the *dgqα3* splice variant of *dgq* (Kain et al. 2008) was targeted to the Gr5a neurons in dgq^{221c}/+ flies (Figure 1C). The response to trehalose in dgq^{221c}/+ flies carrying both *Gr5a-GAL4* and *UAS dgqα3* was significantly higher than in dgq^{221c}/+ flies ($UAS\ dgq\alpha3;\ dgq^{221c}/Gr5a\text{-}GAL4$; Figure 1C; $P < 0.001$), indicating that the trehalose phenotype in dgq^{221c}/+ flies can be rescued by expression of *UAS dgqα3* exclusively in the Gr5a GRNs. Taken together with the *dgq* RNAi data, we conclude that reduced trehalose responses in dgq^{221c}/+ flies can be attributed to low level of $G_q\alpha$ in the Gr5a GRNs.

This together with previous data from Ueno et al. (2006) support the idea that 2 different G proteins-$G_q\alpha$ and Gsα-collaborate in trehalose sensing in *Drosophila.*

Behavioral response to trehalose in PLC mutants—*plcβ21c*

Several studies have shown that the downstream effector of $G_q\alpha$ signaling is phospholipase Cβ (PLCβ) (Devary et al. 1987; Lee et al. 1990; Scott et al. 1995). There are 2 genes encoding PLCβ in the *Drosophila* genome, referred to as *norpA* (Bloomquist et al. 1988) and *plc21C* (Shortridge et al. 1991). The $plc21c^{P319}$ mutant allele is a viable hypomorph, with a P-insert in the first intron of *plc21c,* whereas $plc21c^{p60a}$ is a small deficiency that removes the 5′ end of *plc21c* and a neighboring essential gene *p60A* (Weinkove et al. 1999). The response to trehalose is affected in both $plc21c^{P319}$/+ (*P319*/+) and $plc21c^{p60a}$/+ heterozygotes (*p60A*/+) (Figure 3A; $P < 0.005$ compared with CS shown in Figure 1A). *P319*/*P319* and *p60A*/*P319* animals showed greatly reduced responses at all concentrations of trehalose tested compared with heterozygotes (Figure 3A; $P < 0.005$). Flies of the genotype $norpA^{P24}$ were unaffected at any concentrations of trehalose tested (data not shown). This was demonstrated previously (Ueno et al. 2006). Hence, the results shown here suggest that *Drosophila* GRNs require *plc21C*, but not *norpA*, for normal responses to trehalose.

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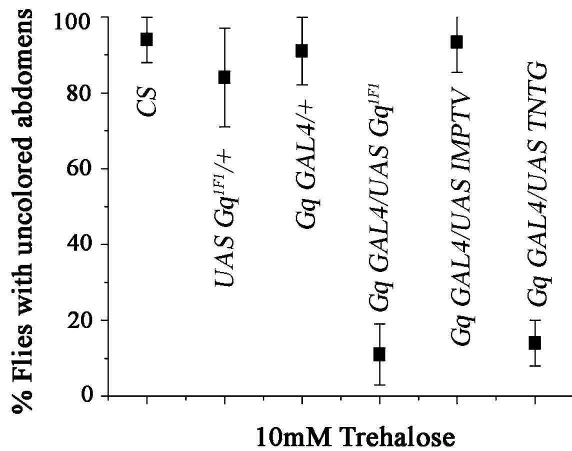

Figure 2 Gq knockdown by RNAi and expression of tetanus toxin in *Gq-GAL4* neurons leads to reduced feeding of trehalose as measured by the feeding plate assay. The feeding response to trehalose in *Gq-GAL4/UAS-Gq^{1F1}* (10–20%) flies is significantly lower than that in *CS*, *Gq-GAL4/+*, and *UAS-Gq^{1F1}/+* (80–90%; P < 0.0001). The response to trehalose in flies expressing the active form of tetanus toxin (*UAS-TNTG*) driven by *Gq-GAL4* (10–20%; *Gq-GAL4/UAS-TNTG*) was significantly lower than when inactive form of tetanus toxin is expressed (`90%; *Gq-GAL4/UAS-IMPTV*; P < 0.0001). Each symbol represents the mean ± SEM of 10 experimental plates for each genotype (100 flies in each plate).

DAG kinase is necessary for normal trehalose responses

The data so far show that trehalose responses in *Drosophila* are significantly attenuated by mutants in *dgq* and *plc21C* genes, similar to the effect of these mutants on olfactory responses (Kain et al. 2008). PLCβ acts on the membrane bound phospholipid, phosphatidyl inositol bis-phosphate to generate 2 second messengers, DAG which remains membrane bound and soluble inositol 1,4,5-trisphosphate ($InsP_3$). To understand which among these 2 second messengers function in taste reception, we measured the response of adults with defects in the gene *rdgA*, which encodes an ATP-dependent DAG kinase, that converts DAG to phosphatidic acid (Inoue et al. 1989). Homozygotes for 2 *rdgA* mutant alleles *rdgA^{1}* and *rdgA^{3}* (P < 0.005 for 10, 100, and 1000 mM trehalose) exhibit reduced responses to trehalose compared with heterozygotes (*rdgA^{1}*/+ and *rdgA^{3}*/+; Figure 3B).

Interestingly, heterozygotes of both, the *plc21βC* alleles (*P319*/+ and *p60A*/+; Figure 3A) as well as *rdgA* alleles, (*rdgA^{1}*/+ and *rdgA^{3}*/+; Figure 3B), showed lowered PER responses as compared with CS (WT) shown in Figure 1A. These differences could arise from variations in the genetic background or could indicate that dosages of *plc21c* and *rdgA* gene products are important for trehalose taste sensing. However, in both cases, the mutants as homozygotes showed significantly lower responses than the heterozygotes (P < 0.005) arguing for a requirement for both PLCβ and rdgA in trehalose sensing. In order to support this conclusion, we used RNAi-mediated knockdown of *plc21c* and *rdgA* using the *Gr5a-GAL4* driver. Expressing *plc21c* or *rdgA* RNAi in Gr5a neurons (*Gr5a-GAL4*/*UAS-RNAiplc21c^{557}* or *Gr5a-GAL4*/*UAS-RNAirdgA^{100}*) showed lowered PER responses compared with controls (Figure 3C; P < 0.005 compared with *UAS-RNAiplc21c^{557}*/+ or *UAS-RNAirdgA^{100}*/+ animals).

In both *rdgA* alleles, proboscis morphology is normal even 6 days after eclosion as judged by examining the morphology of the proboscis and sensillae present at the light microscopic level (data not shown) as described for antennae (Kain et al. 2008) but unlike in eyes which undergo significant retinal degeneration (Masai et al. 1993), the proboscis morphology appeared normal. However, the conclusion that reduced sensitivity to trehalose is unlikely to be due to neurodegeneration requires analysis at a higher resolution.

The role of the $InsP_3$ receptor in sugar sensing has been documented by different groups in vertebrates and invertebrates. It is been shown that targeted expression of $InsP_3$ sponge and $InsP_3$ double stranded RNA impairs sugar taste sensation in *Drosophila* as well in other diptera (Koganezawa and Shimada 2002; Usui-Aoki et al. 2005). We tested if $InsP_3R$ plays any role during trehalose taste transduction in *Drososphila*. *itpr* gene transcripts were knocked down in the Gr5a GRNs with the help of an RNAi construct (*Gr5a-GAL4*/*UAS-itprRNAi*; Agrawal et al. 2009). We observed that *Gr5a-GAL4*/*UAS-itprRNAi* animals exhibit lowered responses compared with control animals of the genotypes *Gr5a-GAL4*/+ and *UAS-itprRNAi*/+ (Supplementary Figure S3; P < 0.05). However, *itpr* gene knockdown affected trehalose sensing to a lesser extent than knockdown of other genes including *dgq*, *plcβ21c*, and *rdgA* (compare Supplementary Figures S3 with Figures 1 and 3), suggesting a modulatory or indirect role for the $InsP_3R$.

Trehalose response in TRPC channel mutants

In *Drosophila* vision, the generation of DAG or a DAG metabolite by PLCβ results in the opening of a class of membrane channels referred to as the transient receptor potential canonical (TRPC) channels (Hardie and Raghu 2001; Hardie 2003). To examine the role of TRPC channels in trehalose sensing, the responses of *trp*, *trpl*, *and trpγ* mutants were measured in behavioral assays. *trp^{343}* is a null allele (Scott et al. 1997), whereas *Trp^{365}* is a gain of function allele at the *trp* gene (Yoon et al. 2000; Hong et al. 2002). Both *trp* loss of function (*trp^{343}*/*trp^{343}*) and gain of function (*Trp^{365}*/*Trp^{365}*) alleles showed significantly lower responses when compared with their heterozygote controls (*trp^{343}*/+ and *Trp^{365}*/+; Figure 4A; P < 0.005). In order to lower the levels of Trpl and Trpγ specifically in Gr5a neurons, we exploited 2 RNAi lines. Both *Gr5a-Gal4*;*UAS-trpl^{571}RNAi* and *Gr5a-GAL4;UAS-trpγ^{338}RNAi* flies showed a strongly reduced response curve to trehalose compared with *Gr5a-GAL4*/+, *UAS-trpl^{571}RNAi*/+, and *UAS-trpγ^{338}RNAi*/+ (Figure 4B; P < 0.005 for 0.01 to 10 mM concentrations and P < 0.0001 for 100 and 1000 mM trehalose) at all the concentrations of trehalose. Null mutants for *trpl* also showed a strong reduction in response to trehalose at all

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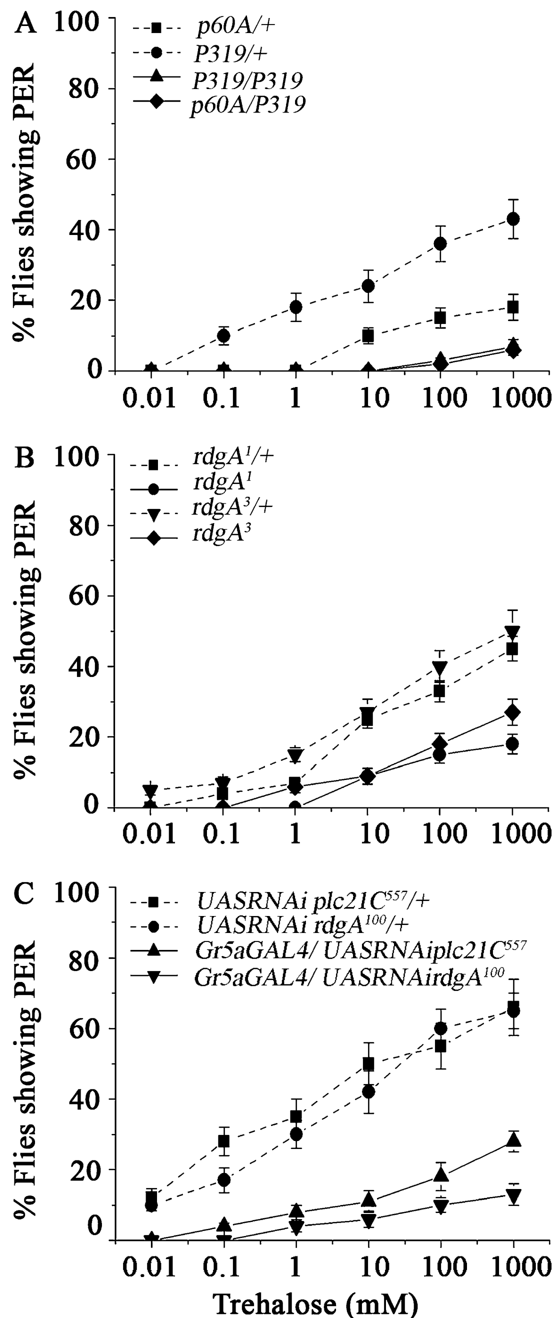

Figure 3 PER of mutants in phospholipid signaling. **(A)** *plcβ21c* deficiency (*p60A*) and mutant (*P319*) alleles. The responses of *P319/P319* and *p60A/P319* flies are significantly lower than *P319/+* heterozygote control animals ($P < 0.005$) at all concentrations of trehalose tested. *p60A/+* and *P319/+* both showed lowered PER responses compare with CS (WT) shown in Figure 1A ($P < 0.005$). But PER of *p60A/+* flies are more lowered compared with *P319/+* animals **(B)** Trehalose sensitivity is reduced in DAG kinase (*rdgA*) mutants. Trehalose response of homozygous mutant alleles of *rdgA* (*rdgA^1/rdgA^1* and *rdgA^3/rdgA^3*) are reduced compared with 2 different heterozygotes (*rdgA^1/+* and *rdgA^3/+*; $P < 0.005$ with *rdgA^1/rdgA^1* and *rdgA^3/rdgA^3* for 10, 100, and 1000 mM trehalose). Just like *plc21c* alleles, *rdgA^1/+* and *rdgA^3/+* both showed lowered PER responses compare with CS (WT) shown in Figure 1A ($P < 0.005$). Both *rdgA* mutants alleles and heterozygote controls behaved in a similar way at lowered concentrations (0.01, 0.1, and 1 mM trehalose). **(C)** *plc21c* and *rdgA* knockdown by RNAi *Gr5a-GAL4* neurons leads to reduced PER responses. Expressing *plc21c* RNAi in Gr5a neurons (*Gr5a-GAL4/UAS-RNAiplc21c^{557}*) showed lowered PER responses just like the *plc21c* mutants alleles ($P < 0.005$ compared with *UAS-RNAiplc21c^{557}/+* animals). *rdgA* RNAi driven by *Gr5a-GAL4* (*Gr5a-GAL4/UAS-RNAirdgA^{100}*) leads to reduced PER responses compared with *UAS-RNAirdgA^{100}/+* animals ($P < 0.005$). Each symbol represents the mean ± SEM of 8–10 experimental sets.

concentrations tested (Figure 4C; $P < 0.0001$ compared with CS). Moreover, flies heterozygous for the *trpl* null allele (*trpl$^-$/+*) exhibit significantly lower responses toward trehalose (Figure 4C). The reduced response of *trpl–/–* animals could be rescued by expression of *UAS-trpl$^+$* in the *Gr5a-GAL4* domain (*trpl–/–; Gr5a-GAL4/UAS-trpl$^+$*) with responses comparable with that of the wild-type controls—*CS*, *Gr5a-GAL4/+*, and *UAS-trpl$^+$/+* (Figure 4C; $P > 0.001$). Interestingly, animals with overexpression of *UAS-trpl$^+$* in Gr5a neurons [*Gr5a-GAL4/UAS-trpl$^+$*] alone showed lower responses to all concentrations of trehalose (Figure 4C). Thus, Gr5a-positive neurons and their response to trehalose appear very sensitive to the level of TRPC channels. Taken together, the results presented here suggest that normal trehalose responses in the sugar sensing neurons of *Drosophila* require PLCβ, its downstream genes encoding a DAG kinase (*rdgA*) and the $InsP_3$ receptor (*itpr*) as well as the membrane channels *trp*, *trpl*, and *trpγ*.

Mutants in the Gq signaling affect trehalose sensing in adults

In order to verify that PER defects in response to trehalose are not developmental but are rather due to signaling defects, we exploited the $GAL80^{ts}$ expression system (McGuire et al. 2003). Temporal expression of *dgq, plc21c, rdgA, trpl,* and *trpγ* RNAi during the very late pupal stages in gustatory neurons showed similar reduction in PER responses as seen with the mutants of these molecules or expressing RNAi in Gr5a neurons. Flies of genotypes 1) *GAL80^{ts}; Gr5a-GAL4/UAS-Gq^{1F1}*, 2) *GAL80^{ts}; Gr5a-GAL4/UAS-RNAiplc21c^{557}*, 3) *GAL80^{ts}; Gr5a-GAL4/UAS-RNAirdgA^{100}*, 4) *GAL80^{ts}; Gr5a-GAL4/UAS-trpl^{571}RNAi*, and 5) *GAL80^{ts};Gr5a-GAL4/ UAS-trpγ^{338}RNAi* were reared at 18 °C ($GAL80^{ts}$-active) and shifted to 29 °C ($GAL80^{ts}$-inactive) at late pupal stages (black pupae) and grown till the sixth day after eclosion. All control animals were treated in the same manner. *GAL80^{ts}; Gr5a-GAL4/UAS-Gq^{1F1}* flies showed lowered PER responses to trehalose (Figure 5A; $P < 0.001$) compared with control animals *GAL80^{ts}; Gr5a-GAL4/+* and *UAS-Gq^{1F1}/+* tested on the same day. This defect could be rescued by introduction of a *UAS-Gq* transgene (Figure 5A; *UAS-dgqα3/GAL80^{ts}; Gr5a-GAL4/UAS-Gq^{1F1}*).

Similarly, reduced PER was observed when *plc21c* and *rdgA* RNAi were expressed very late in the pupae (Figure 5B). Both *GAL80^{ts}; Gr5a-GAL4/UAS-RNAiplc21c^{557}* and *GAL80^{ts}; Gr5a-GAL4/UAS-RNAirdgA^{100}* showed lowered

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PER compared with controls—*UAS-RNAiplc21c*^{557}/+ and *UAS-RNAirdgA*^{100}/+ (Figure 5B; $P < 0.005$).

Late expression of *trpl* and *trpγ* RNAi constructs in the Gr5a neurons showed reduced PER as well at all the concentrations of trehalose (Figure 5C). *GAL80*^{ts}; *Gr5a-GAL4*/*UAS-trpl*^{571}*RNAi* and *GAL80*^{ts}; *Gr5a-GAL4*/*UAS-trpγ*^{338}*RNAi* animals showed significantly lowered PER compared with *UAS-trpl*^{571}*RNAi* /+ and *UAS-trpγ*^{338}*RNAi* /+ (Figure 5C; $P < 0.005$). Results from RNA-mediated knockdown experiments support our data from mutants that suggest that the Trpl channel is necessary for trehalose sensing in adult flies. The defect in the *trpl*$^{-}$ homozygous mutants could be rescued by temporal expression of the *trpl*$^{+}$ (Figure 5C; *GAL80*^{ts}; *trpl–/–*; *Gr5a-GAL4*/*UAS-trpl*$^{+}$). These animals responded to trehalose in a manner comparable with wild type ($P > 0.001$). Interestingly, overexpression of wild-type *trpl* (*UAS-trpl*$^{+}$) in Gr5a neurons also leads to reduced PER responses compared with *UAS-trpl*$^{+}$/+(*GAL80*^{ts}; *Gr5a-GAL4*/*UAS-trpl*$^{+}$ Figure 5C; $P < 0.005$). The mechanism underlying this phenotype requires further investigation.

These data together provide compelling evidence that several molecules in the dGq pathway are necessary for trehalose sensing in the adult fly. Temporal knockdown of *dgq*, *plcβ21c*, *rdgA*, *trpl*, and *trpγ* in mature Gr5a neurons (after late pupae) leads to significant defects in trehalose sensing by the proboscis extension test.

Discussion

In this study, we determined that the *Drosophila* gene encoding Gqα, *dgq,* is expressed in GRNs where its effect is on the behavioral response to sugars. We show that normal levels of Gqα are required to evoke responses to trehalose in the Gr5a-positive GRNs. Defects in behavior could, however, arise from a lesion at any step of the circuit from sensory transduction to integration of information or a motor output. We were able to delimit the effects on the sensory neuron by use of cell-specific RNAi-mediated

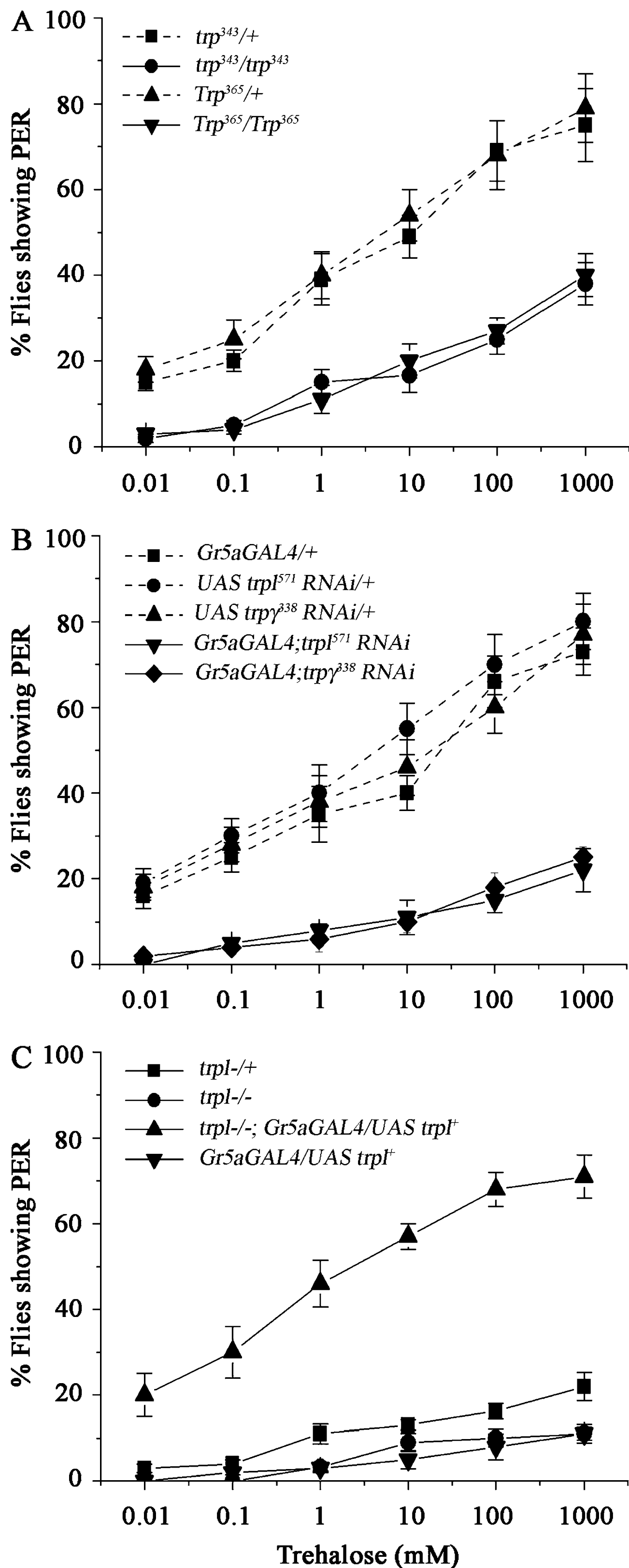

Figure 4 PER for trehalose in *trp*, *trpl*, and *trpγ* mutant and RNAi-expressing flies. **(A)** The loss (*trp*^{343}/*trp*^{343}) and gain of function (*Trp*^{365}/*Trp*^{365}) mutant alleles of *trp* show reduced responses at all concentrations of trehalose tested as compared with heterozygote control animals (*trp*^{343}/+ and *Trp*^{365}/+; $P < 0.005$). **(B)** The PER of test animals expressing RNAi driven by *GR5a-GAL4* (*Gr5a-GAL4*/*UAS-trpl*^{571}*RNAi* and *Gr5a-GAL4*/*UAS trpγ*^{338}*RNAi*) are significantly lower than control animals (*Gr5a-GAL4*/+, *UAS-trpl*^{571}*RNAi*/+, and *UAS-trpγ*^{338}*RNAi*/+; $P < 0.005$ for 0.01 to 10 mM concentrations and $P < 0.0001$ for 100 and 1000 mM trehalose) at all concentrations of trehalose tested. **(C)** The response to trehalose is highly sensitive to levels of Trpl. Heterozygote and homozygous *trpl* animals both exhibit deficient PER at all the tested concentrations of trehalose. Overexpression of wild-type *trpl* (*UAS-trpl*$^{+}$) in Gr5a neurons (*Gr5a-GAL4*/*UAS-trpl*$^{+}$) showed lower responses toward trehalose just like the *trpl* null allele. Rescue is obtained when wild-type *trpl* is expressed in Gr5a neurons in *trpl* null mutant background (*trpl–/–; Gr5a-GAL4/UAS-trpl*$^{+}$). The rescue PER response are comparable with control flies ($P > 0.001$ compared with *Gr5a-GAL4*/+ and CS). Each data point represents the mean ± SEM of 10 experimental sets.

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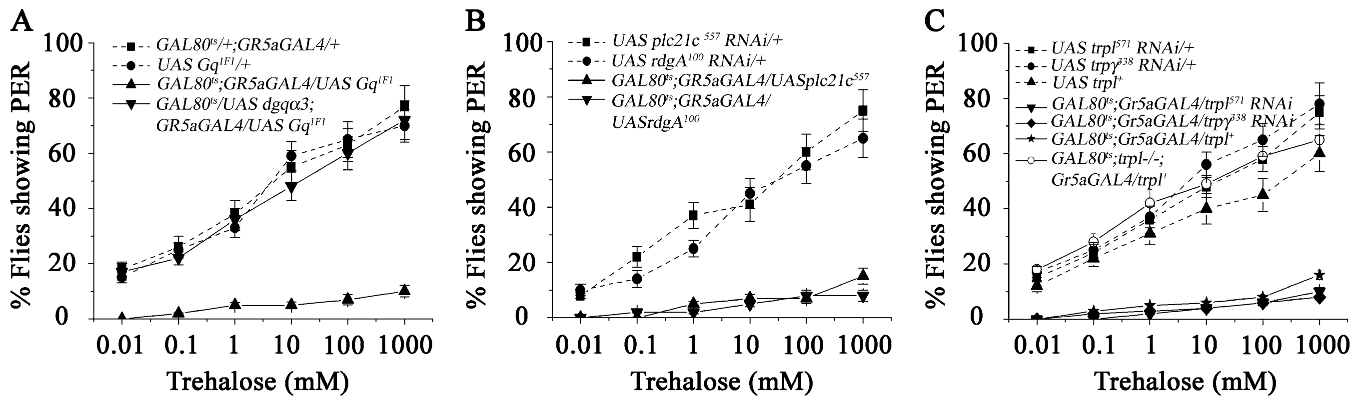

Figure 5 Defects in signaling cause reduced PER responses in mutants of Gq signaling pathway. Temporal expression of *dgq*, *plc21c*, *rdgA*, *trpl*, and *trpγ* RNAi during the very late pupal stages in Gr5a gustatory neurons showed lowered PER. Control and experimental flies were reared at 18 °C and shifted to 29 °C at late pupal stages (black pupae) and grown till the sixth day after the eclosion. **(A)** *GAL80^{ts}; Gr5a-GAL4/UAS-Gq^{1F1}* flies showed lowered PER responses to trehalose (Figure 5A; $P < 0.001$) compared with control animals *GAL80^{ts}; Gr5a-GAL4/+* and *UAS-Gq^{1F1}/+* tested on the same day. Temporal rescue of *dgq* was achieved by expressing *UAS dgqα3* in the GAL80^{ts} background (*UAS dgqα3/GAL80^{ts}; Gr5a-GAL4/UAS-Gq^{1F1}*) showed normal responses just like the control animals (Figure 5A; $P > 0.001$). **(B)** Similarly, reduced PER were observed when *plc21c* and *rdgA* RNAi were expressed very late in the pupae. Both *GAL80^{ts}; Gr5a-GAL4/UAS-RNAiplc21c^{557}* and *GAL80^{ts}; Gr5a-GAL4/UAS-RNAirdgA^{100}* showed lowered PER compared with controls—*UAS-RNAiplc^{557}/+* and *UAS-RNAirdgA^{100}/+* ($P < 0.005$). **(C)** Late expression of *trpl* and *trpγ* RNAi constructs in the Gr5a neurons showed reduced PER as well at all the concentrations of the trehalose. *GAL80^{ts}; Gr5a-GAL4/UAS-trpl^{571}RNAi* and *GAL80^{ts}; Gr5a-GAL4/UAS-trpγ^{338}RNAi* animals showed significantly lowered PER compared with *UAS-trpl^{571}RNAi /+* and *UAS-trpγ^{338}RNAi /+* ($P < 0.005$). Overexpression of wild-type *trpl* (*UAS-trpl^{+}*) in Gr5a neurons also leads to reduced PER responses compared with *UAS-trpl^{+}/+* ($P < 0.005$). Rescue of *trpl* phenotype was obtained by expressing *UAS-trpl^{+}* in the GAL80^{ts} background (*GAL80^{ts}; trpl−/−; Gr5a-GAL4/UAS-trpl^{+}*). *GAL80^{ts}; trpl−/−; Gr5a-GAL4/UAS-trpl^{+}* showed normal responses comparable with control animals ($P > 0.001$). Each data point represents the mean ± SEM of 8–10 experimental sets.

knockdown of different gene. Results from these experiments allow us to suggest that trehalose sensing in *Drosophila* occurs through a $G_q\alpha$-mediating transduction pathway coupled to the Gr5a receptor. This conclusion requires to be strengthened by data from electrophysiological measurement of neural activities of GRNs in response to sugars. Such an analysis still requires to be done. Our genetic data provides further evidence that molecules downstream of Gq signaling such as PLCβ, DAG Kinase, $InsP_3R$, and TRP channels also play a part in trehalose sensing in adult *Drosophila.*

Gq signaling is likely to cooperate with other G proteins in sugar sensitivity

After identifying *Drosophila* gustatory receptors as GPCRs, Clyne et al. (2000) postulated that taste transduction in *Drosophila* is very likely mediated by heterotrimeric G-proteins. Recently, 2 different G-protein subunits of heterotrimeric G-proteins, Gγ1 for sucrose (Ishimoto et al. 2005) and Gsα for trehalose (Ueno et al. 2006) have been reported as required for sugar perception in *Drosophila*. The Gγ1 gene is shown to be expressed largely in one class of GRNs. In null mutants of *Gsα*, the response to trehalose is strongly reduced (Ueno et al. 2006) but not abolished suggesting that other G proteins may collaborate in trehalose sensing. We have shown that signaling through $G_q\alpha$ is also involved in trehalose reception in the Gr5a-expressing neurons. The mechanisms by which multiple transduction pathways act within a single neuron requires further investigation.

A surfeit of signaling pathways and intermediates in chemosensory neurons

A great deal of controversy still exists about the mechanisms underlying chemosensory transduction in *Drosophila.* Recent studies have shown that the odorant receptors in insects exist in an unusual topology with the proposed G-protein domain lying on the extracellular side of the membrane (Benton et al. 2006). More recently, Sato et al. (2008) demonstrated that Or/Or83b heterodimers can act as ligand-stimulated nonselective ion channels. Such a mechanism would elicit a rapid response although the absence of an amplification step through G proteins is likely to make direct transduction through a ligand-gated receptor less sensitive. This signaling mechanism appears to reside in olfactory neurons together with Gαs (Wicher et al. 2008) and Gαq (Kain et al. 2008) transduction modes that possibly operate for higher sensitivities of odorant detection.

The mechanisms of taste transduction are also likely to involve multiple signaling processes. Our genetic data lead us to propose that molecules that are postulated to act downstream of $G_q\alpha$ such as PLCβ, DAG Kinase, and TRPC channels, participate in trehalose sensing in the G5a neurons. This could mean that DAG or a DAG metabolite act as a second messenger in taste perception. This is supported by previous biochemical studies that show that a Gq/PLC-mediated

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pathway is involved in Gr5a-initiated signaling in *Drosophila* S2 cells (Chyb et al. 2003).

Our data also supports previous findings that $InsP_3$ may act as an intermediary in taste neurons (Usui-Aoki et al. 2005) in *Drosophila*. The existence of a PLC/IP3-mediating pathway in sugar-taste signaling has already been shown in the fleshfly *Boettcherisca peregrina* (Koganezawa and Shimada 2002) and the guanosine-3',5'-cyclic monophosphate/nitric oxide pathway in the blowfly (*Phormia regina*) (Amakawa et al. 1990; Murata et al. 2004). The 3′-5′-cyclic adenosine monophosphate pathway may be involved in sugar-taste perception in the frog, rat, and pig (Avenet and Lindemann 1987; Striem et al. 1989; Naim et al. 1991), whereas a recent study on T1R2/T1R3 gustatory sugar receptors of the mouse supports involvement of the PLC pathway (Miyoshi et al. 2001; Zhang et al. 2003). Additional comparative studies are necessary to elucidate the diversity of molecular mechanisms of sugar-taste signaling in various animals.

A central role for TRP, TRPL, and TRPγ is well established in *Drosophila* visual transduction (Montell et al. 1985; Montell and Rubin 1989; Niemeyer et al. 1996; Reuss et al. 1997; Scott et al. 1997; Xu et al. 1997, 2000). However, a role for the TRPC (canonical group) class of channels in sugar taste transduction in *Drosophila* has not been shown previous to this work. *painless*, the fly homolog of mammalian TRPA1/ANKTM1, the ankyrin group of TRP channels is been shown to mediate the wasabi response in *Drosophila* (Al-Anzi et al. 2006).

The role played by multiple signaling systems and channels in sugar sensing needs further investigation. Anatomical and electrophysiological experiments have demonstrated the presence of 2–4 chemosensory neurons innervating a single taste sensillum (Falk et al. 1976; Rodrigues and Siddiqi 1978; Nayak and Singh 1983; Arora et al. 1987). Of these, only one neuron is thought to be sugar sensing and the others respond to bitter, salt, and water, respectively. It is unclear how the large number of sugars discriminated by adult *Drosophila* are encoded in the activities of sugar neurons. The requirement of a myriad of signaling mechanisms within the same cell needs to be understood.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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