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Review

Molecular neurophysiology of taste in *Drosophila*

H. Ishimoto and T. Tanimura *

Department of Biology, Graduate School of Sciences, Kyushu University, Ropponmatsu, Fukuoka 810-8560 (Japan), Fax: +81 92 726 4625, e-mail: tanimura@rc.kyushu-u.ac.jp

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Abstract. The recent identification of candidate receptor genes for sweet, umami and bitter taste in mammals has opened a door to elucidate the molecular and neuronal mechanisms of taste. *Drosophila* provides a suitable system to study the molecular, physiological and behavioral aspects of taste, as sophisticated molecular genetic techniques can be applied. A gene family for putative gustatory receptors has been found in the *Drosophila* genome. We discuss here current knowledge of the gustatory physiology of *Drosophila*. Taste cells in insects are primary sensory neurons whereupon each receptor neuron responds to either sugar, salt or water. We found that particular tarsal gustatory sensilla respond to bitter compounds. Electrophysiological studies indicate that gustatory sensilla on the labellum and tarsi are heterogeneous in terms of their taste sensitivity. Determination of the molecular bases for this heterogeneity could lead to an understanding of how the sensory information is processed in the brain and how this in turn is linked to behavior.

Key words. Taste receptor; gustatory receptor neuron; *Drosophila*; electrophysiology; enhancer trap; Gal4/UAS.

Introduction

From bacteria to humans, the ability to detect chemical information in the external environment is essential for survival. Volatile substances are recognized by olfactory sensory neurons in which a large number of olfactory receptors (ORs) are expressed. Olfaction is a vital facility that enables organisms to detect foods, predators and mates. In contrast, gustation is necessary to determine whether soluble substances are nutritive or aversive foods. In insects, gustation also has a role in mate recognition in courtship behavior and in the choice of oviposition sites. *Drosophila* offers several advantages to explore the mechanism of gustation at different levels of an organism. In *Drosophila*, as in other insects, taste substances are recognized by bipolar gustatory receptor neurons (GRNs) [1, 2] whose axons project directly to the central nervous system (CNS). To determine the coding mechanism for taste in the CNS, it is necessary to identify the function of each GRN and to investigate its central projection pattern. In *Drosophila*, an olfactory receptor (*Or*) gene family consisting of at least 60 genes was identified by searching the genomic sequence [3–6]. The odor ligands for OR43a were identified by employing a heterologous expression system and overexpression experiments in vivo [7, 8]. Dobritsa et al. isolated mutants for *Or* genes and showed that *Or*22a and *Or*47a are necessary for sensing ethyl butyrate and pentyl acetate, respectively [9]. The projection pattern of olfactory receptor neurons expressing a particular OR gene has been studied by employing molecular genetic tools [9–11]. Compared with the degree of progress made in olfactory research, the molecular mechanisms of gustation are less well understood. With the recent identification of putative gustatory receptor genes, it might now be possible for the receptor mechanism of taste to be elucidated [12–14]. To this end, it will probably be necessary to combine both molecular and physiological approaches. We introduce

* Corresponding author.

here a summary of recent progress made on the physiology and molecular biology of taste in *Drosophila*.

Taste organs, gustatory receptor neurons and feeding behavior

In humans, taste modalities are categorized into sweet, bitter, salty, sour and umami. Taste cells in vertebrates are specialized epithelial cells that are innervated by taste neurons. Previous studies showed that a single taste cell in mouse responds to compounds belonging to multiple taste modalities [15, 16]. However, recent molecular studies suggest that a single taste cell responds to one taste modality [17–20]. In insects, taste cells are primary sensory neurons and directly send axons to the CNS, as is the case with olfactory receptor neurons of both mammals and insects. Pioneering work using larger flies, *Phormia* and *Boettcherisca*, provided helpful knowledge of the foraging and chemosensory electrophysiology of flies [21, 22]. *Drosophila melanogaster* provides an excellent experimental system to study the taste receptor mechanisms, as approaches employing behavioral, electrophysiological and molecular genetic analyses can be applied and integrated. Taste reception in *Drosophila* is mediated by gustatory sensilla on the labellum, legs, wings and genitalia in the adult fly (fig. 1a–c) [1, 2, 23]. To date, there have been no physiological studies on the sensilla of the wings and genitalia. In total, 31 gustatory sensilla are located on each half of the labellum. Gustatory sensilla

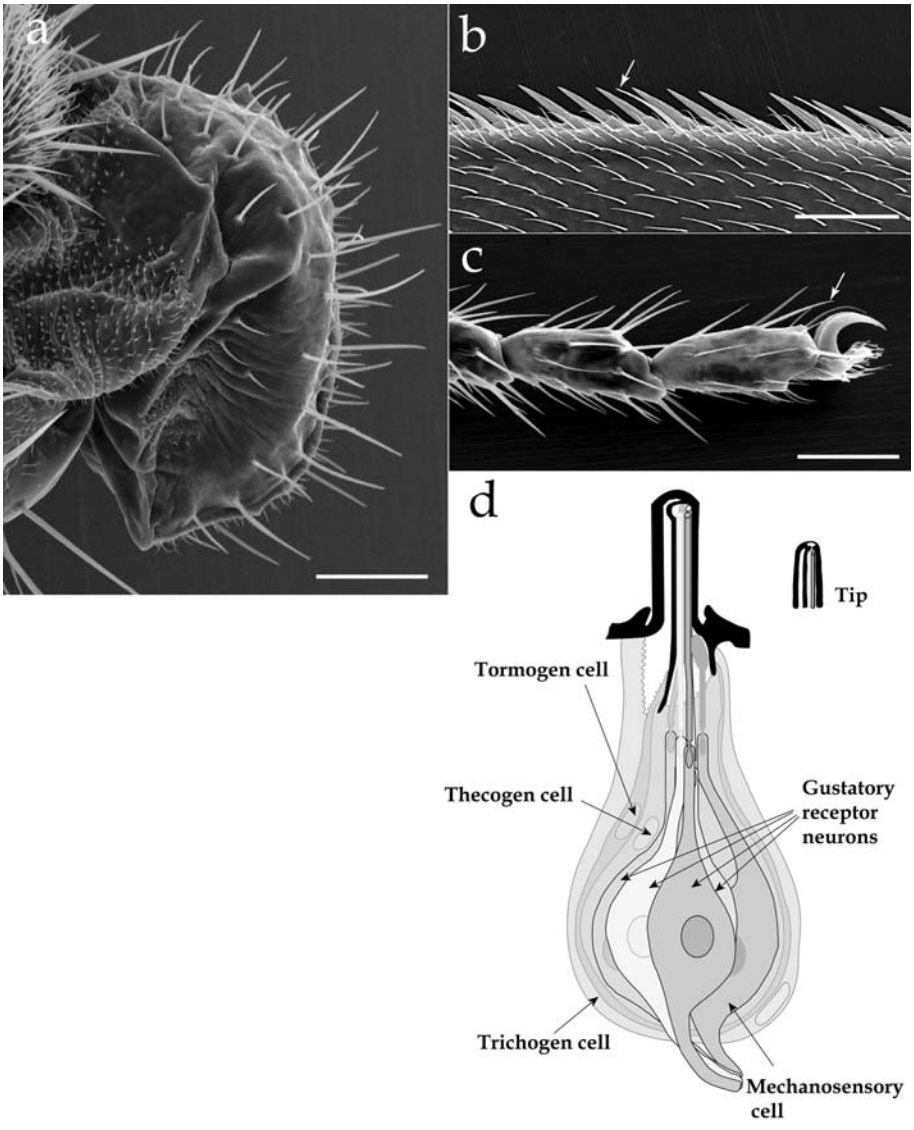

Figure 1. Morphology of *Drosophila* taste organs. (*a*) Labellum, (*b*) wing and (*c*) tarsus. Arrows in (*b*) and (*c*) indicate gustatory sensilla. (*d*) A schematic diagram of a typical chemosensillum. GSNs are surrounded by accessory cells, named the thecogen, tormogen and trichogen cells. Scale bars represent 50 μm.

on the labellum are classified into three types: L-, S- and I-type, based on their shape and location. S- and L-type sensilla house four GRNs, while I-type sensilla have two GRNs (fig. 1d). GRNs in insects are specialized in that each neuron responds exclusively to either sugar, water, or low or high concentrations of salt. The respective GRNs are called S, W, L1 and L2 cells [2]. S cells are sugar receptor cells and respond to mono-, di- and trisaccharides, but do not respond to artificial sweeteners. W cells are specialized to respond to water. Water cells seem to have evolved in insects that must contend with water loss from the cuticle. It is known that the activity of the water receptor neurons is inhibited by high osmolarity, but nothing is known about the molecular mechanism of the water receptor. L1 and L2 cells respond to low and high concentrations of salt, respectively. The significance of the presence of two separate cell types for salt is not known. Low concentrations of salt may be a positive signal for flies, while high concentrations of salt may be an aversive signal. Since the spike frequency of the L2 cells to high concentrations of salt is low and its dose dependence is not evident, there may be other stimulants for L2. Axons of labellar GRNs connect directly with interneurons in the subesophageal ganglion (SOG), where gustatory information is processed [2, 24]. The dendrites of GRNs are located in the lumen within the sensillum, separate from the second lumen filled with sensilla lymph. Most gustatory sensilla on the labellum have a two-pronged tip, with one of the tips holding the sensillum pore. On the other hand, single-tipped sensilla have a pore at the end of each sensillum. About 30 sensilla are located on the tibia and tarsi of each leg, with prothoracic legs having more sensilla than meso- and metathoracic legs, and male prothoracic legs having about three times more sensilla than those of females. Axons of GRNs in the tarsi project to the thoracic-abdominal ganglion. Neuroanatomical studies revealed the neuronal projection patterns of GRNs in the labellum or tarsi using horseradish peroxidase as a neurotracer [25–28]. The structural feature of GRNs is especially suitable for electrophysiological studies since, using the tip-recording method, it is possible to record nerve impulses originating from GRNs by stimulating a chemosensillum. Nerve impulses originating from each GRN can be discriminated by their firing intervals and magnitude (fig. 2) [29, 30].

For behavioral analyses several methods are available to measure the taste sensitivity of flies. Tanimura et al. developed a two-choice preference test to measure taste sensitivity to sugars. They defined a genetic dimorphism of taste sensitivity to trehalose using this method [31, 32]. In the two-choice preference test, flies are allowed to feed between two kinds of sugar-agar solutions, each colored with a different food dye. The flies were able to discriminate a remarkably small difference of sweetness between the two solutions and consumed the sweeter one. Since the taste sensitivity is measured in comparison with a control sugar in this test, taste sensitivity for an individual tastant should be measured by another method. The use of a food dye enables the intake of an individual tastant to be quantified by measuring the absorbance of homogenized flies after the ingestion of a colored solution. Taste responses can also be measured by a proboscis extension reflex (PER) test. The PER test measures the taste response to a single tastant. In this test, the prothoracic tarsus is touched with a drop of sugar solution. If a fly perceives the stimulus, it extends its proboscis. This test uses populations of flies and shows that the proportion of responding flies increases as the sugar concentration is raised. The PER depends on the satiety of the flies. PER tests can also be done by bi-

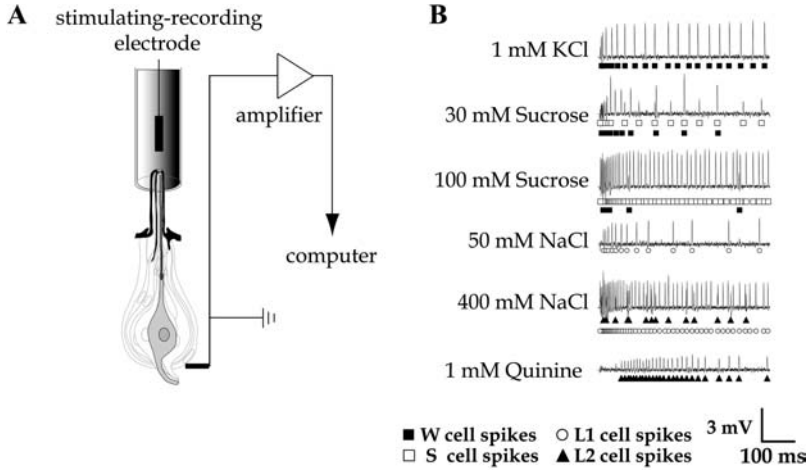

Figure 2. Electrophysiological recordings from *Drosophila* chemosensilla. (*A*) A schematic diagram of the tip-recording method. The electrode is used for both stimulating and recording. (*B*) Spike firing pattern of each GRN recorded from a labellar chemosensillum. Each trace indicates impulses during the first 500 ms after stimulation. Filled squares show W cell spikes, open squares show S cell spikes, open circles show L1 cell spikes and filled triangles show L2 cell spikes.

lateral stimulation of the legs. Flies retract the proboscis when one leg is stimulated with a sugar solution while the other leg is stimulated with a high concentration of salt. The advantage of the *Drosophila* behavioral assay is that taste sensitivity can be easily quantified, and a difference in the taste sensitivity can be compared among strains. Furthermore, these behavioral assays can be used to screen for taste mutants.

Multiple taste receptors for sugars and the *Gr* gene family

Previous studies have indicated that there are at least three separate receptor sites for sugars on the membrane of the sugar receptor neurons of *Drosophila*. First, proteolytic treatment of gustatory sensilla eliminated the nerve response to fructose without affecting responses to other sugars [29]. This finding indicated the presence of a specific receptor for fructose. Second, mutants showing reduced responses to specific sugars with a glucopyranoside moiety have been reported [31, 33, 34]. These studies suggested the presence of separate receptor sites for glucopyranosides. Third, genetic dimorphism was found in the taste sensitivity for disaccharide trehalose among the laboratory strains [32]. The genetic dimorphism is controlled by a single gene, named *Tre,* on the X chromosome [31]. Gene dosage studies suggested that the *Tre* gene product might be a receptor protein for trehalose [32]. Most laboratory strains have either a high or a low sensitivity to trehalose. Such a dimorphism also exists in flies in natural populations. These three receptor sites have been tentatively named F, G and T sites. Sucrose is composed of glucose and fructose, and trehalose is a disaccharide of glucose. It is interesting to note that the fructose moiety of sucrose does not seem to bind to the F site, in the same way that the glucose moiety of sucrose does not bind to the T site. Thus, there must be a strict stereospecificity between a ligand and a putative sugar receptor. It is also interesting to note that trehalose possesses a unique structure.

Receptors for sweet and bitter taste are believed to belong to the family of G-protein-coupled receptors (GPCRs). In mammals, putative taste receptors (TRs) have been identified [17]. T1R2, T1R3 and T1R1 have been demonstrated to mediate sweet and umami tastes by expressing these receptors in cultured cells [19, 35, 36]. In *Drosophila*, a *Gr* gene family that includes 70 putative gustatory receptors has been found using a computer algorithm to search the genome database [12–14, 37]. In *Anopheles gambiae*, 76 putative gustatory receptors (AgamGPRgrs) were found using bioinformatic approaches [38]. Phylogenic analysis between Agam GPRgrs and *Gr*s showed that there is likely to be a common ancestor among *Diptera* gustatory receptors. In *Drosophila*, a GPCR gene that is expressed in GRNs was reported to control taste sensitivity to trehalose [39]. Transgenic analysis subsequently showed that one of the *Gr*s is needed to recognize the sugar trehalose [40, 41]. The function of other *Gr*s is not known.

In order to examine the function of putative gustatory receptors, it is important to demonstrate their expression in the taste organ. The expression of several *Gr*s was examined in adult and larval gustatory organs. Clyne et al. analyzed the expression of 19 *Gr*s using reverse transcription polymerase chain reaction (RT-PCR) and confirmed that 18 *Gr*s are expressed in gustatory receptor neurons in the labellum [12]. Transcriptional levels of most *Gr*s are too weak to be detected by in situ hybridization, meaning that the Gal4/upstream activation sequence (UAS) system may help to detect the gene expression if an effective promoter sequence is available. Dunipace et al. and Scott et al. independently established Gal4 strains that contain *Gr* promoter::Gal4 element [13, 14]. They investigated expression patterns of *Gr*s using a marker gene, UAS-green fluorescent protein (GFP) or UAS-*lacZ*, and confirmed that their expression is localized to gustatory receptor neurons, but the expression among sensilla was spatially limited. Surprisingly, one *Gr* is expressed in the olfactory organ. In this way, 12 transgenic strains that covered 10 *Gr*s were examined, and the expression of 9 *Gr*s was confirmed in the labellum and the legs. Hiroi et al. reexamined the expression of *Gr*s using 6 out of 12 *Gr* promoter::Gal4 strains. By tracing the GFP-expressing dendrite, they could identify the sensillum innervating a particular neuron. They found that *Gr*s are mainly

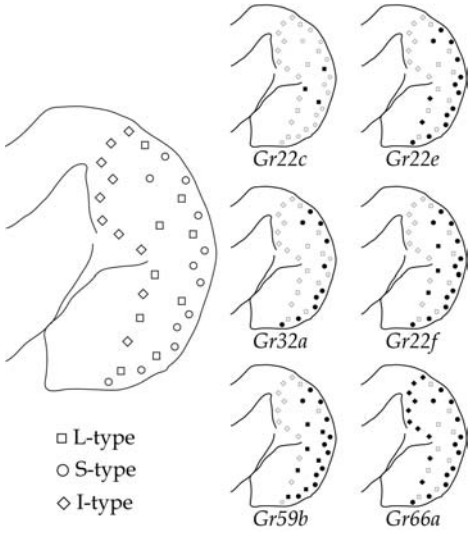

Figure 3. The expression profile of *Gr*s in Gr-Gal4/UAS-GFP. The arrangement of each chemosensillum is indicated by symbols. The square indicates the position of L-type sensilla. The diamond and the circle indicate the positions of I-type and S-type sensilla, respectively. The expression pattern of each *Gr* is indicated by the black coloring. Most of the *Gr*s are expressed in S-type sensilla.

expressed in the S-type sensilla (fig. 3) [42]. Two out of 6 *Gr*s were expressed in a part of the L-type sensilla, although significant differences in physiological response among the L-type sensilla have not been found so far. Taste responses to sugars are different for each of the three types of sensilla [42]. L-type sensilla give good responses to sugars, while I-type sensilla show poor responses to sugars. S-type and L-type sensilla both respond to sucrose but do not respond well to glucose and trehalose. At present, a relationship cannot be found between the expression pattern of *Gr*s and the variations in sugar sensitivity. There remains a possibility that the expression pattern of *Gr* promoter::Gal4 does not reflect the original expression pattern, or that the Gal4 expression level is too low to be detected. Also, *Gr*s may interact with unknown compounds. We do not have reasons for why there are so many *Gr* genes. In mammals, a recent study indicated that taste receptors function as heterodimers of GPCRs belonging to the T1R family. T1R2/T1R3 is activated by sweet compounds with different chemical structures, whereas T1R1/T1R3 is the umami taste receptor and interacts with most of the amino acids [35, 36]. If these T1Rs are the only sweet and umami receptors, then the ligand-receptor system in gustation is quite different from that in olfaction. Furthermore, it remains unresolved as to whether *Gr*s of *Drosophila* function as heterodimers.

'Bitter' taste in *Drosophila*

A bitter taste sensation is critical for organisms to avoid toxic substances. Since bitterness is a psychological term for humans, we will use the word 'deterrent' for insects. Deterrent cells have a functional role for herbivorous insects in the recognition of noxious secondary plant substances and are known to be involved in host-plant interactions [43]. The silkworm, for example, is a monophagous insect, but one mutant strain has a wide range of diet. The deterrent cells in this mutant were found to have lost their sensitivity to some of the deterrent compounds tested [44]. In *Manduca sexta,* although dietary exposure to caffeine desensitizes deterrent cells to caffeine, dietary exposure to salicin or aristolochic acid does not desensitize the deterrent cells to those compounds [45]. In this way, a deterrent cell may express multiple receptors for different deterrent compounds. A deterrent compound, quinine, was used as a repellant in previous behavioral experiments with *Drosophila* [46, 47]. However, while it is known that quinine inhibits the activity of sugar receptor neurons, no data have yet been published showing that a GRN responds to deterrent compounds. If *Drosophila* has a receptor cell for deterrents, it provides an attractive system to investigate the molecular mechanism of deterrent sensitivity.

Using the two-choice preference test, we examined the effect of deterrents on sugar reception and found several compounds that inhibited the sugar response. We also found that stimulation of tarsal sensilla with deterrents inhibited the PER elicited by sugar stimulation. These behavioral results suggest the presence of deterrent cells on the leg. Previous electrophysiological work was done on the L-type labellar sensilla, since other gustatory sensilla, that is, the S- and I- type sensilla on the labellum, are not always easily accessed by an electrode and can give poor responses [42]. Likewise, with the exception of one study, gustatory sensilla on the legs have not been used for electrophysiological recordings in *Drosophila* [48]. We recently found that specific sensilla on the prothoracic legs respond to compounds that are known as bitter for humans or deterrent for other insects and identified the responding cells as L2 cells [49]. It is interesting to note that the L2 cells may detect noxious information for flies, since they also respond to high concentrations of salts. In L2 cells, spikes evoked by deterrent compounds occur with a latency, the length of which is dependent on the concentration of the deterrent compounds. Deterrent compounds also inhibit the activity of S and W cells, with the same latency as that observed in L2 cells. This means that S, W and L2 cells are likely to have a common receptor mechanism for deterrent compounds [49]. In gustatory sensilla located on the terminal tarsal segments, sensillum 5b responds to quinine, and another type, 5s, responds to berberine, with both types responding to denatonium and strychnine. These physiological data suggest that there are separate receptors in a GRN for the respective deterrent compounds. We also found that the S- and I-type sensilla on the labellum respond to deterrent compounds [M. Hiroi et al., unpublished]. Some *Gr* genes are expressed in one of the GRNs at the base of S-type sensilla.

We may now ask whether *Gr*'s function as receptor proteins for deterrent compounds or whether alternative mechanisms are involved. A family of candidate receptors (T2Rs) for bitter compounds was identified in humans and mice [18, 50]. T2Rs are located on a genomic region that is genetically linked to loci that influence the perception of bitter taste. The study, which employed in situ hybridization experiments, suggested that multiple T2R genes are expressed in a single taste cell. So far, heterologous expression analysis indicates that the function of two human T2Rs and one mouse T2R is to interact with different bitter compounds [51]. One of the *Gr* genes is specifically expressed on the sensilla housing a deterrent-sensitive cell. Further experiments are needed to confirm the role of the *Gr*.

Molecular dissection of the taste transduction pathway

A heterologous expression system can be used to investigate the ligand specificity of putative gustatory receptors.

Even though the intrinsic signaling mechanism for GPCRs is not known, the signaling machinery present in cultured cells can be utilized by expressing a suitable type of G protein and then monitoring changes in the intracellular Ca^{2+} concentration using a fluorescent dye. In mammals, the heterodimer of T1R2 and T1R3 was shown using a heterologous expression system to function as a sweet receptor [19, 35]. T1R2 and T1R3 are coexpressed in taste receptor cells in the papilla lingualis. The heterologous expression system, however, has several problems. First, the efficiency of the translocation of receptor proteins to the plasma membrane is a critical factor. There is a requirement of accessory proteins for integration of the receptor protein into the plasma membrane in *Drosophila* and *Caenorhabditis elegans* [52–54]. Though it is uncertain whether the results obtained in the heterologous expression precisely reflect the function of receptors in vivo, an alternative approach, such as gene silencing or conditional gene knockout, is required.

In mammals, several molecules, such as Gα, phospholipase C and phosphodiesterase, as well as the inositol 1,4,5-triphosphate receptor and the transient receptor potential-like channel, have been proposed to be involved in the taste transduction pathway [55]. α-Gustducin, which is a subset of heterotrimeric G protein, has been shown to be involved in sweet and bitter taste transduction [56, 57]. Recently, Zhang et al. used a knockout mouse to confirm that phospholipase C-β_2 (PLCβ_2) is essential for sweet and bitter signal transduction [20]. They also showed that a transient receptor potential-like channel (TRPM5) is involved in both the sweet and bitter taste signaling pathways. The signal transduction pathways in insects are yet to be determined but may be different from those in vertebrates, as in the case of the visual system [58].

The above proposals are based on the assumption that the molecular bases of taste receptors involve GPCRs. However, a completely different hypothesis has been proposed. Murakami and Kijima recorded taste responses from a sensory process in the flesh fly using the patch clamp method [59]. They cultured taste sensory processes from labellar gustatory sensilla of the flesh fly and found the existence of a sucrose-gated channel-type receptor on the distal membrane of the sensory process of the sugar receptor cell. The channel is directly activated by sugars and transduces taste information without the mediation of any secondary messengers or G proteins. At present, there are no lines of evidence to exclude this ligand-gated ion channel hypothesis, but molecular studies are necessary to prove the existence of such a molecule.

Molecular genetic approaches to identify genes expressed in GRNs

To understand the neural pathway of taste information processing, we need to identify each functional class of GRNs, S, W, L1 and L2 cells, and to trace the central projection of each GRN. To this end, a genetic marker is needed to label each gustatory neuron. In *Drosophila*, the enhancer trap method can be used to identify genes expressed in a specific group of cells [60]. This molecular genetic technique is based on the property of the P-element transposon, which mediates integration of a reporter gene into the genome. The expression of the reporter gene is affected by the enhancer activity around the integration site. The expression of a gene that is nearest to the integration site of the reporter gene can then be trapped based on the expression pattern of the reporter gene. Strains in which a reporter gene is expressed in the gustatory receptor neurons have been screened and used to investigate the neural pathway and taste information processing mechanisms. Rodrigues et al. identified at the behavioral level a loss-of-function mutant of *malvolio* (*mvl*) that showed reduction of sugar taste acceptance and increased avoidance of salt [61]. In this mutant, P[w^+-*lacZ*] element is inserted upstream of the transcriptional start site of the *mvl* gene. The *mvl* gene is expressed in adult GRNs and in larval chemosensory neurons, antennomaxillary complex (AMC). The *mvl* gene encodes a membrane protein that belongs to a group of natural resistance-associated macrophage proteins (Namps) or transporter. Since the nerve response of GRNs did not change in the *mvl* mutant, the *mvl* gene may function in the neuronal processing or discrimination of gustatory information. Nakamura et al. reported that the defective proboscis extension response (*dpr*) gene is required for the salt response [62]. In normal flies, a sugar solution mixed with a certain concentration of salt does not induce PER, but this inhibition was not observed in a *dpr* mutant strain. The *dpr* gene is expressed in a subset of GRNs and encodes for a transmembrane protein that belongs to the protein family having Ig repeats. The molecular mechanism of the *dpr* mutant phenotype is not clear, but the *dpr*-Gal4 strain provides a marker for the L1 or L2 cells. Further studies are necessary to clarify how the cell adhesion molecule has modified the function of the sugar receptor mechanism.

The second generation of the enhancer trap system is mediated by the Gal4 element, which encodes for a transcriptional factor of yeast [63]. In this system, Gal4 is randomly integrated into the *Drosophila* genome in the same way as the enhancer trap system. Gal4 directs the expression of any gene downstream to UAS elements. Thus, the combination of Gal4 and a UAS strain permits ectopic expression of a different cell marker gene, such as GFP or *lacZ* (fig. 4A). Balakireva et al. reported a Gal4 enhancer trap strain, *Voila*[1], that shows abnormal courtship and a defect in salt taste behavior [64, 65]. In the *Voila*[1] strain, Gal4 is expressed in the adult CNS and GRNs and in the larval AMC. Molecular analysis revealed that the P[Gal4] transposon is inserted upstream of the *prospero* gene, which encodes a transcription fac-

tor. Grosjean et al. demonstrated that the *prospero* gene is required for the normal development of the nervous system [66]. We have recently identified water GRNs (W cells) using the Gal4 enhancer trap system (fig. 4B) (Inoshita et al., in preparation). We screened Gal4 enhancer trap strains by monitoring GFP expression and found a strain in which Gal4 is expressed in one of four GRNs at the base of every gustatory sensillum on the labellum. When the neurotransmission of the Gal4-expressing neurons was inhibited by the targeted expression of *shibire*^{ts1}, which inhibits synaptic vesicle endocytosis under restrictive temperature conditions [67], PER to water was dramatically reduced. In the Gal4 enhancer trap system, the Gal4 expression generally conforms to the enhancer activity of flanking genes near the P[Gal4] insertion site. In this case, flanking genes are not expressed in W cells. However, this Gal4 strain is useful to mark W cells. W cells can be ablated by the targeted expression of a cell death gene that is under the control of UAS. Functional identification of all the GRNs would help in the investigation of the axonal projections of these neurons and the neural mechanism of gustatory information processing.

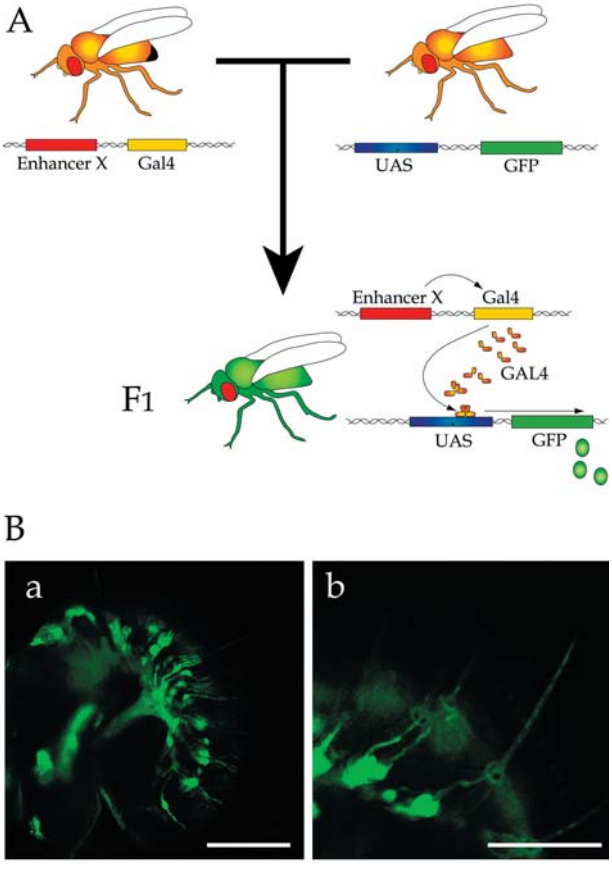

Figure 4. (*A*) Gal4/UAS system. By crossing a Gal4 enhancer trap strain with a strain having UAS-GFP, GFP is expressed in the F_1 flies in a spatially and temporally restricted manner. (*B*) (*a*) and (*b*) W cells can be visualized with the aid of GFP. GFP is observed in one of four GRNs at the base of each chemosensillum. Scale bars represent 50 µm in (*a*) and 25 µm in (*b*).

Perspective

Recent physiological studies in *Drosophila* show that gustatory sensilla in both the labellum and the tarsi are not homogeneous in terms of taste sensitivity to different compounds. For example, the profile of sugar sensitivity is different among the three types of labellar sensilla, while gustatory sensilla on the tarsus show distinct responses to different kinds of bitter compounds. There are several sensilla on the tarsus that do not respond to any stimuli examined so far, which suggests that mechanisms could be in place for the identification of other, unknown stimulants [48]. The expression pattern or expression level of *Gr*s may cause the heterogeneity or the signal transduction pathway to be different, for example, being mediated by a ligand-gated ion channel. The behavioral significance of the heterogeneity is an interesting problem to be solved. In *Drosophila*, olfactory receptor neurons expressing the same receptor gene project to a specific glomerulus in the antennal lobe [6, 14, 68]. In the gustatory system, it is thought that a glomerulus-like unit does not exist in the SOG. To determine the neural processing of taste information, it is necessary to characterize GRNs functionally and to make a projection map of them. Moreover, the function of Gal4-expressing GRNs can be disrupted using the Gal4-UAS system. A neurotoxin gene, cell death genes and genes for modulating neuronal activity can be used under the control of UAS. UAS-EKO (electrical knockout) can be used to block potassium conductance in targeted cells [69]. Among these effector genes, *shibire*^{ts1} is only available for conditional inhibition. Conditional inhibition of GRNs, on the other hand, is highly effective in avoiding developmental aberrations and lethality. The activity pattern of olfactory neurons in the fly brain can be monitored in vivo using a microscopy technique and a calcium-sensitive fluorescent protein such as cameleon or G-CaMP [10, 11]. This technique can be applied to visualize the activity of GRNs. To determine taste reception mechanisms at the molecular level, a targeted gene suppression technique is required. Specific gene expression can be suppressed by double-stranded RNA (dsRNA) in various organisms [70]. Using the Gal4-UAS system, it is possible to constitutively interfere with a tissue-specific gene based on the RNAi mechanism (GAL4*i*). Kalidas and Smith demonstrated olfactory neuron-specific gene targeting using the GAL4*i* technique [71]. They disrupted the function of dGqα, which is part of the signal transduction pathway of olfaction, and the fly exhibited abnormal olfactory behavior. The GAL4*i* technique is an effective method to determine the function of *Gr*s and the putative molecules

of taste signal transduction. We believe that integrated multilateral approaches employing behavioral, electrophysiological, modern genetic, and molecular biological techniques should provide powerful tools to study the transduction of taste mechanisms.

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