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Drosophila sweet taste receptor*

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Abstract: Like the *Sac* locus controlling sugar sensitivity in mice, the taste gene *Tre* of the fruitfly *Drosophila* was discovered in wild populations as a genetic dimorphism controlling gustatory sensitivity to a sugar trehalose. By activating a P-element transposon near the gene locus we obtained induced *Tre* mutations and analyzed the associated changes in gene organizations and the mRNA expressions. The analysis showed that *Tre* is identical to *Gr5a*, a gene that belongs to a novel seven-transmembrane receptor family expressed in chemosensory neurons and predicted to encode chemosensory receptors. Thus, *Gr5a* is a candidate sweet taste receptor in the fly. An amino acid substitution in the second intracellular loop domain was identified to be functionally correlated with the genetic dimorphism of *Tre*. Since *Tre* controls sweet taste sensitivity to a limited subset of sugars, other *Gr* genes phylogenetically related to *Tre* may also encode sweet taste receptors. Those candidate sweet taste receptors, however, are phylogenetically distinct from vertebrate sweet taste receptors, suggesting that the sweet taste receptors in animals do not share a common origin.

INTRODUCTION

The sugar content of food is one of the most important sensory pieces of information that induces or controls feeding behavior throughout animals. In food-deprived flies, a feeding response, or proboscis extension reflex, can be triggered by stimulating gustatory receptor neurons with sugar solutions [1]. The gustatory organs of the fly are chemosensory hairs that are distributed on the mouthpart, leg tips, or the wing margins. In each hair, a total of usually four receptor neurons are innervated and send their dendrites to the tip opening of the hair and also send their axons to the brain or to the thorax ganglion [2]. One of the four neurons in the hairs commonly found on the mouth or the legs specifically responds to sugars [1]. Thanks to the simple structure of the fly gustatory organs, it is possible to record and analyze the electrophysiological response of single gustatory neurons, and, in fact, the first simultaneous recording of the receptor potential and the action potential of a single sensory neuron was successful with the sugar-sensitive neuron of the fly [3].

Sweet substances for the fly

The stimulating effectiveness for flies among the sweet substances including oligosaccharides, glycosides, or polyols was first extensively studied in the blowfly by Dethier [4]. There is a striking similarity between sweet substances for flies and humans: for example, only pentoses or hexoses are sweet for flies; mono-, di-, and trisaccharides are sweet; D isomers tend to be sweeter than L isomers. The struc-

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tural and conformational requirements of sweetness for flies were reviewed by Kijima [5] and by Pflumm [6]. These analyses, as well as other studies of the synergistic or inhibitory interactions [7], cross adaptation between sugars [8], or the chemical or enzymatic pretreatment of the receptor neurons [9] led to the hypothesis that the molecular machinery of sugar reception involves more than two receptor molecules (or binding sites) with different ligand-binding specificity.

Drosophila gene controlling gustatory sugar sensitivity

A different line of evidence supporting the fact that multiple types of receptors are involved in the fly sweet taste response came from our physiological and genetic analysis of gustatory sugar sensitivity in the fruitfly *Drosophila melanogaster* [10–12]. We found that some wild strains show decreased gustatory sensitivity to trehalose while they show almost normal sensitivity to other sugars. The peripheral gustatory receptor neurons are involved for the difference [12]. The gustatory sugar sensitivity was determined by a behavioral feeding experiment and also by electrophysiological analysis. We found that about a log-unit higher trehalose concentrations are necessary to induce the same gustatory responses in the insensitive strains. Genetic analysis showed that a single gene located on the distal X chromosome is responsible for the genetic dimorphism [10,11]. The gene was named *Tre* (*Trehalose sensitivity*), and the allele responsible for the spontaneous mutation in wild populations leading to low trehalose sensitivity was designated as Tre^{01} (http://flybase.bio.indiana.edu/).

SWEET TASTE RECEPTOR OF THE FLY

Molecular identification of the sweet taste receptor gene

Although the trehalose sensitivity gene *Tre* was considered a candidate gene encoding a gustatory receptor molecule or a molecule involved in the transduction specific to a subset of sugars, no molecular information was available at that time.

The *Drosophila* databases and the genome projects [13], however, now provide us not only the sequence data, but also many cDNA clones and transposon insertions for a substantial number of genes or locations throughout the genome. Since *Tre* was precisely mapped within a small region on the salivary X chromosome, we investigated several P-element transposon insertions [14] within the region to induce mutations of *Tre*. Among them, an insertion *EP(X)496* was found to induce *Tre* mutations at a considerably high frequency when the transposon is activated to be imprecisely excised out, resulting in a miniature deletion of the flanking DNA sequences. Among the transposon excisions, flies carrying *Tre* mutations were behaviorally screened by a simple two-choice feeding preference test between 20 mM trehalose and 2 mM sucrose solutions. We recovered a total of 22 induced *Tre* mutations out of about 350 transposon excisions.

Figure 1 shows some examples of the *Tre* mutations with apparent decrease of the preference to trehalose solution in the two-choice feeding test. While most parent flies showed strong preference to 20 mM trehalose solution, all the induced mutants with the transposon-excisions showed an opposite preference to 2 mM sucrose solution due to the decrease in the sensitivity to trehalose. An advantage of using transposon as a mutagen is that the insertion site on the genome can be determined easily by the transposon sequence tag. We, therefore, investigated the DNA sequences near the insertion site in the mutant genome. Figure 2 illustrates changes in the genomic sequences of the three mutations. Note that in all three cases the deletion disrupts a gene, which was previously known as *Gr5a*. *Gr5a* is one of the members of the candidate chemosensory receptor gene family recently identified through the genome-wide screen of the genes with seven-transmembrane domains [15–18]. It belongs to the gustatory receptor (GR) subfamily with about 60 gene members, some of which are known to be expressed in the mouth or the leg gustatory receptor neurons. Their molecular functions as gustatory or olfactory receptors, however, have yet to be investigated.

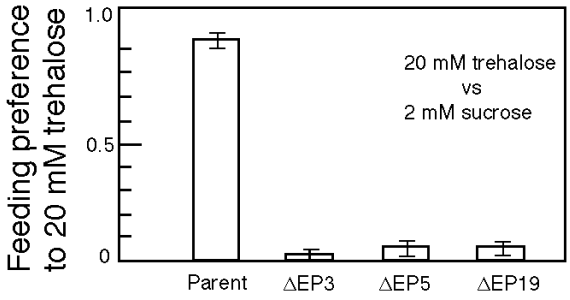

Fig. 1 Feeding preference in wild-type parent and the induced mutant strains. Flies deprived of food for 24 h were given a choice of two sugar solutions—20 mM trehalose and the control 2 mM sucrose solution—each stained with different dyes. After 1 h, flies choosing each solution were counted by inspecting the color of the digestive organs filled with the consumed solutions. For most wild-type parent flies, 20 mM trehalose solution is sweeter than 2 mM sucrose, while most mutant flies defective in the gustatory sensitivity to trehalose show the opposite preference choosing 2 mM sucrose.

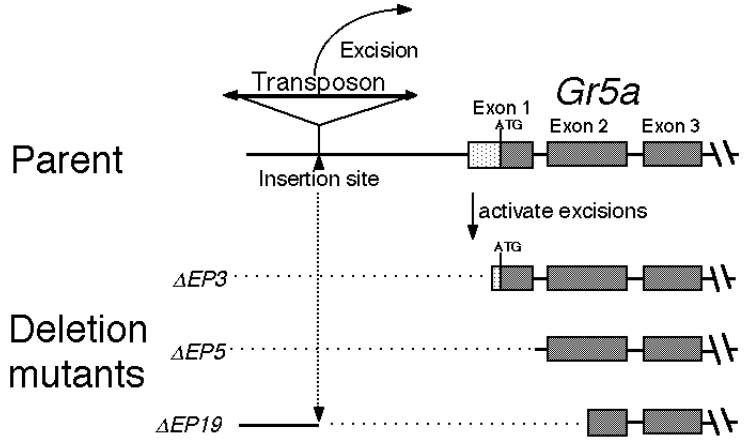

Fig. 2 Induced miniature deletions of the genomic DNA by imprecise excisions of a transposon. In the genome of the parent flies the transposon is inserted near *Gr5a* without disrupting the gene. By the excisions, the gene organization of *Gr5a* was disrupted due to the deletions as illustrated by broken lines.

The experimental details are described elsewhere [19]. One deletion (*ΔEP3*) had no promoter sequence with a truncated 5' leader. The others (*ΔEP5* and *ΔEP19*) had no promoter, no 5' leaders, and truncated coding sequences in the *N*-terminal regions (Fig. 2). Mutations *ΔEP3* and *ΔEP5* also disrupt an adjacent gene *CG3171*, which is not illustrated in Fig. 2 but is located near the insertion site. *CG3171* is a G protein-coupled receptor with unknown function and belongs to the rhodopsin superfamily. Ishimoto et al. suggested that *CG3171* is the taste receptor gene *Tre* responsible for gustatory trehalose sensitivity [21]. The following results, however, provide several lines of evidence against their conclusion.

They carried out a similar P mutagenesis experiment using *EP(X)496* and reported that all the deletions were associated with the disruption of the adjacent *CG3171* gene [21]. Our analysis [19], however, showed that the deletions induced by the P-element do not always disrupt *CG3171* as is the case for *ΔEP19*.

Ishimoto et al. also reported that *CG3171* is specifically expressed in taste receptor cells. Our Northern blot and reverse transcription-polymerase chain reaction (RT-PCR) analysis, however, failed

to support their observations and showed that *CG3171* is a ubiquitous gene expressed in nongustatory tissues or throughout the developmental stages [19]. We also showed that the expression of *CG3171* mRNA was not always disrupted among the *Tre* mutants. On the other hand, the RT-PCR analysis of *Gr5a* using wild-type and *poxn* mutant flies, where taste receptor neurons are transformed to nonneural cells, showed that *Gr5a* is expressed specifically in taste receptor neurons.

Another result by Ishimoto et al. [21], which was inconsistent with that of Dehanukar et al. [20], as described below, is that an ectopically introduced *CG3171* construct modifies the gustatory sensitivity to trehalose. Ishimoto et al. described an experiment with a transformant carrying a *Tre* mutation and an ectopic *CG3171* cDNA sequence under the control of a heat shock promotor. Although the gustatory preference of the transformant tested by a two-choice test between 80 mM trehalose and 2 mM sucrose solutions apparently changed by a heat shock treatment, the gustatory sensitivity remained as low as in the *Tre* mutant flies according to their data on the concentration–preference relationship (Fig. 2B [21]). Therefore, the transformant was not rescued. Systematic behavioral and physiological analyses using transformants with ectopically introduced *CG3171* and *Gr5a* were recently carried out by Dehanukar et al. to investigate whether one or the other gene affects gustatory sensitivity to trehalose [20]. They prepared constructs with a long genomic fragment fully covering the two genes with or without a stop-codon mutation in each coding region. The constructs were introduced to *ΔEP5* (double mutants of *CG3171* and *Gr5a*) or *ΔEP19* (single *Gr5a* mutant) flies that we provided for the experiment. By gustatory preference tests and electrophysiological recordings from the sensory neurons, they clearly showed that *Tre* mutations can be rescued only by wild-type *Gr5a* gene but not by wild-type *CG3171*.

Another independent line of evidence for *Gr5a* being identical to *Tre* was obtained by us from nucleotide sequence analysis of *CG3171* and *Gr5a* in wild populations [19]. As we will discuss in the next section, we identified a single nucleotide polymorphism (SNP) in the spontaneous *Tre* mutation that substitutes an amino residue of *Gr5a* resulting in low gustatory sensitivity to trehalose. The polymorphic sites leading to amino residue substitutions in the coding region of *CG3171*, on the other hand, were shown to be irrelevant to the gustatory phenotype. Taken together, both we [19] and Dehanukar et al. [20] concluded that the sugar sensitivity locus *Tre* is identical to *Gr5a* on the genome.

Sweet taste receptor protein

The spontaneous mutation Tre^{01} is commonly found in wild *Drosophila* populations. Analysis of *Gr5a* mRNA in Tre^{01} strains revealed that, unlike the transposon-induced *Tre* mutations, the *Gr5a* mRNA is normally expressed in the spontaneous mutants. We, therefore, suspected that there is a substitution of an amino acid that leads to the modification of gustatory sensitivity. A genomic DNA of 1.7 kbp in length covering the *Gr5a* coding region was analyzed for possible polymorphism. Based on the analysis, the amino acid sequence of a wild type *Gr5a* (*Tre*) is shown in the top row of Fig. 3. Among many wild and laboratory Tre^{+} and Tre^{01} strains there were a total of 25 single nucleotide polymorphisms (SNPs) within the exons and 19 SNPs plus two single nucleotide insertions/deletions in the introns of *Gr5a* gene (Genbank accessions numbers AB066619-24). Among the SNPs in the exons, we found four nonsynonymous SNPs, Val19Ile, Met23Ile, Leu216His, and Ala218Thr, as shown in Fig. 3. While the former three SNPs were not correlated with the gustatory phenotype, the Ala218Thr polymorphism was found to be perfectly correlated with the gustatory phenotype in all the 14 strains we investigated: all Tre^{+} strains had an alanine at the 218^{th} position, while all Tre^{01} strains had a substitution with threonine (Genbank accessions numbers AB066619-066640). Since the statistical significance of the correlation is highly significant ($p < 0.0001$), we concluded that the decrease of sugar sensitivity in Tre^{01} is due to amino acid substitution. The candidate sweet taste receptor encoded by *Tre*, or *Gr5a*, has 444 predicted amino residues with seven transmembrane domains as illustrated by shaded areas in Fig. 3.

The transposon-induced mutations in *Tre* led to severely decreased amounts of mRNA. The *N*-terminal coding domains of the *Gr5a* are also deleted in *ΔEP5* and *ΔEP19*. Therefore, those mutations are

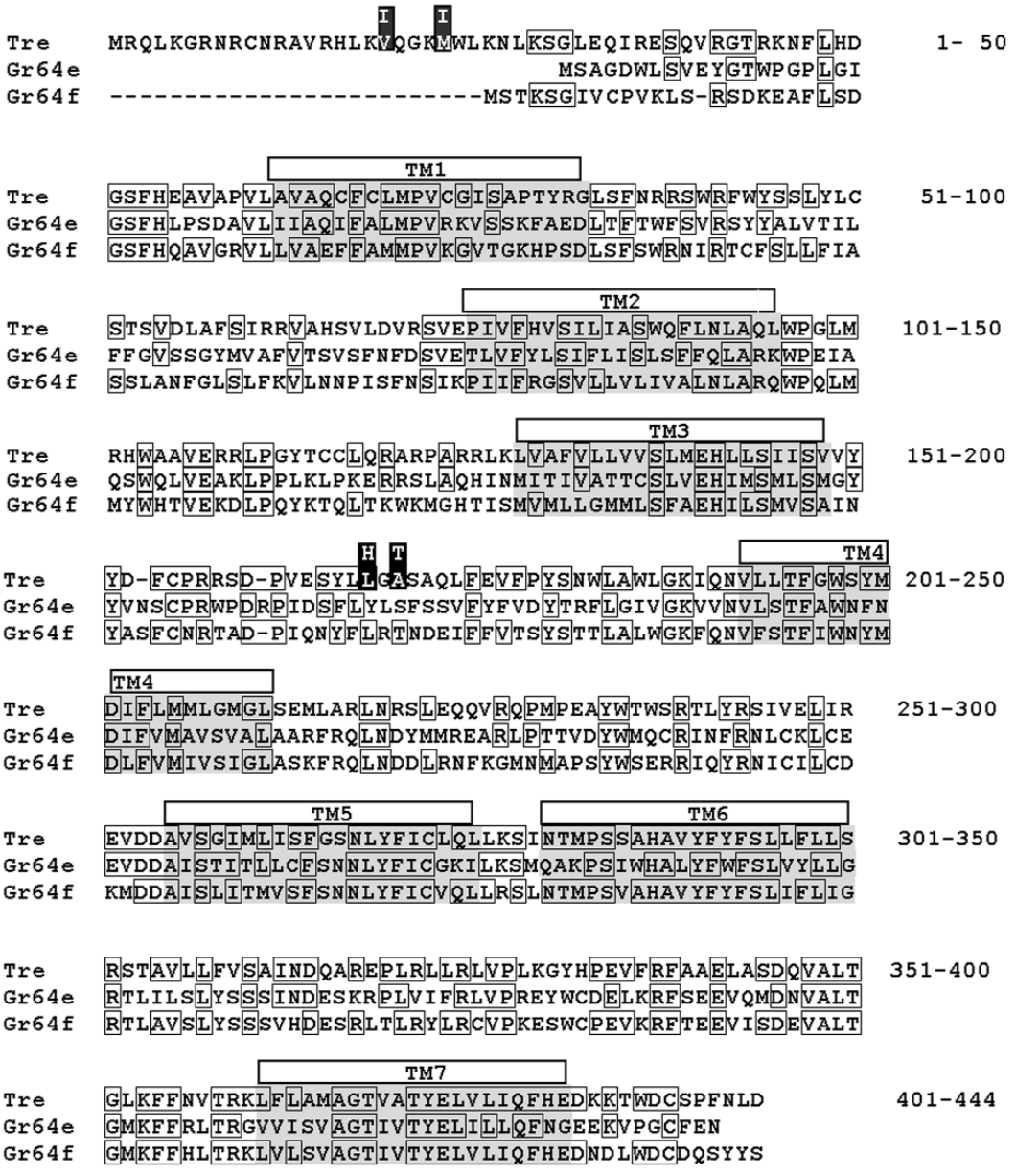

Fig. 3 Predicted amino acid sequence of the sugar receptor encoded by *Tre* aligned with the sequences of the two candidate sugar receptors encoded by *Gr64e* and *Gr64f.* Predicted transmembrane regions TM1 to TM7 are shaded in gray. Four polymorphic amino residues in the *Tre* sequence are highlighted and shown at the corresponding sites. Numbers at the right are the residue numbers from *N*-terminal methionine for *Tre*.

considered to be null mutations producing no or very low amounts, if any, of functional sugar receptors. Nevertheless, the mutations did not totally abolish the response to trehalose and also gustatory sensitivity to sucrose and other sugars, suggesting that the receptor encoded by *Tre* does not explain the

whole sugar spectrum. Therefore, other receptor(s) with different sugar specificity may also be involved for the sweet taste response in *Drosophila*.

Among the candidate gustatory receptor genes, there are a total of seven genes showing sequence similarity to *Tre*. Among them, the most suitable candidates may be *Gr64f* or *Gr64e*, because they share 43 % or 35 % identity, respectively, to *Tre*. The amino residue sequences of *Gr64f* and *Gr64e,* according to Amrein's research group (http://genetics.mc.duke.edu/faculty/amrein.htm) are aligned against *Tre* and shown in the middle and bottom rows of Fig. 3. Residues that are shared with *Tre* are shown in boxes. Transmembrane segments, especially segments 4–7, are more conserved than other domains among the three *Gr*s. The second intracellular loop and the second and third extracellular loops are also conserved domains. Although not shown in Fig. 3, five other genes belonging to the same subfamily—*Gr64a, Gr64b, Gr64c, Gr64d,* and *Gr61a*—may also be candidates for sweet taste receptors.

Molecular mechanism of sweet taste response

The candidate olfactory and gustatory receptors that belong to the large chemosensory receptor family in *Drosophila* show little overall homology to any other known G protein-coupled receptors and contain no functionally known domains. The vertebrate sweet taste receptors, on the other hand, belong to family C G protein-coupled receptors with a large *N*-terminal domain which accommodates the binding sites for the ligand molecules [22]. Since *Tre* and other candidate chemosensory receptors have short *N*-terminal domains as in family A receptors, the main binding domain may be located in the transmembrane segments or in the extracellular loops as were identified for some receptors belonging to this class [22]. The conserved domains of the transmembrane segments 4–7 or the second and the third extracelluar loops in *Tre*, *GR64e,* and *f* may be involved in binding ligand molecules.

The amino acid substitution leading to the decrease in sugar sensitivity in Tre^{01} is located at the 218^{th} residue in the predicted second intracellular loop domain and is unlikely to be involved in the binding with sugar ligands. Rather, the second or the third intracellular loop is known to be important in the interaction with or activating G protein, as is suggested in rhodopsins or metabotropic glutamate receptors [23–25]. Ala218 may, therefore, be involved in the interaction with a G protein, which is schematically illustrated in Fig. 4.

A total of five genes that encode the alpha subunit of the heterotrimeric G proteins exist in the *Drosophila* genome. Lee et al. [26] and Scott et al. [27] showed that one of the G alpha genes, *Galpha49B*, encoding a *Drosophila* Gq homolog, is functionally involved in the phototransduction.

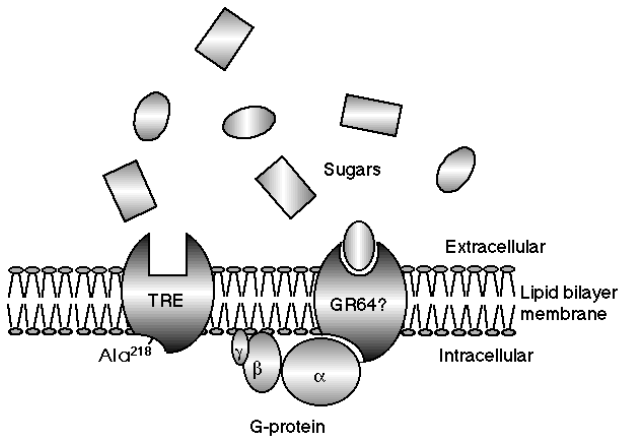

Fig. 4 Speculative mechanism of the sweet taste transduction in *Drosophila*.

Talluri et al. [28] showed that a splice variant form, which is distinct from the visual Gq isoforms, is expressed in the olfactory and gustatory neurons. Although the transduction mechanism in the invertebrate gustatory system has yet to be elucidated, the identification of the gustatory receptor in the present study will provide clues to the molecular understanding of sweet taste in *Drosophila*.

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