Construction of a taste-blind medaka fish and quantitative assay of its preference–aversion behavior

Y. Aihara†, A. Yasuoka‡, S. Iwamoto§, Y. Yoshida†, T. Misaka† and K. Abe*†

†Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.
‡Department of Biological Engineering, Maebashi Institute of Technology, Maebashi, and
§Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. E-mail: aka7308@mail.ecc.u-tokyo.ac.jp

On capturing food in their mouth, animals immediately need to identify whether it contains nutritious or toxic compounds. However, any tastant, nutritious or toxic, is received by taste buds located at the peripheral end of the taste system (Kinnamon et al. 1985; Northcutt 2004). Information relating to taste stimuli is transmitted to taste nerves and then to the central nervous system. The behavioral response to this taste signal transmission is either ingestion or ejection.

Recent studies have uncovered the molecular mechanisms underlying the initial step in taste reception. Mammalian T1Rs and T2Rs, which are G-protein coupled receptors (GPCRs), are expressed in taste bud cells (Adler et al. 2000; Hoon et al. 1999). T1R heteromers are activated by sugars and amino acids, which are preferable tastants, while T2Rs are activated by alkaloids that elicit aversive behavior (Chandrashekar et al. 2000; Nelson et al. 2001, 2002). T1Rs and T2Rs, when activated, transduce taste signals to G-proteins (Kusakabe et al. 2000; Wong et al. 1996) and then to the effector enzyme, namely phospholipase C-β 2 (PLC-β 2) (Adler et al. 2000). Because PLC-β2 knockout mice have reduced sensitivity to both T1R and T2R ligands (Zhang et al. 2003), this enzyme may be located at a critical point in the signaling pathways for the transduction of preferable and aversive tastes. Interestingly, these taste signaling pathways seem to be conserved in a wide variety of vertebrates ranging from mammals to fish (Ishimaru et al. 2005; Yasuoka et al. 2004).

Small fish species such as zebrafish (Danio rerio) and medaka fish (Oryzias latipes) are recognized as useful model animals for studying the involvement of the nervous system in sensory perception as their nervous systems consist of a small number of cells (Chapouton & Baby-Cuif 2004; Schier et al. 1996). In addition, the small genome sizes and low redundancy of gene function are advantages when investigating sensory signaling at the molecular level (Crollius & Weissenbach 2005; Furutani-Seiki et al. 2004). Fish perceive amino acids, nucleotide-related substances and carbonic acids as taste stimulants (Kasumyan & Doving 2003; Kohbara et al. 1992; Marui & Caprio 1992; Valentinic & Caprio 1994; Yoshii et al. 1979). Their taste nerves also respond to quinine hydrochloride, caffeine and denatonium benzoate, which are perceived as bitter by humans (Chandrashekar et al. 2000; Ogawa et al. 1997). We have recently reported that fish T1Rs and T2Rs are activated by amino acids and denatonium, respectively (Oike et al. 2007), suggesting that fish and mammals share a common mechanism for taste discrimination. The fish is a useful model organism for studying the vertebrate taste system. In particular, the development of a quantitative assay system for evaluating taste preference–aversion behavior in small fish species is required. Here, we report the development of such a system that is applicable to...
medaka fish, and the construction of a transgenic, taste-blind phenotype in the species for confirming the utility of this system.

Materials and methods

Fluorescent dye-labeled food

The preferred tastant solution (AN solution) contained 100 mM glycine, 100 mM L-serine, 100 mM L-proline, 100 mM monosodium L-glutamate and 20 mM inosine monophosphate (IMP) disodium salt. The aversive tastant solution was composed of 100 mM denatonium benzoate (DN solution). An aqueous phase solution was prepared by dispersing 15 ml of tastant solution or water (for NT food) in 300 ml of 2% sorbitan monoooleate (Emasol O-120V; Kao, Tokyo, Japan) at 60°C. Approximately 30 g of tripalmitin was melted at 70°C and stirred for 5 min and mixed with 1.5 ml of 10 mM DiIC12(3) (Molecular probes D-383, Invitrogen, Carlsbad, CA, USA) ethanol solution to form a uniform molten mass (lipid phase). This was then poured into the aqueous phase at 60°C and stirred for 5 min at 10 000 r.p.m., using vacuum emulsification equipment (PVQ-3UN; Mizuho Industrial, Osaka, Japan). To prevent the development of coalesced droplets in the emulsion, 150 g of potato starch was added and the mixture was stirred quickly to encourage gelatinization. The mixture was then steamed for 5 min to allow the starch to solidify, and the sample was cooled rapidly in liquid nitrogen and then dried under a vacuum. The dried sample was milled and particles with a small diameter of 150–212 µm were selected. This size is slightly smaller than the mouth size of juvenile fish. To prepare diluted DN food, NT food was spread on a stainless steel sieve, dipped in denatonium solution or water (for NT food) and immediately dried. The dried mass was milled into particles with a diameter of 150–212 µm.

Food intakes of wild-type medaka fish

We performed the assay four times and selected fish for further analysis with eye diameters of 460–560 µm as this measurement was closely proportional to their body size. The sample size for each test was calculated based on a type I error rate of 5% and a type II error rate of 20%. The between-food differences in mean FI values were analyzed by Dunnett’s multiple comparison test using K-Pt or 3.0 (KyensLab, Tokyo, Japan).

Figure 1: An experimental design for evaluating the feeding behavior of medaka fish. (a) As they grow, medaka fish change their feeding behavior. After hatching at around 9 days postfertilization (dpf), the larvae begin to ingest a powdered diet as well as nutrients in the yolk sac, which is depleted at around 12 dpf. After 30 dpf, juvenile fish begin to ingest both a powdered diet and a live food (Artemia salina). Under laboratory conditions, the fish mature at around 60 dpf. (b) Preparing medaka fish for the behavioral assay. After hatching, larvae were placed in the maintenance system and fed the powdered diet until 19 dpf. They were then isolated in a Petri dish, starved for 24 h and subjected to the behavioral assay using fluorescently labeled food.

Quantification of ingested food

The fluorescence intensity (FI) values by integrating pixel values from images of the abdominal region using PHOTOSHOP CS2 (Adobe Systems, San Jose, CA) and the SCION IMAGE Beta 4.0.2 software (Scion, Frederick, MD, USA; http://www.scioncorp.com/). A standard curve generated from InSpeck™ orange fluorescent microspheres (Invitrogen) was used to process the data, which were then submitted for statistical analysis. To extract DiIC12(3) from the bodies of the fish, each was homogenized in 200 µl of extraction solution (75% ethanol and 1% acetic acid) and centrifuged. This procedure was repeated, the 200 µl of supernatant from each extraction was pooled and the resulting 400 µl solution was filtered using Ultrafree-MC HV (Millipore, Billerica, MA, USA). A 100-µl aliquot was subjected to reverse-phase high-performance liquid chromatography (HPLC) analysis using a Shodex Rapak RP18-415 column (4.6 × 150 mm, 6 µm; Showa Denko K. K., Tokyo, Japan). The sample was eluted with a solution containing 50% methanol and 1% acetic acid at a flow rate of 1 ml/min at ambient temperature. DiIC12(3) was detected using a fluorescence detector with an excitation wavelength of 550 nm and an emission wavelength of 604 nm. The fluorescence was quantified based on the integrated peak area of the chromatogram with a retention time of 8.4 min. To obtain standards, the same analyses were performed against a given amount of NT food.

GpS47C transgenic fish

Rat GpS 2 cDNA was obtained using the method described by Kusakabe et al. (2000). The cDNA fragment from the initiation codon to the Ser 47 codon was mutagenized using a Takara LA Taq PCR kit (Takara Bio, Tokyo, Japan) and the primers 5'-gcgatatcatgctggcactccgtgagccgca-3’ and 5'-gcgatatctggcactccgtgagccgca-3’, producing an ApaLI recognition site. The cDNA fragment from the Ser 47 codon to the termination codon was also mutagenized using the same kit and the primers
Aihara et al.

5'-ggatatctcagagaagggacagtctctca-3' and 5'-ggatatcgtgcActctcctc-
tgatlccagc-3', producing also an ApaLI recognition site. These frag-
ments were digested by ApaLI and EcoRV and subcloned into the 
EcoRV site of pBluescript II SK+I (Stratagene, La Jolla, CA, USA) to 
obtain full-length GαS47C cDNA. The cDNA was excised by 
EcoRV and inserted into the BamHI site of pmpfLCb2-1.6 kb (Aihara 
et al. 2007) to produce a pmpfLCb2-1.6 kb-GαS47C fragment. 
*pmpfLCb2-1.6 kb-EGFP<Kan>- (Aihara et al. 2007) was used as a 
backbone vector. The SV40 polyadenylation signal for the rat 
GalpαsS47C transcript was first subcloned into the Sal site of 
pmpfLCb2-1.6 kb-EGFP<Kan>- (Aihara et al. 2007) and then excised by 
NotI and Smal and inserted into the Xhol site of pmpfLCb2-1.6 kb-EGFP<Kan>- (Aihara et al. 2007), linking the SV40 polyadenylation 
signal to produce the final construct (Fig. 4a). Injection of the trans-
gene and establishment of transgenic lines were performed using the 
method described previously (Aihara et al. 2007). Briefly, three G0 fish 
exhibiting the green fluorescent protein (GFP) signal were crossed 
with wild-type fish to obtain the F1 generation. Two lines were found 
to inherit the transgene, and the one exhibiting the most intense GFP 
signal was crossed with wild-type fish to produce the F2 and F3 
generations. Mainly F3 fish were used in the food-preference–aversion 
assay. The siblings exhibiting no GFP signal were used as wild-type 
controls.

In situ hybridization

The digoxigenin-labeled antisense riboprobes were synthesized using the partial cDNA fragment encoding medaka PLC-Cβ2 (Ilie 369 to Ser 616, accession number AB254242) and full-length rat GαS47C 
cDNA as templates. The head portion of each adult fish was removed 
and fixed in phosphate-buffered saline (PBS) containing 4% neutral-
ized paraformaldehyde for 18 h at 4°C. They were then dehydrated in 
95% ethanol for 4 h at room temperature, decalcified in 0.5M EDTA 
and fixed in phosphate-buffered saline (PBS) containing 4% neutral-
ized paraformaldehyde for 18 h at 4°C, embedded in Tissue–Tek O.C.T. compound (Sakura Finetechical Co., Ltd., Tokyo, Japan) in vapors of liquid nitrogen and 
cryosectioned at a thickness of 4 μm. 

Results

Preparing fish for the feeding assay

Medaka fish change their feeding behavior during growth. 
After hatching, the larvae consume a powdered diet along 
with the nutrients in the yolk sac (Fig. 1a). The yolk sac 
disappears at around 12 dpf, and the larvae become increas-
ingly more responsive to food. Supporting this, we detected 
more intense plcβ2 expression in the taste bud cells at this 
stage than at the 9-dpf stage (unpublished data). The larvae 
also show food preferences. They examine an object on the 
surface of the water by biting into it and, depending on their 
preference, either ingest or eject it. Fish are maintained on 
a powdered diet until 30 dpf and are then supplied with 
plankton as the main feed for the rest of their lives (Fig. 1a).

It seems that 20 dpf juvenile fish were suitable for use in 
our feeding assay as they were sufficiently developed to 
exhibit constant feeding behavior, yet possessed a transpar-
rent body that allowed fluorescence observations of the 
gastrointestinal tract. At 19 dpf, fish were isolated from the 
maintenance system, starved for 24 h in a dish, supplied with the 
fluorescently labeled food for 150 seconds in a new dish 
and subjected to the evaluation of food intake as follows 
(Fig. 1b).

Designing fluorescently labeled food and its detection 
in the gastrointestinal tract of medaka fish

It is obvious that medaka fish utilize their visual and olfactory 
systems to search for food. They are usually more attracted to 
food floating on the surface of the water than that at the 
bottom, possibly because of the optical contrast between the 
food and the surface. This implied that it was necessary to 
design a food that possessed buoyancy in order to lure the 
fish. Fish also respond to chemical stimuli from a distance. 
For example, if a solution containing amino acids is dropped 
into the water, they respond by swimming vigorously and 
searching for food (Carr et al. 1977; Hara 2006; Lindsay & 
Vogt 2004). This results from the rapid diffusion and detection 
of the chemicals by the fish olfactory system. Because our 
aim was to evaluate the behavior triggered by taste stimuli, 
we required a food that retained its chemical compounds until 
it came into contact with the fish’s taste buds. Our porous 
food matrix, consisting of starch, detergent and lipids (Fig. 2a, i), 
satisfied both these demands. The resulting food had a lower 
specific gravity than water, and its multiple cavities 
retained tastants and allowed them to be released in response 
to mechanical stimuli. In addition, the amphipathic 
matrix was labeled with a fluorescent dye, namely DiIC12(3), 
to allow quantification of the amount of food ingested 
(Fig. 2a, ii and iii).

Fish were fed the experimental food at 20 dpf and 
observed under a fluorescence microscope. The red fluores-

cence of DiIC12(3) could be detected in the abdominal region 
of fish that consumed the food (Fig. 2b, upper panels) but not 
in those that did not (Fig. 2b, lower panels). This experiment 
was performed in an entirely noninvasive manner, and 
therefore, the fluorescence was detected only in the gastro-
intestinal tract and clearly not on the body surface. Based on
the fluorescence image of each fish, we were able to obtain a FI value by integrating pixel values. To confirm that this FI value represents the amount of food ingested by the fish, we compared the FI values with the amount of DiIC12(3) extracted from the body of the fish \( (n = 30) \). Fish were fed the foods containing tastants as described in the next section, and the FI value for each fish was measured. Subsequently, DiIC12(3) was extracted from the body of each fish by an organic solvent for quantification by HPLC analysis. As shown in Fig. 2c, a positive correlation was observed between the FI values and the amount of food ingested \( (r^2 = 0.78) \).

**Effect of the tastants present in the fluorescently labeled food on fish behavior**

Several varieties of tastants were added to the food to examine their effect on the feeding behavior of fish. Previous reports have shown that the taste system in fish is sensitive to amino acids and nucleotide-related substances (Kasumyan & Doving 2003; Kiyohara et al. 1975; Kohbara et al. 1992). We therefore used a mixture of glycine, L-serine, L-proline, L-glutamate sodium salt and IMP disodium salt as preferable tastants. In contrast, few studies describe the taste nerve responses to aversive tastants, such as quinine hydrochloride, caffeine and denatonium benzoate (Ogawa et al. 1997). With regard to behavioral responses, it is reported that pellets flavored with quinine hydrochloride are rejected by goldfish (*Carassius auratus*) (Lamb & Finger 1995) and we observed that zebrafish consumed less food containing denatonium benzoate than that containing no tastants (Oike et al. 2007). Because medaka fish have been seen to strongly avoid a solution of denatonium benzoate that is a potent bitter chemical for mammals, this compound was used as an aversive tastant (data not shown). The tastants were mixed with food materials (see Materials and Methods), and the resulting products were designated as amino acid–IMP (AN food) and denatonium foods (DN food). Food containing no tastants (NT food) was used as the control. With regard to the initial sequences of feeding behavior, no between-food differences were observed.

Typical fluorescence images of fish after food ingestion are shown in Fig. 3a. Stronger intensities of fluorescence were detected in the fish that were fed AN food (panels in the AN column) than in those fed NT food (panels in the NT column). In contrast, only faint fluorescence images were observed for the group fed DN food (panels in the DN column). When the mean FI values were compared by Dunnett’s multiple comparison test, significant differences were observed between the AN food group and the NT food group \( (t = 2.68, df = 101, k = 3, P = 0.016) \) and also between the NT food group and the DN food group \( (t = -2.99, df = 101, k = 3, P = 0.007) \). These data clearly indicate that medaka fish prefer AN food and are averse to DN food. Next, we prepared foods...
containing denatonium benzoate at lower concentrations by dipping NT food into a \(10^{-8}\), \(10^{-6}\) or \(10^{-4}\) \(\text{m}\) denatonium benzoate solution (see Materials and Methods). The foods were designated as \(10^{-8}\) DN, \(10^{-6}\) DN or \(10^{-4}\) DN food and subjected to the behavioral assay together with NT food as a control, which was prepared by dipping NT food into water. The amount of ingested food decreased as the denatonium benzoate concentration increased, and a significant difference was detected between NT food and \(10^{-4}\) DN food at \(P < 0.05\) (Dunnett’s multiple comparison test).

**Generation of transgenic fish expressing a dominant-negative mutant of G\(_i2\) in taste receptor cells**

To examine whether the observed food preference behavior was dependent on the taste system of medaka fish, we used a transgenic approach to inhibit the taste receptor signal in vivo. In mammals and teleosts, two discrete subsets of taste-receptor-expressing cells are present; the T1R and T2R subsets. These two subsets of cells also express PLC-\(\beta\), a common taste signal effector enzyme (Adler et al. 2000; Ishimaru et al. 2005; Zhang et al. 2003). In vitro studies have shown that PLC-\(\beta\) can be activated by both G-protein \(\alpha\) subunits of the alphaq family and \(\beta/\gamma\) subunits that have lower specificities (Hepler et al. 1993; Rhee 2001; Smrcka & Sternweis 1993). In mammals, however, taste receptor cells predominantly express G\(_{\alpha\text{qJ}}\) and G\(_{\alpha\text{q}}\) that lack the potency to activate PLC-\(\beta\) by themselves (Kusakabe et al. 2000; McLaughlin et al. 1992). Therefore, it is highly possible that PLC-\(\beta\) is activated by \(\beta/\gamma\) subunits released from G\(_{\alpha}\) in taste receptor cells (Blake et al. 2001; Huang et al. 1999). We hypothesized that the inhibition of G\(\beta/\gamma\)-dependent activation of PLC-\(\beta\) in medaka taste receptor cells may cause a defect in taste signal transduction. To address this, we employed a rat G\(_{\alpha\text{q}}\) dominant-negative mutant (G\(_{\alpha\text{q}}\text{S47C}\)) that was developed for the purpose of suppressing the GPCR signal to PLC-\(\beta\) by GTP-independent and irreversible binding to G\(\beta/\gamma\) (Slepak et al. 1995). The G\(_{\alpha\text{q}}\text{S47C}\) cDNA was fused to the medaka plc-\(\beta\) promoter that can induce transgene expression specifically in taste receptor cells (Fig. 4a) (Aihara et al. 2007). The construct also contained the gfp gene, under the control of the same promoter, to facilitate the optical identification of transgenic fish. As a result, the F\(_3\) transgenic larvae exhibited GFP signals in the oropharyngeal region (Fig. 4b), exactly reproducing the pattern obtained with the transgene containing a single promoter and gfp reporter (Aihara et al. 2007). Next, we examined whether these transgenes were expressed in the same way as the endogenous plc-\(\beta\) gene, by sequential observation of GFP fluorescence and the in situ hybridization signal (Fig. 4c). The upper panels of Fig. 4c show the colocalization of GFP and the endogenous plc-\(\beta\) transcript, while the lower panels confirm the coexpression of the two transgenes, namely gfp and g\(_{\alpha\text{q}}\text{S47C}\). No signal was observed in wild-type fish sections hybridized with the rat g\(_{\alpha\text{q}}\text{S47C}\) probe (data not shown), and therefore, cross-hybridization to endogenous medaka g\(_{\alpha}\) genes was negligible. The transgenic line established was named G\(_{\alpha}\text{S47C}\), and these fish were subjected to the feeding assay.

**Taste preference of G\(_{\alpha}\text{S47C}\) transgenic fish**

The taste preference of G\(_{\alpha}\text{S47C}\) fish for AN food was examined (Fig. 5a), and the FI values for AN and NT foods...
were measured in transgenic and wild-type siblings. Two-way ANOVA with genotype and food type as factors showed a significant interaction between factors [interaction \( F_{1,112} = 3.98, P < 0.05 \); genotype \( F_{1,112} = 0.08, P > 0.05 \); food \( F_{5,112} = 7.37, P < 0.01 \)]. When the consumption for AN food and NT food was compared, a significant difference was detected in the case of wild-type fish (Aspin–Welch’s \( t \)-test, \( t = -0.50, df = 50, P < 0.05 \)) but not in transgenic fish (\( t = -3.37, df = 48, P < 0.01 \)). G2S47C and wild-type fish were examined for their aversive behavior toward \( 10^{-4} \) DN food (Fig. 5b). The FI values for \( 10^{-4} \) DN and NT# food were measured in transgenic and wild-type siblings. As expected, the transgenic fish ingested a certain amount of \( 10^{-4} \) DN food. In addition, the use of two-way ANOVA with genotype and food type as factors showed evidence of a significant interaction \( [F_{1,74} = 5.33, P < 0.05] \); genotype \( F_{1,74} = 2.17, P > 0.05 \); food \( F_{1,74} = 25.2, P < 0.001 \). When the consumption for \( 10^{-4} \) DN food and NT# food was compared, no significant difference was detected in the case of transgenic fish (Aspin–Welch’s \( t \)-test, \( t = 1.97, df = 30, P > 0.05 \)). In contrast, wild-type fish showed a highly statistically significant tendency to reject \( 10^{-4} \) DN food (\( t = 5.08, df = 22, P < 0.001 \)). Taken together, it is concluded that G2S47C transgenic fish had lost the ability to show food preferences and aversion because of the inhibition of inner-cellular taste signal transduction.

**Discussion**

In this study, we have generated a transgenic medaka fish in which taste receptor-dependent PLC-β2 activation is inhibited. We have also developed an assay system that facilitates the quantification of the food preference behavior of this species. The transgenic fish exhibited a taste-blind phenotype, which showed neither a preference for food containing an amino acid–IMP mixture nor an aversion to that containing denatonium benzoate. This paper is the first to describe the genetic manipulation of the taste signaling pathway and to evaluate the behavioral effect of this manipulation in a fish model.

The feeding behavior of fish consists of multiple sequential steps: (1) noticing food, (2) approaching it, (3) capturing it and (4) ingesting/ejecting it. Both the olfactory and the taste systems are involved in steps 1, 2 and 3 (Bardach et al. 1967; Valentincic & Caprio 1994), while the taste system plays a major role in step 4 (Atema 1971). Previous studies that evaluated taste preferences in fish were performed by observing food sorting behavior or food consumption (Kasumyan & Morsy 1996; Lamb & Finger 1995), but these methods had experimental limitations with regard to the quantification of food intake. In the present study, we confronted this limitation by incorporating the fluorescent probe into the food, thereby allowing a direct measurement of the amount of food ingested. Moreover, the transparency of the juvenile fish allowed us to observe the food in the gastrointestinal tract and quantitatively assess its amount by fluorescence imaging (Fig. 2). In the sequence of feeding behavior, tastants stimulate olfactory as well as taste systems and indeed, compared with their taste nerves, fish olfactory nerves respond to amino acids at a lower threshold (Hara 1994). In this study, we intended to efficiently stimulate only the fish taste system. Because medaka fish seek food floating on the surface of the water, our food particles were designed to contain fine cavities for buoyancy. Moreover, these cavities, with the aid of tripalmitin, served to retain the
tastants. The lipid also prevented the food from dissolving in the water. These physical characteristics allowed the tastants to stimulate the taste buds of the fish the moment it bit into the food. This design of the food matrix is also applicable for use with different tastants. For example, organic acids and salts are candidate materials for use in future experiments. The matrix is amphipathic, and therefore also allows the creation of foods containing fatty acids as tastants, whose receptor molecule still remains to be identified.

An amino acid–IMP mixture was used as a preferable tastant, and denatonium benzoate was used as an aversive tastant for medaka fish (Fig. 6a). These tastants also stimulate the mammalian taste system in which T1R and T2R function as taste receptors at the peripheral end of the system. T1R and T2R exhibit mutually exclusive expression patterns and different response profiles in the taste buds, and it is therefore likely that these taste receptors are responsible for discriminating between preferable and aversive tastes (Mueller et al. 2005). Despite the sharp contrast between the functions of these two receptors, they are both coexpressed with the same effector molecule, PLC-β2. It has previously been shown that mice lacking the plc-β2 gene have neither a preference for amino acids nor an aversion to denatonium benzoate (Zhang et al. 2003). This result resembles the phenotype exhibited by G_{2}S47C transgenic fish in the current study (Fig. 5). We have recently shown that fish T1R is activated by all four amino acids used in this study, namely glycine, L-serine, L-proline and L-glutamate sodium salt, and that fish T2R is activated by denatonium benzoate (Oike et al.)

![Figure 5: Differences in the feeding behavior between transgenic fish and wild-type fish.](image)

(a) The feeding behavior for AN food. Bars represent the mean FI values with standard errors. Twenty-nine transgenic and 28 wild-type fish were used for the statistical analysis of NT and AN foods. A significant between-food difference was observed only in wild-type fish (**P < 0.01). (b) The feeding behavior with 10^{-4} DN food. For statistical analysis, 19 transgenic fish were used for both NT and 10^{-4} DN foods, and 18 and 19 wild-type fish were used for NT and 10^{-4} DN foods, respectively. A significant between-food difference was observed only in wild-type fish (**P < 0.01).

![Figure 6: Transgenic medaka fish with suppressed PLC-β2 activation in taste receptor cells lose their ability to discriminate between taste modalities.](image)

(a) Our behavioral assay system revealed that medaka fish prefer foods containing amino acids and IMP (red stars) and that they are averse to those containing denatonium benzoate (cyan circles), which are perceived as bitter tasting by mammals. (b) The transgenic medaka fish constructed in the present study was found to be taste blind. Because the G_{2}S47C mutant (G_{2}^{*}) expressed in the taste receptor cells inhibits the signal transduction to PLC-β2, the fish were no longer able to recognize preferable or aversive tastes.
Taste-blind medaka and its feeding behavior

Hara, T.J. (2006) Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. J Fish Biol 68, 825–826.

Hepler, J.R., Kozasa, T., Smrcka, A.V., Simon, M.I., Rhe, S.G., Sternweis, P.C. & Gilman, A.G. (1993) Purification of Sf9 cells and characterization of recombinant G alpha and G11 alpha. Activation of purified phospholipase C isozymes by G alpha subunits. J Biol Chem 268, 14367–14375.

Hoon, M.A., Adler, E., Lindemeyer, J., Battey, J.F., Ryba, N.J.P. & Zuker, C.S. (1999) Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 96, 541–561.

Huang, L., Shanker, Y.G., Dubauser, J., Zheng, J.Z., Yan, W., Rosenweig, S., Spielman, A.I., Max, M. & Margolskee, R.F. (1999) G_{13} colocalizes with gustducin in taste receptor cells and mediates IP_3 responses to bitter denatonium. Nat Neurosci 2, 1055–1062.

Ishimaru, Y., Okada, S., Naito, H., Nagai, T., Yasuoka, A., Matsumoto, I. & Abe, K. (2006) Two families of candidate taste receptors in fishes. Mech Dev 122, 1310–1321.

Kasumyan, A.O. & Doving, K.B. (2003) Taste preferences in fishes. Fish Fish 4, 289–347.

Kasumyan, A.O. & Morsy, A.M.H. (1996) Taste sensitivity of common carp Cyprinus carpio to free amino acids and classical taste substances. J Ichthyol 36, 391–403.

Kinnamon, J.C., Taylor, B.J., Delay, R.J. & Roper, S.D. (1985) Ultrastructure of mouse vallate taste buds. I. Taste cells and their associated synapses. J Comp Neurol 235, 48–60.

Kiyohara, S., Hidaka, I. & Tamura, T. (1975) Gustatory response in cyprinid fish. J Comp Physiol 94, 275–294.

Kohbara, J., Michel, W. & Caprio, J. (1992) Responses of single facial taste fibers in the channel catfish, Ictalurus punctatus, to amino acids. J Neurophysiol 68, 1012–1026.

Kumazawa, T., Brand, J.G. & Teeter, J.H. (1998) Amino acid–activated channels in the catfish taste system. Biophys J 75, 2757–2766.

Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arii, S., Emori, Y. & Abe, K. (2000) Comprehensive study on G protein alpha-subunits in taste bud cells, with special reference to the occurrence of Gsalpha2 as a major Galpha species. Chem Senses 25, 525–531.

Lamb, C.F. & Finger, T.E. (1999) Gustatory control of feeding behavior in goldfish. Physiol Behav 63, 483–488.

Lindsay, S.M. & Vogt, R.G. (2004) Behavioral responses of newly hatched zebrasid Danio rerio to amino acid chemostimulants. Chem Senses 29, 93–100.

Marui, T. & Caprio, J. (1997) Teleost gustation, in Fish chemoreception, pp. 391–403.

McLaughlin, S.K., McKinnon, P.J. & Margolskee, R.F. (1992) Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 357, 563–565.

Mueller, K.L., Hoon, M.A., Erlenbach, I., Chandrashekar, J., Zuker, C.S. & Ryba, N.J.P. (2005) The receptors and coding logic for bitter taste. Nature 434, 225–229.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Raiby, N.J.P. & Zuker, C.S. (2001) Mammalian sweet taste receptors. Cell 106, 381–390.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L.X., Zhao, G., Ryba, N.J.P. & Zuker, C.S. (2002) An amino-acid taste receptor. Nature 416, 199–202.

Northcutt, R.G. (2004) Taste buds: development and evolution. Brain Behav Evol 64, 198–206.

Ogawa, K., Marui, T. & Caprio, J. (1997) Quinine suppression of single facial taste fiber responses in the channel catfish. Brain Res 769, 263–272.

Olive, H., Nagai, T., Furuyama, A., Okada, S., Aihara, Y., Ishimaru, Y., Marui, T., Matsumoto, I., Misaka, T. & Abe, K. (2007) Characterization of ligands for fish taste receptors. J Neurosci 27, 5584–5592.

Rhee, S.G. (2001) Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem 70, 281–312.

Scheer, A.F., Neuhaus, S.C.F., Harvey, M., Malicki, J., Solnica-Krezel, L., Stainier, D.Y.R., Zwartkruis, F., Abdellah, S., Stemple, D.L.,
Aihara et al.

Rangini, Z., Yang, H. & Driever, W. (1996) Mutations affecting the development of the embryonic zebrafish brain. Development 123, 165–178.

Slepak, V.Z., Katz, A. & Simon, M.I. (1995) Functional analysis of a dominant negative mutant of $G_{i2}$. J Biol Chem 270, 4037–4041.

Smrcka, A.V. & Sternweis, P.C. (1993) Regulation of purified subtypes of phosphatidylinositol-specific phospholipase C beta by G protein alpha and beta gamma subunits. J Biol Chem 268, 9667–9674.

Valentincic, T. & Caprio, J. (1994) Consummatory feeding behavior to amino acids in intact and anosmic channel catfish Ictalurus punctatus. Physiol Behav 55, 857–863.

Wong, G.T., Gannon, K.S. & Margolskee, R.F. (1996) Transduction of bitter and sweet taste by gustducin. Nature 381, 796–800.

Yasuoka, A., Aihara, Y., Matsumoto, I. & Abe, K. (2004) Phospholipase C-beta 2 as a mammalian taste signaling marker is expressed in the multiple gustatory tissues of medaka fish, Oryzias latipes. Mech Dev 121, 985–989.

Yoshii, K., Kamo, N., Kurihara, K. & Kobatake, Y. (1979) Gustatory responses of eel palatine receptors to amino acids and carboxylic acids. J Gen Physiol 74, 301–317.

Zhang, Y., Chandrashekar, J., Mueller, K.L., Cook, B., Zuker, C.S., Hoon, M.A., Ryba, N.J.P. & Wu, D. (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112, 293–301.

Acknowledgments

The authors are indebted to Dr. Michiko Watanabe for technical assistance and pertinent discussions. This study was partially supported by a grant-in-aid for JSPS Fellows (to Y.A.), Scientific Research (S) (to K.A.), and Young Scientists (A) (to T.M.) from the Japan Ministry of Education, Culture, Sports, Science and Technology.

Genes, Brain and Behavior (2008) 7: 924–932