Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb20

Studies on Taste: Molecular Biology and Food Science

Keiko ABE

To cite this article: Keiko ABE (2008) Studies on Taste: Molecular Biology and Food Science, Bioscience, Biotechnology, and Biochemistry, 72:7, 1647-1656, DOI: 10.1271/bbb.70690

To link to this article: https://doi.org/10.1271/bbb.70690

Published online: 22 May 2014.

Biosci. Biotechnol. Biochem., **72** (7), 1647–1656, 2008

Award Review

Studies on Taste: Molecular Biology and Food Science

Keiko ABE

Department of Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Online Publication, July 7, 2008
[doi:10.1271/bbb.70690]

Taste is indispensable for vertebrate to find a proper way of living by selection of foods at their discretion. It is also a mainstay in the construction of human culture and the food industry, but no systematic information is available regarding the molecular logic of taste signaling and associated chemical entities. Against this backdrop, our research had bumble beginnings in the 1990s and then traced a unique path of development revealing major signaling pathways involving G protein-coupled receptors, Gαi2, PLC-β2, IP_3R3, PLA2IIa, TRPM5, KCNQ1, *etc.* The validity of our studies on the molecular biology of taste was verified by material science in the case of an enigmatic protein, neoculin, which converts sourness to sweetness. The study should provide new information for better understanding of taste-taste interactions which are important in food design.

Key words: taste reception; taste signaling; taste preference-aversion; neoculin; human sweet taste receptor

Organisms are exposed to a variety of exogenous signals in their environments. They convert them to cognitive endogenous signals to select ways of surviving. Sensory systems work for this purpose, playing crucial roles in normalizing life processes. The sense of taste is directly involved in the feeding behaviors of animals. It is also a mainstay of our dietary culture and a basis of the food industry. This is one of the main reasons why the sense of taste has attracted a great deal of biological, social, and industrial interest.

The molecular science of taste had humble beginnings in the late 20th century. It traced a unique path of development in accordance with modern advances in molecular and cell biology. A great degree of international development of this science resulted after the time (2004) when the Nobel Prize of Medicine and Physiology was awarded to Buck and Axel[1] who identified the molecular entity of olfactory receptors as G-protein-coupled receptors (GPCRs) with a seven-transmembrane topology.

Early in the 1990s the author's group presented a paper which was the first to report that taste receptors are GPCR proteins.[2,3] Taking advantage of this, we launched into taste science based on both molecular biology and food science, in the hope of giving shape to the paradigm of agricultural chemistry which is characterized by the collaboration of chemistry and biology and by the integration of basic and applied studies.

The present article is described by the stratification of (1) taste bud cells and intracellular signaling, (2) construction of primary culture of taste bud cells, (3) universality of taste signaling mechanisms in vertebrates, (4) genomics of signal transduction from gustatory neurons to the central nervous system, and (5) the molecular logic of taste-taste interaction and its application to food design. The item (5) is unique and particularly important as a material science verifying the validity of the proceeding studies (1)–(4). It deals with a new sweet protein neoculin we found in a tropical fruit. Our previous studies[4–14] on oryzacystatin as the first well-defined cystatin of plant origin were of great use in pursuing this neoculin study. In the present paper, all these items are described in the above order of numbering.

I. Taste Bud Cells and Intracellular Signaling

As reported by Zuker's group,[15–19] two lines of taste-receiving GPCR proteins, T1R and T2R, residing on the surfaces of sensory cells in each taste bud have been recognized. The signals generated upon reception of tastants as first messengers activate the coupling G proteins and effectors, producing second messengers that excite taste cells by depolarization. The excitation results in transmitting the signals to gustatory neurons synapsed with the taste cells. A variety of molecular entities must intervene in this signal transduction-transmission process.

It is known that hetero-oligomeric T1R2-T1R3 responds to sweetness and T1R1-T1R3 to amino acid

This review was written in response to the author's receipt of The Japan Bioscience, Biotechnology, and Agrochemistry Society Award in 2007.
Correspondence: Fax: +81-3-5841-8006; E-mail: aka7308@mail.ecc.u-tokyo.ac.jp

taste or umami, while T2R series of taste receptors respond to bitterness.[15–19] All these receptors are GPCR proteins functioning in coupling with G proteins, among which gustducin (Ggust)[20–22] is involved in receiving bitter tastants.

We found that while, cells expressing T2Rs as bitter taste receptors possess Ggust exclusively, this G protein is not necessarily expressed in cells having T1R molecules as sweet or umami taste receptors. Using the technique of *in situ* hybridization, we found that the α-subunit of another G protein, Gαi2, was clearly expressed in T1R-possessing cells.[23,24] The use of these techniques also demonstrated that the effector phospholipase C-β2 (PLC-β2)[25,26] was the molecule that recognized G protein activation, *i.e.*, GTP/GDP exchange, resulting in a dissociation of the α-subunit from the $\beta\gamma$ subunits. This finding led us to predict that PLC-β2 activation can induce phosphoinositol (PI) turnover, with the formation of inositol trisphosphate (IP_3) and diacylglycerol (DG) from phosphatidyl trisphosphate (PIP2). IP_3 reacts with an endoplasmic membrane receptor to accelerate calcium ion flow into the cytosol. We observed that PLC-β2 and IP_3 receptor 3 share a common taste cell.[26] All these results indicate that T1R-expressing cells are distinct from T2R-expressing cells. Despite this, all the cells were found commonly to express Gαi2, PLC-β2 effector, and IP_3 receptor 3 distinctly. A series of these events causes the enhancement of the intracellular concentration of Ca^{2+}, which accelerated opening the gate of the tangent receptor potential (TRP) M5 channel[27,28] for cell membrane depolarization. We thus found that a diversity of exogenous taste signals gave rise to simple, cognitive endogenous signals that were then transduced and transmitted intracellularly and trans-synaptically (Table 1). Our findings described in summary form are i) the existence of PI turnover machinery; ii) the coexistence of TRPM5 activation-associated arachidonic acid cascade factors PLA2IIA,[29] monoacyl-glycerol lipase (MGL), and cyclo-oxygenase 2 (COX2),[30] and iii) the expression of electric potential-dependent potassium channels (KCN)Q1 and H2 as repolarization cascade factors.[31] KCNQ1 was expressed in most taste bud cells. This strongly suggests that, besides sweetness-, umami-, and bitterness-receiving cells, some unidentified taste cells exist, participating in reversible depolarization-repolarization circuits. KCNQ1-expressing cells were found to contain candidate sour taste receptor (PKD2L1)-expressing cells.[32–34] Other chemical entities which we have found in taste bud cells include CAP,[35] CNG channel,[36] and G proteins[37] and taste bud-specific maker proteins.[38]

Table 1. Molecules as Players Involved in Taste Signaling

Player	1st messenger		
	Sweetness	Umami	Bitterness
Receptor	T1R2+T1R3	T1R1+T1R3	T2Rs
G protein	Gi2		Ggust/Gi2
Effector	Phospholipase Cβ2 (PLCβ2)		
2nd messenger	Inositol 1,4,5-tris phosphate (IP_3), Diacylglycerol (DAG)		
Channel	IP_3 receptor type 3 (IP_3R3)		
Ca^{2+}	Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)		
Depolarization (modulator)	Transiant receptor potential M5 channel (TRPM5) (Arachidonic acid)		
Repolarization	Voltage-dependent potassium channel Q1 (KCNQ 1)		

II. Construction of Primarily Cultured Taste Bud Cells

To understand the molecular and cell biology of taste reception and signaling, it is indispensable to observe the functional expression of taste-related genes in taste bud cells. The observation necessitates constructing a primary cell culture system. Many studies[39–42] have been attempted of taste-bud cell culturing, with unsuccessful results. To break through the hurdles bringing about the failures, we carried out experiments to solve fundamental problems. First we characterized matrigel, fibronectin, and laminine as matrix elements involved in the connection of cultured taste bud cells,[43] and found proper conditions for enhancing their extracellular Ca^{2+}-dependent connectivity.[44] Cultured taste bud cells we thus constructed from rat circumvallated papillae were alive for up to 1 week, which was long enough for us to pursue our planned experiments.[45] Also, the cells were found to express taste cell-intrinsic signaling molecules T1R1, Ggust, and PLC-β2. We then tried adenovirus-aided introduction of α-adrenergenic receptor as a GPCR protein that structurally resembled the taste receptors T1R and T2R. The resulting cultured taste bud cells were found to have acquired the ability to receive noradrenalin, with a significant increase in intracellular $[Ca^{2+}]$.[45] Thus the representative taste signaling molecules were expressed in the cultured cells, as in the case *in vivo*. It was also possible to introduce taste-related genes for functional expression. The cultured taste bud cells should find use as a new tool in taste signaling analysis, and in the near future as a taste sensor that faithfully and intelligibly reflects the human sense of taste.

III. Universality of Taste Signaling Mechanisms in Vertebrates

It is recognized as appropriate in the investigation of the universal nature of vertebrates to use fish as the prototype of mammals. A good example is offered by medaka and zebrafish as internationally accepted model animals.[46,47] We have performed analysis of α1A-adrenoreceptor,[48] olfactory receptors,[49–52] and other GPCRs,[53,54] and have searched in fish databases

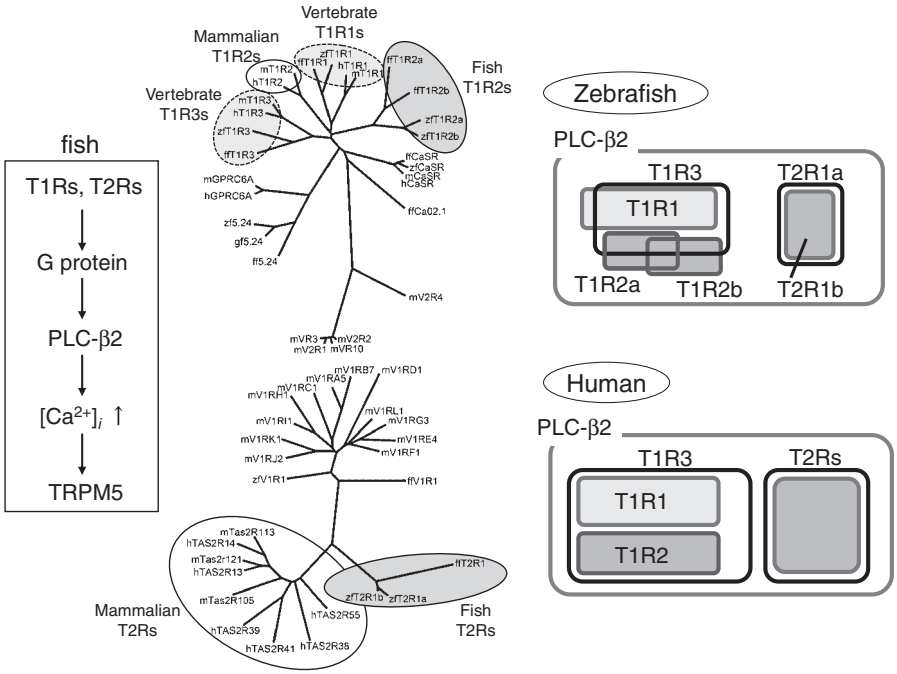

Fig. 1. Fish and Human Taste Receptors and Their Expression Profiles in PLC-β2 Expressing Cells.

and e-genetic data, which provided us with information on candidate taste receptors.[55] Studies of these candidates using the *in situ* hybridization technique revealed that fish as well as mammals possess T1Rs and T2Rs, which are expressed in mutually segregated cells. It was also found that the fish T1R1s and T1R3s show high degrees of amino acid similarities to mammalian T1R1s and T1R3s, respectively. On the other hand, fish T1R2s showed almost equal degrees of similarity to mammalian T1R1s and T1R2s. In T1R2, there is 31–34% identity between fish and mammal (Fig. 1). We analyzed receptor-ligand relationships with the use of HEK 293 cells made to express these molecules.[56] From consideration of the results with special reference to tastant reception and feeding behaviors, it was inferred that, while T1R2-T1R3 functions as a sweet-taste receptor in humans and other mammals which generally prefer sweetness, this receptor in fish responded to amino acid taste rather than to sugar taste. This discrepancy between fish and mammals suggests that taste receptors have undergone molecular evolution to adapt to environmental nutrients such as proteins and amino acids in the water and to those such as starch and sugars on land (Fig. 2). It is known that in mammals the taste receptors T1R1, T1R2, and T1R3 are of single gene. We found that although T1R1 and T1R3 were of a single gene in fish, there were two members of T1R2 in zebrafish, and at least three members of T1R2s in medaka (Fig. 1). In addition, the T1R2 members were coupled with T1R3 to form a variety of heterodimers that function as amino acid receptors. The profiles of the amino acids received varied depending on the combination of these heterodimers. Fish may receive and ingest amino acids sometimes as a protein source and sometimes as an energy source *via* gluconeogenesis. On the other hand, T2R receptors in fish and in mammals responded to denatonium (a bitter tastant) with aversion[56] (Fig. 2). In terms of molecular entities functioning in the downstream of taste reception, fish were similar to mammals. In fish the important signaling marker PLC-β2[57] is involved in opening the PI turnover-associated TRPM5 channel[58] (Fig. 1). Similarities between fish and mammals, including humans, were thus found in taste reception and subsequent signal transduction (Fig. 2). In fish as well as in humans, taste signals are transmitted by the facial and glossopharyngeal nerves to the brain where the tastes are recognized, and this is reflected in dietary behaviors.[59]

It is likely that taste signaling is a life process common to many vertebrates, and that a high degree of universality exists with respect to molecular mechanisms. Our study with fish provides a good example of the well-known paradigm in biology, "Use model animals to know humans." Technically, the use of fish such as medaka is more favorable than that of mammals whose taste reception-transduction mechanisms are similar but whose taste-information treatment in neural networks is much more complex.[59]

As a new approach, we designed a quantitative assay system applicable to medaka for quantification of taste reception (input) and taste preference-aversion as reflected in it feeding behavior (output). Constructing a coordinated input-to-output assay system should facilitate observation of likes and dislikes as to a given food and the measurement of food intake. We prepared a unique, floating diet that contained controlled amounts

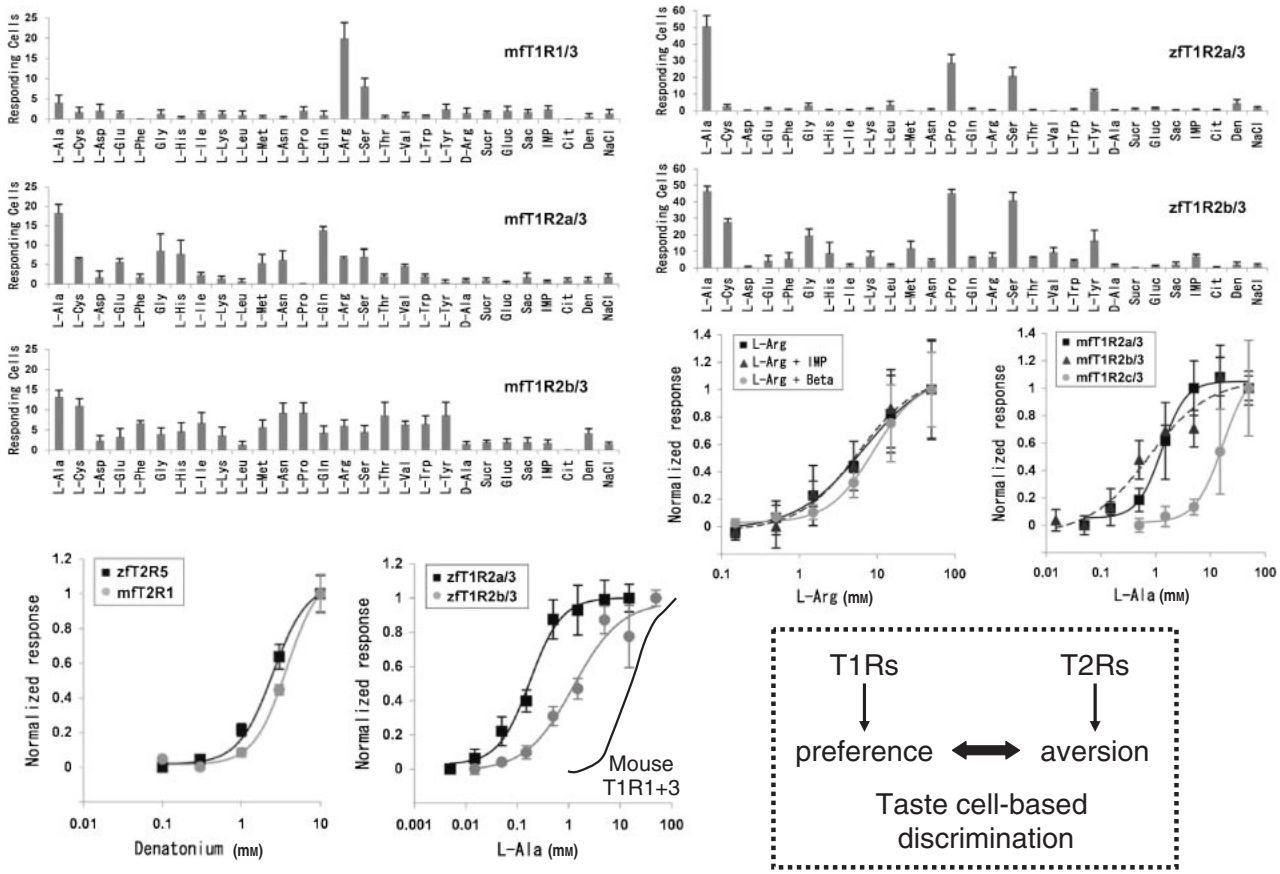

Fig. 2. Fish Taste Receptors and Their Ligands.

of various tastants and a given amount of fluorescent label, $DiIC_{12}$, in order to quantify the ingested food by fluorometry of the digestive tract[60] (Fig. 3A). The use of this device in preference-aversion assays revealed that it was possible to introduce exotic genes into T1R-T2R-expressing cells of medaka by the use of a PLC-β2 transcription-regulatory region of 1.6 kbp[61] (Fig. 3B). With this transgenic system, we succeeded in constructing a medaka mutant that expresses Gi2S47C[62] coupled with T1R-T2R.[60] This mutant was expected to inhibit the activation of PLC-β2 downstream of the amino acid taste receptor (T1R) and the bitter taste receptor (T2R) (Fig. 3B). As expected, the mutant became sensory-blinded as a result of losing both amino acid-preference and bitterness-aversion mechanisms (Fig. 3C). The use of this taste-blind fish in the elucidation of taste signaling mechanisms might be useful in the analysis of sensory recognition-feeding behavior relationships in mammals including humans[60] (Fig. 3D).

IV. Genomics of Signal Transduction from Taste Nerves to the Brain

In mammals, tastants are received at the taste bud position (input) and the consequently generated signals are transduced to gustatory neurons. The taste signals are finally recognized in the brain (output) for discrimination between likes and dislikes. However, little has been clarified in research at the molecular level. Against this backdrop, we launched into an investigation of somatosensory nerves that respond to pain, temperature, and irritative tastants such as capsaicin, as well as gustatory neurons that respond to the five basic tastes, sweetness, sourness, saltiness, bitterness and umami. We analyzed the gene expression profiles of each neuron in cranial sensory ganglia containing trigeminal, geniculate, petrosal, and nodose ganglia.[63] The use of DNA microarray and histochemical techniques was successful in identifying a number of candidate genes showing neuron type-specific expression in the peripheral ganglia (Fig. 4).[64–66] We also applied these techniques to parabrachial nucleus (PBN) to find molecular markers useful for the characterization of taste-signaling specific to PBN. It was possible to accumulate PBN-intrinsic information at the genetic level.

Wheat germ agglutinin (WGA) is available as a visible trans-synaptic tracer.[67,68] We established transgenic mouse lines in which WGA was expressed in sweet/umami-responsive taste receptor cells under the control of mouse T1R3 gene promoter/enhancer.[69] As a result, WGA protein was transferred from taste cells to a subset of gustatory neurons in the geniculate and nodose/petrosal ganglia, and was clearly observed in a subpopulation of NST neurons. This technique gave a

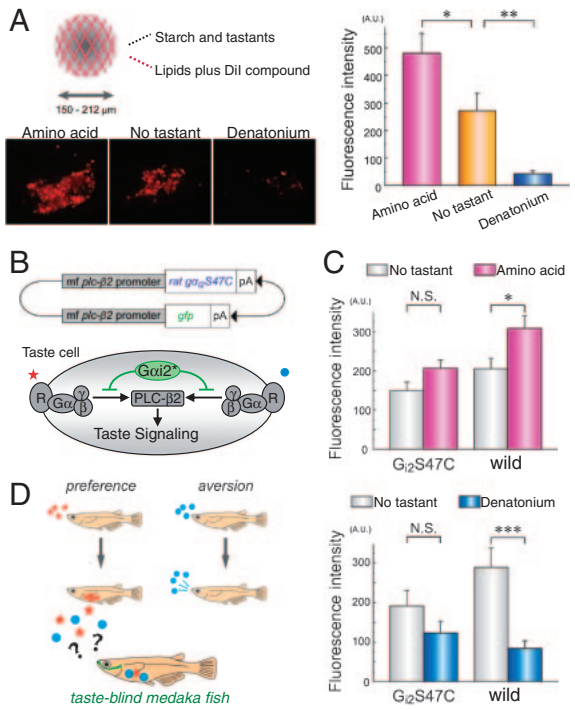

Fig. 3. Construction of an Assay System for Quantification of Feeding Behaviors of Model Fish and Evaluation of Taste Responses of Gi2S47C-Transgenic Fish.

clue to the taste coding process, and was found useful in verifing the hypothesized labeled line theory,[70] which states that sweet-, sour-, salt-, bitter- and umami-transducing pathway lines from taste cells to the central gustatory area *via* gustatory neurons are independent. Our study should also contribute to so-called sensory systems biology in the near future.

V. Molecular Logic of Taste-Taste Interactions and Its Application to Food Design

There are various cases of taste-taste interactions. Food scientists and technologists have long paid a great deal of attention to taste modification. Examples are offered by a sweet tastant that attenuates sourness and by a sour tastant that potentates sweetness, but no information is yet available to explain the molecular logic of such antagonistic-synergistic taste phenomena.

We have found and isolated a sweet protein, neoculin, from the fruit of a tropical plant, *Curculigo latifolia*, which grows wildly in West Malaysia.[71] We then determined the primary structure of neoculin by molecular cloning.[71] Neoculin shows conversion of sourness to sweetness in the presence of acids, *e.g.*, citric acid. This means that the taste of sour lemon can be changed into a sweet orange-like taste when noeculin is

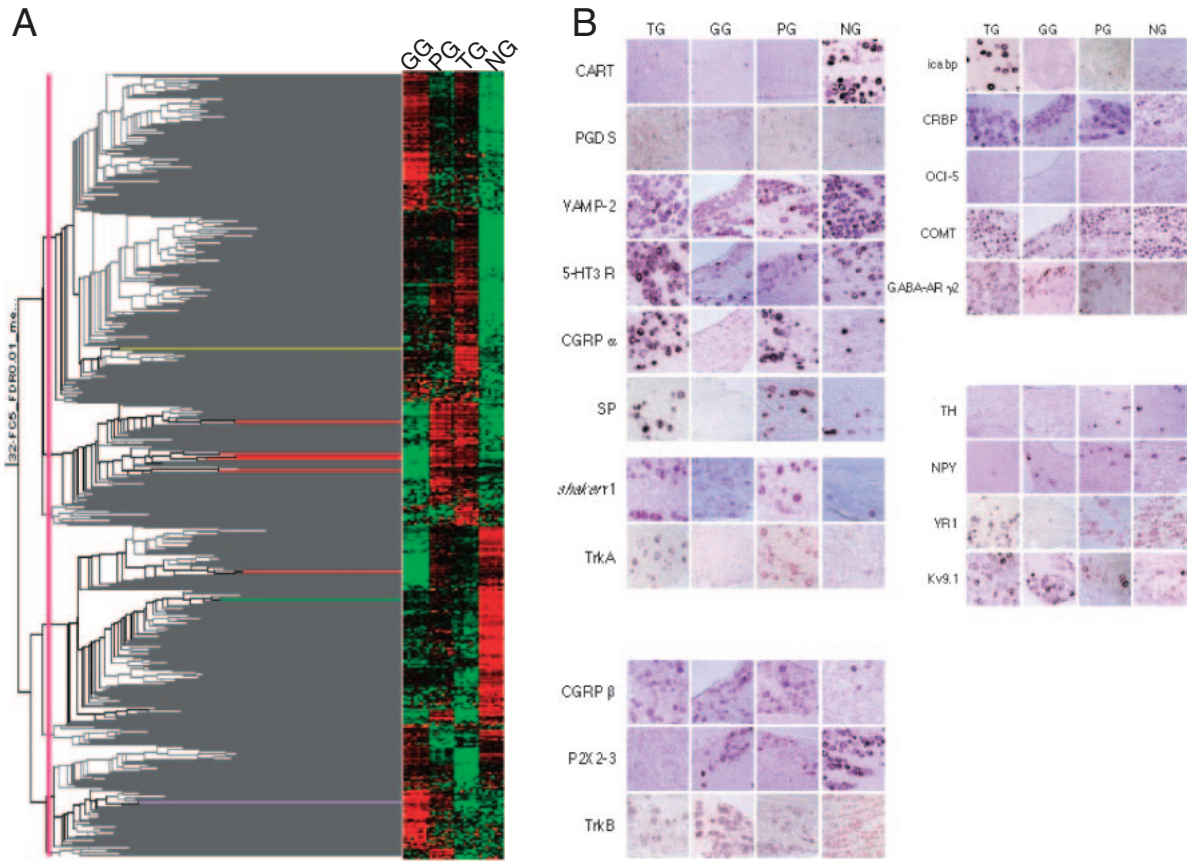

Fig. 4. Genes Expressed in Four Cranial Ganglions Related to Gustatory Stimulation.
A, Hierarchical cluster analysis; B, Genes extracted by cluster analysis and their expression in TG, GG, PG, and NG.

added. Acidification of the gustatory environment to pH 4 or lower makes neoculin taste about 400 times as sweet as sugar on a weight basis and about 40,000 times as sweet on a molar basis, with a decrease in sourness. Structurally, this protein is a heterodimer of an acidic subunit (NAS) and basic subunit (NBS), both being about 10 kDa in molecular mass.[71] We established an *Aspergillus oryzae*-aided expression system, producing sensory-active NAS-NBS as a secretory protein that resembled wild neoculin in most respects.[72]

The sensory properties of neoculin, *viz.*, sweetness and taste-modifying activity, are recognized almost exclusively by humans. We constructed a cultured human-embryonic kidney (HEK) cell system by introducing the human sweet taste receptor hT1R2-hT1R3 together with chimeric Gα protein, which functioned in coupling.[73] Challenged with neoculin at acidic pH, the HEK cells responded, enhancing its intracellular [Ca^{2+}]. For quantitative measurement we used a calcium imaging method, and found that the measurements depended on the concentration of the acid used as a ligand and on the pH value of the culture medium. Also, the HEK cell responses were regulated to a greater or lesser extent depending on the concentration of lactisole which is used as an hT1R2-hT1R3 inhibitor.[73] The cell assay system worked, faithfully simulating in sensory tests conducted with well-trained panel members. This *in vitro* assay is expected to be applied in the objective evaluation of sweet tastants in general.

Biochemically, elicitation of the sensory activity of neoculin required the N-terminal extracellular domain of T1R3.[74] This receptor-ligand interaction accorded with the pH-dependent sensory activity of neoculin, *viz.*, activation at pH 6 or lower and inactivation at pH 8 or higher.[73] This changing pattern closely resembled the imidazole titration curve, suggesting the involvement of histidine residues that can undergo protonation-deprotonation at pK values of about pH 6. X-ray crystallography with a pure neoculin preparation revealed that the five histidine residues were exposed to the surface.[75] This permits trapping of the protons supplied by the acid, attenuating its sourness (Fig. 5). To characterize the potent sweetness of neoculin itself, we adopted molecular dynamics simulation, and inferred that NAS and NBS interacted tightly with each other to form a closed quaternary structure at neutral pH, while at acidic pH the interaction became loose, shifting toward an open form[75] (Fig. 5). The possible closed-open state equilibrium is reflected in the pH-dependent, reversible change in sweetness (Fig. 5).

The validity of this conclusion was verified by recent studies of ours with a neoculin variant engineered by replacement of all the histidine residues (H) with alanine (A).[76] The HA variant tasted sweet even at pH 8 and, on top of that, the sweetness held pH-independent. At all the pH levels investigated, the variant was found to interact with hT1R2-hT1R3 (Fig. 6) and, the sweetness elicited at such a pH level was completely inhibited in

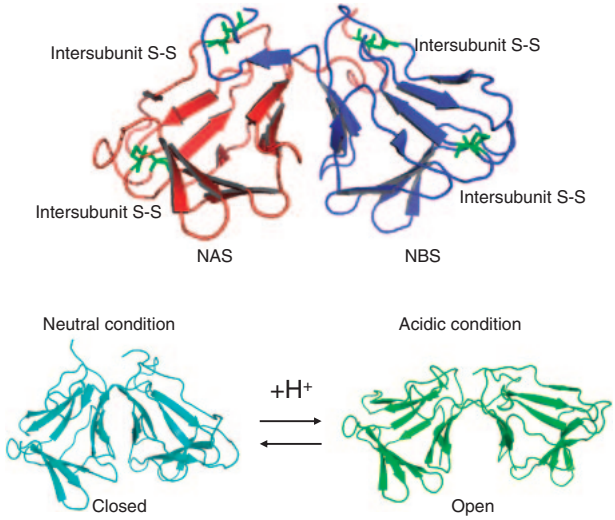

Fig. 5. X-Ray Crystallography and Molecular Dynamics Simulation of Neoculin and Its Possible Conformationed Change.

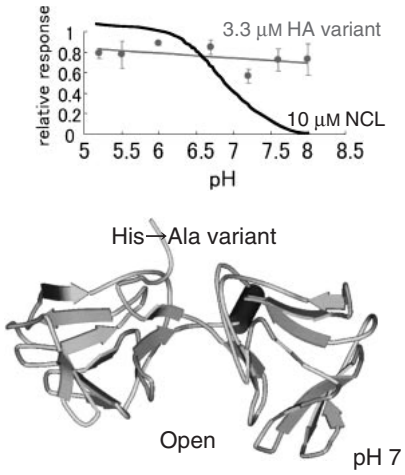

Fig. 6. Neoculin HA Variant with pH-Independent Sweetness and Its Deduced Conformation.

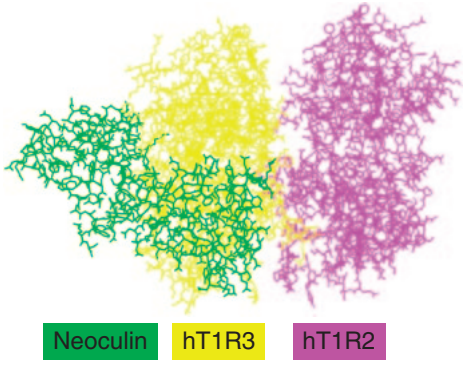

Fig. 7. One of the Docking Models with Sweet-Taste Receptor T1R2-T1R3.

the presence of neoculin. This finding indicates that even under alkaline conditions, neoculin acts as an antagonist to interact with hT1R2-hT1R3, while it acts as an agonist potentiating its own sweetness under acidic conditions. These data provide a molecular logic explaining the taste attenuation-protentiation event.

Of phytophysiological interest is that neoculin can be recognized as having evolved from plant mannose lectins,[77–79] since it has a certain degree of amino acid sequence similarity to garlic lectin for example. However, no blood-coagulating activity exists any longer in neoculin. This is extremely important in view of food safety. To reconfirm the wholesomeness of neoculin, we investigated its effect on Caco-2 cells by the DNA microarray method. Fortunately, no significant result was obtained as to gene expression profiles.[80] Neoculin should eventually find use as a non-caloric, non-glycemic functional food material for anti-diabetic use.[81]

VI. Perspectives

One of the most important attributes of food is its palatability which is triggered on the reception of tastants at the taste bud position. We have focused our studies on taste reception and subsequent signal transduction, demonstrating that the mechanisms of taste preference aversion in vertebrates share a common principle. In addition to the life science of the sense of taste, a methodology of material science was introduced to determine the molecular logic of enigmatic taste-taste interaction events in the case of a taste-modifying protein, neoculin, as a ligand. The use of X-ray crystallography and molecular dynamics simulation was successful in proposing a taste receptor-ligand docking model (Fig. 7). Also, we recognized the possibility that neoculin acts as a taste agonist and antagonist in a pH-dependent manner. There are also a variety of taste-taste interactions and complex taste coding mechanisms in the brain. Elucidating these points should contribute to explaining the psychological cognition of the sense of taste. Our molecular studies have unveiled major segments of the taste-sensory events in the body, and should provide information for a better understanding of food palatability. Both life and material sciences at the molecular level are promising for solving a number of problems confronting food scientists.

Acknowledgments

It is a great pleasure for me to win the prize awarded for our studies on tastants from the point of view of food science and on the sense of taste from the point of view of molecular biology as a life science. All the outcomes of studies are fruits created with the aid of my collaborators, university staff, researchers, students, and graduates in the Biological Function Laboratory, Department of Applied Biological Chemistry, and the Endowed Chair of Functional Food Genomics in the same Department at The University of Tokyo. Outcomes have also been provided by a great many collaborators in universities, industry, government ministries, and other places. I am indebted to all those who were and are involved in collaboration. Finally, my sincere thanks our given to Professor Soichi Arai, Tokyo University of Agriculture, who suggested the theme of taste and has encouraged me throughout my studies.

References

1) Buck, L., and Axel, R., A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, **65**, 175–187 (1991).
2) Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y., and Arai, S., Multiple genes for G protein-coupled receptors and their expression in lingual epithelia. *FEBS Lett.*, **316**, 253–256 (1993).
3) Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y., and Arai, S., Primary structure and cell-type specific expression of a gustatory G protein-coupled receptor related to olfactory receptors. *J. Biol. Chem.*, **268**, 12033–12039 (1993).
4) Abe, K., Emori, Y., Kondo, H., Suzuki, K., and Arai, S., Molecular cloning of a cysteine proteinase inhibitor of rice (oryzacystatin): homology with animal cystatins and transient expression in the ripening process of rice seeds. *J. Biol. Chem.*, **262**, 16793–16797 (1987).
5) Abe, K., Emori, Y., Kondo, H., Arai, S., and Suzuki, K., The NH2-terminal 21 amino acid residues are not essential for the papain-inhibitory activity of oryzacystatin, a member of the cystatin superfamily: expression of oryzacystatin cDNA and its truncated fragments in *Escherichia coli*. *J. Biol. Chem.*, **263**, 7655–7659 (1988).
6) Kondo, H., Abe, K., Nishimura, I., Watanabe, H., Emori, Y., and Arai, S., Two distinct cystatin species in rice seeds with different specificities against cysteine proteinases: molecular cloning, expression, and biochemical studies on oryzacystatin-II. *J. Biol. Chem.*, **265**, 15832–15837 (1990).
7) Kondo, H., Abe, K., Emori, Y., and Arai, S., Gene organization of oryzacystatin-II, a new cystatin superfamily member of plant origin, is closely related to that of oryzacystatin-I but different from those of animal cystatins. *FEBS Lett.*, **278**, 87–90 (1991).
8) Watanabe, H., Abe, K., Emori, Y., Hosoyama, H., and Arai, S., Molecular cloning and gibberellin-induced expression of multiple cysteine proteinases of rice seeds (oryzains). *J. Biol. Chem.*, **266**, 16897–16902 (1991).
9) Abe, M., Abe, K., Kuroda, M., and Arai, S., Corn kernel cysteine proteinase inhibitor as a novel cystatin superfamily member of plant origin: molecular cloning and expression studies. *Eur. J. Biochem.*, **209**, 933–937 (1992).
10) Kondo, H., Ijiri, S., Abe, K., Maeda, H., and Arai, S., Inhibitory effect of oryzacystatins and a truncation mutant on the replication of poliovirus in infected Vero cells. *FEBS Lett.*, **299**, 48–50 (1992).
11) Misaka, T., Kuroda, M., Iwabuchi, K., Abe, K., and Arai, S., Soyacystatin, a novel cysteine proteinase inhibitor

in soybean, is distinct in protein structure and gene organization from other cystatins of animal and plant origin. *Eur. J. Biochem.*, **240**, 609–614 (1996).
12) Irie, K., Hosoyama, H., Takeuchi, T., Iwabuchi, K., Watanabe, H., Abe, M., Abe, K., and Arai, S., Transgenic rice established to express corn cystatin exhibits strong inhibitory activity against insect gut proteinases. *Plant Mol. Biol.*, **30**, 149–157 (1996).
13) Kudo, N., Nishiyama, M., Sasaki, H., Abe, K., Arai, S., and Tanokura, M., Crystallization and preliminary X-ray diffraction studies of a rice cysteine proteinase inhibitor, oryzacystatin-I. *J. Biochem.*, **123**, 568–570 (1998).
14) Nagata, K., Kudo, N., Abe, K., Arai, S., and Tanokura, M., Three-dimensional solution structure of oryzacystatin-I, a cysteine proteinase inhibitor of the rice, *Oryza sativa L.* japonica. *Biochemistry*, **39**, 14753–14760 (2000).
15) Hoon, M. A., Adler, E., Lindemeier, J., Battey, J. F., Ryba, N. J. P., and Zuker, C. S., Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell*, **96**, 541–551 (1999).
16) Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekar, J., Ryba, N. J. P., and Zuker, C. S., A novel family of mammalian taste receptors. *Cell*, **100**, 693–702 (2000).
17) Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., Zuker, C. S., and Ryba, N. J. P., T2Rs function as bitter taste receptors. *Cell*, **100**, 703–711 (2000).
18) Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J. P., and Zuker, C. S., Mammalian sweet taste receptors. *Cell*, **106**, 381–390 (2001).
19) Zhao, G. Q., Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., Ryba, N. J. P., and Zuker, C. S., The receptor for mammalian sweet and umami taste. *Cell*, **115**, 255–266 (2003).
20) McLaughlin, S. K., Mckinnon, P. J., and Margolskee, R. F., Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature*, **357**, 563–569 (1992).
21) Wong, G. T., Gannon, K. S., and Margolskee, R. F., Transduction of bitter and sweet taste by gustducin. *Nature*, **381**, 796–800 (1996).
22) He, W., Danilova, V., Zou, S., Hellekant, G., Max, M., Margolskee, R. F., and Damak, S., Partial rescue of taste responses of α-gustducin null mice by transgenic expression of α-transducin. *Chem. Senses*, **27**, 719–727 (2002).
23) Kusakabe, Y., Abe, K., Tanemura, K., Emori, Y., and Arai, S., GUST27 and closely related G protein-coupled receptors are localized in taste buds together with Gi-protein α-subunit. *Chem. Senses*, **21**, 335–340 (1996).
24) Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arai, S., Emori, Y., and Abe, K., Comprehensive study on G protein α-subunits in taste bud cells, with special reference to the occurrence of Gαi2 as a major Gα species. *Chem. Senses*, **25**, 525–531 (2000).
25) Asano-Miyoshi, M., Abe, K., and Emori, Y., Co-expression of calcium signaling components in vertebrate taste bud cells. *Neurosci. Lett.*, **283**, 61–64 (2000).
26) Asano-Miyoshi, M., Abe, K., and Emori, Y., IP3 receptor type 3 is co-expressed with PLCβ2 in a subset of rat taste bud cells that includes the cells expressing two types of taste receptor, T1R and T2R. *Chem. Senses*, **26**, 259–265 (2001).
27) Zhang, Y., Hoon, M. A., Chandrashekar, J., Mueller, K. L., Cook, B., Wu, D., Zuker, C. S., and Ryba, N. J. P., Coding of sweet, bitter, and umami taste: different receptor cells sharing similar signaling pathways. *Cell*, **112**, 293–301 (2003).
28) Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Perez, C. A., Shigemura, N., Yoshida, R., Mosinger, B., Glendinning, J. I., Ninomiya, Y., and Margolskee, R. F., Trpm5 null mice respond to bitter, sweet, and umami compounds. *Chem. Senses*, **31**, 253–264 (2006).
29) Oike, H., Matsumoto, I., and Abe, K., Group IIA phospholipase A2 is co-expressed with SNAP-25 in mature taste receptor cells of rat circumvallate papillae. *J. Comp. Neurol.*, **494**, 876–886 (2006).
30) Oike, H., Wakamori, M., Mori, Y., Nakanishi, H., Taguchi, R., Misaka, T., Matsumoto, I., and Abe, K., Arachidonic acid can function as a signaling modulator by activating the TRPM5 cation channel in taste receptor cells. *Biochim. Biophys. Acta*, **1761**, 1078–1084 (2006).
31) Ohmoto, M., Matsumoto, I., Misaka, T., and Abe, K., Taste receptor cells express voltage-dependent potassium channels in a cell age-specific manner. *Chem. Senses*, **31**, 739–746 (2006).
32) Huang, A. L., Chen, X., Hoon, M. A., Chandrashekar, J., Guo, W., Trankner, D., Ryba, N. J. P., and Zuker, C. S., The cells and logic for mammalian sour taste detection. *Nature*, **442**, 934–938 (2006).
33) Ishimaru, Y., Inada, H., Kubota, M., Zhuang, H., Tominaga, M., and Matsunami, H., Transient receptor potential family members PKD1L3 and PKD2L1 from a candidate sour taste receptor. *Proc. Natl. Acad. Sci. USA*, **103**, 12569–12574 (2006).
34) Lopez-Jimenez, N. D., Cavenagh, M. M., Sainz, E., Cruz-Ithier, M. A., Battey, J. F., and Sullivan, S. L., Two members of the TRP family of ion channels, Pkd1l3 and Pkd2l1, are co-expressed in a subset of taste receptor cells. *J. Neurochem.*, **98**, 68–77 (2006).
35) Ishimaru, Y., Yasuoka, A., Asano-Miyoshi, M., Emori, Y., and Abe, K., An actin binding protein, CAP, is expressed in a subset of rat taste bud cells. *Neuroreport*, **12**, 233–235 (2001).
36) Misaka, T., Kusakabe, Y., Emori, Y., Gonoi, T., Arai, S., and Abe, K., Taste buds have a cyclic nucleotide-activated channel (CNGgust). *J. Biol. Chem.*, **272**, 22623–22629 (1997).
37) Kusakabe, Y., Yamaguchi, E., Tanemura, K., Kameyama, K., Chiba, N., Arai, S., Emori, Y., and Abe, K., Identification of two α-subunit species of GTP-binding proteins, Gα15 and Gαq, expressed in rat taste buds. *Biochim. Biophys. Acta*, **1403**, 265–272 (1998).
38) Asano-Miyoshi, M., Kusakabe, Y., Abe, K., and Emori, Y., Identification of taste tissue-specific cDNA clones from a subtraction cDNA library of rat circumvallate and foliate papillae. *J. Biochem.*, **124**, 927–933 (1998).
39) Farbman, A. I., Differentiation of taste buds in organ culture. *J. Cell Biol.*, **52**, 489–493 (1972).
40) Mbiene, J. P., Maccallum, D. K., and Mistretta, C. M., Organ cultures of embryonic rat tongue support tongue and gustatory papilla morphogenesis *in vitro* without intact sensory ganglia. *J. Comp. Neurol.*, **377**, 324–340

(1997).
41) Barlow, L. A., A taste for development. *Neuron*, **22**, 209–212 (1999).
42) Morris-Wiman, J., Brinkley, L., and Sego, R., An *in vitro* model for the study of taste papillae morphogenesis using branchial arch explants. *Brain Res. Brain Res. Protoc.*, **5**, 172–181 (2002).
43) Kishi, M., Sano, K., Asano-Miyoshi, M., Tsukamoto, Y., Emori, Y., and Abe, K., Identification of β and γ subunits of laminins localized in the basement membrane of rat circumvallate papillae. *Biosci. Biotechnol. Biochem.*, **67**, 1154–1156 (2003).
44) Kishi, M., Emori, Y., Tsukamoto, Y., and Abe, K., Changes in cell morphology and cell to cell adhesion induced by extracellular Ca^{2+} in cultured taste bud cells. *Biosci. Biotechnol. Biochem.*, **66**, 484–487 (2002).
45) Kishi, M., Emori, Y., Tsukamoto, Y., and Abe, K., Primary culture of rat taste bud cells that retain molecular markers for taste buds and permit functional expression of foreign genes. *Neuroscience*, **106**, 217–225 (2001).
46) Hyodo-Taniguchi, Y., and Egami, N., Establishment of inbred strains of the medaka *Oryzias latipes* and the usefulness of the strains for biomedical research. *Zool. Sci.*, **2**, 305–316 (1985).
47) Fetcho, J. R., and Liu, K. S., Zebrafish as a model system for studying neuronal circuits and behavior. *Ann. NY Acad. Sci.*, **16**, 333–345 (1998).
48) Yasuoka, A., Abe, K., Arai, S., and Emori, Y., Molecular cloning and functional expression of the a1A-adrenoceptor of medaka fish, *Oryzias latipes*. *Eur. J. Biochem.*, **235**, 501–507 (1996).
49) Yasuoka, A., Endo, K., Asano-Miyoshi, M., Abe, K., and Emori, Y., Two subfamilies of olfactory receptor genes in medaka fish, *Oyzias latipes*: genomic organization and differential expression in olfactory epithelium. *J. Biochem.*, **126**, 866–873 (1999).
50) Asano-Miyoshi, M., Suda, T., Yasuoka, A., Ohshima, S., Yamashita, S., Abe, K., and Emori, Y., Random expression of main and vomeronasal olfactory receptor genes in immature and mature olfactory epithelia of *Fugu rubripes*. *J. Biochem.*, **127**, 915–924 (2000).
51) Irie-Kushiyama, S., Asano-Miyoshi, M., Suda, T., Abe, K., and Emori, Y., Identification of 24 genes and 2 pseudo genes coding for olfactory receptors in Japanese loach, classified into 4 subfamilies: a putative evolutionary process for fish olfactory receptor genes by comprehensive phylogenetic analysis. *Gene*, **325**, 123–135 (2004).
52) Yasuoka, A., Emori, Y., and Abe, K., Addition of signal leader sequences to the N-termini of olfactory receptor proteins enhances their expression in *Xenopus* oocyte. *Biosci. Biotechnol. Biochem.*, **64**, 1688–1695 (2000).
53) Yasuoka, A., Abe, K., Saigo, K., Arai, S., and Emori, Y., Molecular cloning of a fish gene encoding a novel seven-transmembrane receptor related distantly to catecholamine, histamine, and serotonin receptors. *Biochim. Biophys. Acta*, **1235**, 467–469 (1995).
54) Yasuoka, A., Hirose, Y., Yoda, H., Aihara, Y., Suwa, H., Niwa, K., Sasado, T., Morinaga, C., Deguchi, T., Henrich, T., Iwanami, N., Kunimatsu, S., Abe, K., Kondoh, H., and Furutani-Seiki, M., Mutations affecting the formation of posterior lateral line system in medaka, *Oryzias latipes*. *Mech. Dev.*, **121**, 729–738 (2004).
55) Ishimaru, Y., Okada, S., Naito, H., Yasuoka, A., Matsumoto, I., and Abe, K., Two families of candidate taste receptors in fishes. *Mech. Dev.*, **122**, 1310–1321 (2005).
56) Oike, H., Nagai, T., Furuyama, A., Okada, S., Aihara, Y., Ishimaru, Y., Marui, T., Misaka, T., and Abe, K., Characterization of ligands for fish taste receptors. *J. Neurosci.*, **27**, 5584–5592 (2007).
57) Yasuoka, A., Aihara, Y., Matsumoto, I., and Abe, K., Phospholipase C-beta 2 as a mammalian taste signaling marker is expressed in the mutiple gustatory tissues of medaka fish, *Oryzias latipes*. *Mech. Dev.*, **121**, 985–989 (2004).
58) Yoshida, Y., Saito, K., Aihara, Y., Okada, S., Misaka, T., and Abe, K., Transient receptor potential channel M5 and phospholipaseC-β2 colocalizing in zebrafish taste receptor cells. *Neuroreport*, **18**, 1517–1520 (2007).
59) Chapouton, P., and Bahy-Cuif, L., Neurogenesis. *Methods Cell Biol.*, **76**, 163–206 (2004).
60) Aihara, Y., Yasuoka, A., Iwamoto, S., Yoshida, Y., Misaka, T., and Abe, K., Construction of a taste-blind medaka fish and quantitative assay of its preference-aversion behavior. *Genes Brain Behav.*, in press.
61) Aihara, Y., Yasuoka, A., Yoshida, Y., Ohmoto, M., Shimizu-Ibuka, A., Misaka, T., Furutani-Seiki, M., Matsumoto, I., and Abe, K., Transgenic labeling of taste receptor cells in model fish under the control of the 5′-upstream region of medaka phospholipase C-beta 2 gene. *Gene Expr. Patterns*, **7**, 149–157 (2007).
62) Slepak, V. Z., Katz, A., and Simon, M. I., Functional analysis of a dominant negative mutant of Gαi2. *J. Biol. Chem.*, **270**, 4037–4041 (1995).
63) Matsumoto, I., Emori, Y., Ninomiya, Y., and Abe, K., A comparative study of three cranial sensory ganglia projecting into the oral cavity: *in situ* hybridization analyses of neurotrophin receptors and thermosensitive cation channels. *Mol. Brain Res.*, **93**, 105–112 (2001).
64) Matsumoto, I., Emori, Y., Nakamura, S., Shimizu, K., Arai, S., and Abe, K., DNA microarray cluster analysis reveals tissue similarity and potential neuron-specific genes expressed in cranial sensory ganglia. *J. Neurosci. Res.*, **74**, 818–828 (2003).
65) Matsumoto, I., Nagamatsu, N., Arai, S., Emori, Y., and Abe, K., Identification of candidate genes involved in somatosensory functions of cranial sensory ganglia. *Mol. Brain Res.*, **126**, 98–102 (2004).
66) Matsumoto, I., Nakamura, S., Emori, Y., Arai, S., and Abe, K., Gene expression profiling of cranial sensory ganglia that transmit food intake stimuli. *BioFactors*, **21**, 15–18 (2004).
67) Yoshihara, Y., Mizuno, T., Nakahira, M., Kawasaki, M., Watanabe, Y., Kagamiyama, H., Jishage, K., Ueda, O., Suzuki, H., Tabuchi, K., Sawamoto, K., Okano, H., Noda, T., and Mori, K., A genetic approach to visualization of multisynaptic neural pathways using plant lectin transgene. *Neuron*, **22**, 33–41 (1999).
68) Horowitz, L. F., Moutmayeur, J. P., Echelard, Y., and Buch, L. B., A genetic approach to trace neural circuits. *Proc. Natl. Acad. Sci. USA*, **96**, 3194–3199 (1999).
69) Ohmoto, M., Matsumoto, I., Yasuoka, A., Yoshihara, Y., and Abe, K., Genetic tracing of the gustatory and trigeminal neural pathways originating from T1R3-

expressing taste receptor cells and solitary chemoreceptor cells. *Mol. Cell. Neurosci.*, in press.
70) Frank, M., An analysis of hamster afferent taste nerve response functions. *J. Gen. Physiol.*, **61**, 588–618 (1973).
71) Shirasuka, Y., Nakajima, K., Asakura, T., Yamashita, H., Yamamoto, A., Hata, S., Nagata, S., Abo, M., Sorimachi, H., and Abe, K., Neoculin as a new taste-modifying protein occurring in the fruit of *Curculigo latifolia*. *Biosci. Biotechnol. Biochem.*, **68**, 1403–1407 (2004).
72) Nakajima, K., Asakura, T., Maruyama, J., Morita, Y., Oike, H., Shimizu-Ibuka, A., Misaka, T., Sorimachi, H., Arai, A., Kitamoto, K., and Abe, K., Extracellular production of a heterodimetric protein, neoculin, with sweet-tasting and taste-modifying activities by *Aspergillus oryzae*. *Appl. Environ. Microbiol.*, **72**, 3716–3723 (2006).
73) Nakajima, K., Asakura, T., Oike, H., Morita, Y., Shimizu-Ibuka, A., Misaka, T., Sorimachi, H., Arai, S., and Abe, K., Neoculin, a taste-modifying protein, is recognized by human sweet taste receptor. *Neuroreport*, **17**, 1241–1244 (2006).
74) Koizumi, A., Nakajima, K., Asakura, T., Morita, Y., Ito, K., Shimizu-Ibuka, A., Misaka, T., and Abe, K., Taste-modifying sweet protein, neoculin, is received at human T1R3 amino terminal domain. *Biochem. Biophys. Res. Commun.*, **358**, 585–589 (2007).
75) Shimizu-Ibuka, A., Morita, Y., Terada, T., Asakura, T., Nakajima, K., Iwata, S., Misaka, T., Sorimachi, H., Arai, S., and Abe, K., Crystal structure of neoculin: insights into its sweetness and taste-modifying activity. *J. Mol. Biol.*, **359**, 148–158 (2006).
76) Nakajima, K., Morita, Y., Koizumi, A., Asakura, T., Terada, T., Ito, K., Shimizu-Ibuka, A., Maruyama, J., Kitamoto, K., Misaka, T., and Abe, K., Acid-induced sweetness of neoculin is ascribed to its pH-dependent agonistic-antagonistic interaction with human sweet taste receptor. *FASEB J.*, in press.
77) Wright, C. S., and Hester, G., The 2.0 A structures of a cross-linked complex between snowdrop lectin and a branched mannopentoose; evidence for two unique binding modes. *Structure*, **4**, 1339–1352 (1996).
78) Endo, T., *O*-mannosyl glycansin mammals. *Biochim. Biophys. Acta*, **1473**, 237–246 (1999).
79) Van Damme, E. J. M., Peumans, W. J., Barre, A., and Roug, P., Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Crit. Rev. Plant Sci.*, **17**, 575–692 (1998).
80) Shimizu-Ibuka, A., Nakai, Y., Nakamori, K., Morita, Y., Nakajima, K., Kadota, K., Watanabe, H., Okubo, S., Terada, T., Asakura, T., Misaka, T., and Abe, K., Biochemical and genomic analysis of neoculin compared to monocot mannose-binding lectins. *J. Agric. Food Chem.*, in press.
81) Shimizu-Ibuka, A., Morita, Y., Nakajima, K., Asakura, T., Terada, T., Misaka, T., and Abe, K., Neoculin as a new sweet protein with taste-modifying activity: purification, characterization, and x-ray crystallography. In "Sweetness and Sweeteners: Biology, Chemistry, and Psychophysics," eds. Weerasinghe, D. K., and Dubois, G. E., American Chemical Society, Inc., Carrboro, pp. 546–559 (2007).