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Molecular Genetic Identification of a Candidate Receptor Gene for Sweet Taste

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A cDNA clone encoding a novel member of the putative taste receptor T1R family, designated T1R3, was isolated from circumvallate papillae of the mouse tongue using degenerate primers. Reverse transcription–polymerase chain reaction analysis showed predominant expression of the receptor in circumvallate papillae. *In situ* hybridization analysis revealed that T1R3 was expressed in a subset of taste receptor cells in taste buds and that the topographic distribution of T1R3 in various taste papillae was different from those of the other T1R members. Genetic mapping of T1R3 with a mouse/hamster radiation hybrid panel located the gene on the distal end of mouse chromosome 4 correlated with the *Sac* locus affecting sweet sensitivity of mice. Our results indicate that T1R3 may serve as the receptor for sweet perception in mice.

Key Words: **taste receptor; taste papillae; taste bud; G protein-coupled receptor; seven transmembrane receptor; cloning; *in situ* hybridization; radiation hybrid mapping; saccharin.**

A variety of taste stimuli are received by taste receptor cells (1). In mammals, taste receptor cells are assembled into taste buds that are distributed in the epithelium of three types of taste papillae on the tongue: fungiform, foliate, and circumvallate papillae, located at the front, lateral edge, and back of the tongue, respectively. Taste stimuli are thought to be transduced via distinct mediators expressed in the apical membrane of those taste receptor cells. Electrophysiological studies suggest that sour and salty tastants modulate the function of taste receptor cells by certain membrane channels (2, 3). Other basic tastes, sweet, bitter, and umami are believed to be detected by G-protein-coupled receptor (GPCR) signaling pathways (4, 5). Recent molecular cloning studies have led to the identification of a number of taste receptor cell-specific GPCRs, which are categorized into T1R and T2R families, and taste-mGluR4 (6). T1R is a putative taste receptor family with homology to V2R, a putative pheromone receptor family (7, 8), and is composed of two members, T1R1 and T1R2, formerly known as TR1 and TR2 (9). Although the expression of T1R is restricted to a subset of taste receptor cells, the ligands and functions of the receptors are still unknown. T2R is a multigene family identified from genetic linkage analysis associated with bitter perception and implicated as receptors of bitter tastants by *in vitro* functional expression assay (10, 11). Taste-mGluR4 is an alternatively spliced form of mGluR4 and is thought to be a candidate for umami receptors because of the truncation of amino terminal region decreases its sensitivity for glutamate to the concentrations known to elicit the sensation of umami (12). In view of the chemical diversity of various tastants, many other unknown taste receptors should be exist as observed in olfactory receptors and vomeronasal receptors (13). Identification and characterization of new members of the T1R would clarify the function of the family and facilitate the complicated molecular mechanism of taste perception. In the present study, we report the isolation of a novel member of T1R, its tissue distribution, topographic distribution in the tongue epithelium, and the chromosomal localization of the gene.

Abbreviations used: GPCR, G-protein-coupled receptor; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-PCR.

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MATERIALS AND METHODS

RNA and cDNA preparation. Six-week-old male mice (C57BL/6NCrj) were killed by cervical dislocation. The tongue was dissected immediately and put into divalent-cation-free Ringer solution (26

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mM $NaHCO_3$, 2.5 mM $NaH_2PO_4 \cdot 2H_2O$, 65 mM NaCl, 20 mM KCl, 1 mM EDTA · 4Na, 20 mM glucose). The Ringer solution containing 2.5 mg/ml collagenase type IV (Worthington Biochemical) and 2 mg/ml elastase (Worthington Biochemical) was injected beneath a circumvallate papilla. After incubation for 15 min at room temperature, the epithelium of the circumvallate papilla was peeled off. Total RNA was isolated using TRIZOL reagent (Life Technologies) and the first strand cDNA was synthesized with oligo(dT)12-15 primers using the reagents of the Superscript kit (Life Technologies).

PCR cloning with degenerate oligonucleotides. Based on the consensus amino acid sequence of mouse T1R and V2R (9), the following degenerate oligonucleotides were synthesized: 5′-GCIGTITA(C/T)GCI(A/G)TIGCICA-3′ (primer TRF1, corresponding to the amino acid sequence of AVYA(I/V)A(H/Q)), 5′-TG(C/T)TG(C/T)TT(C/T)GAITG(C/T)IT-3′ (primer TRF2, corresponding to the amino acid sequence of CCF(D/E)C(I/L/V)) and 5′-A(A/G) (A/G/T)ATIA(C/T) (A/G)CA(C/T)TTIGG-3′ (primer TRR1, corresponding to the amino acid sequence of PKC(F/Y) (I/M/V)I). Using primers TRF1 and TRR1, a first round PCR of the circumvallate papillae cDNA was carried out with 40 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 1 min. About 1.3 kbp fragments were isolated and used for the template of a second nested PCR, which was carried out under the same conditions using primers TRF2 and TRR1. The resulting ~900 bp fragment was isolated, cloned into pGEM-T Easy (Promega) and sequenced. To clone the full-length cDNA, 5′-RACE was performed using FirstChoice RLM-RACE kit (Ambion) and 3′-RACE was performed using oligo(dT)-adaptor-primed with circumvallate papillae cDNA as essentially described (14). Primers used for 3′-RACE were oligo(dT)-adapter primer, 5′-GGCCACGCGTCGACTAGTAC(T)17-3′ and adapter primer, 5′-GGCCACGCGTCGACTAGTAC-3′. The sequence data of the full-length cDNA clone was submitted to the DDBJ/EMBL/GenBank Data Libraries under the Accession No. AB049994.

RT-PCR analysis. Total RNA was prepared from various mouse tissues by the method described above. Two pairs of oligonucleotide primers were synthesized for the analytical PCR amplification of reverse transcribed mRNAs for T1R3 and β-actin; 5′-CTACCCTGGCAGCTCCTGGA-3′ and 5′-CAGGTGAAGTCATCTGGATGCTT-3′ for T1R3, 5′-ATCGTGGGCCGCTCTAGGCACC-3′ and 5′-CTCTTTGATGTCACGCACGATTTC-3′ for β-actin. First strand cDNA reverse transcribed from 100 ng of total RNA was amplified with the primer pairs. The PCR conditions were 30 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s for each primer pair.

In situ hybridization. Partial cDNAs of mouse T1R1 and T1R2 were obtained by RT-PCR using the primers; 5′-GCCTTCAAGGACGTGGTGCC-3′ and 5′-AGGCTGAAGGTGACACATTT-3′ for T1R1, 5′-AACTGTAGCTCTCTGCTGCC-3′ and 5′-GTAATGAACTTGGCTTCGTT-3′ for T1R2, respectively. The amplified fragments were cloned into pGEM-T Easy and used as templates for synthesis of RNA probes. The plasmid containing TRF2/TRR1 fragment in pGEM-T Easy was used for the template of T1R3. Digoxigenin-labeled RNA probes for T1R1, T1R2, and T1R3 were synthesized using SP6/T7 Transcription Kit (Roche Molecular Biochemicals). Tissue was obtained from 6-week-old male mice (C57BL/6NCrj), frozen in OCT compound, sectioned into 5 μm slices and attached to APS-coated glass slides. Hybridization was performed as described by Kusakabe *et al.* (15). All *in situ* hybridizations were carried out at high stringency (5× SSC, 50% formamide, 72°C). Signals were detected using anti-digoxigenin Fab fragments conjugated with alkaline phosphatase and developing substrates, 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Roche Molecular Biochemicals) and nitroblue tetrazolium (NBT, Roche Molecular Biochemicals).

Chromosomal mapping of T1R members. The mapping of genes for mouse T1R1, T1R2 and T1R3 was performed with the mouse/hamster radiation hybrid panel (Research Genetics). Three PCR primer pairs were designed from the mouse T1R1, T1R2 and T1R3 cDNA sequences; 5′-CCGTTGAGGAGATAAACAACTCCACAGCTC-3′ and 5′-GGGCTCAGCAGGGCAGCAGTGGTGA-3′ for T1R1, 5′-ACAACTGTAGCTCTCTGCTGCCCGGCGT-3′ and 5′-GGAGAGAATGTTGGACACGGTGATGGCGG-3′ for T1R2, and 5′-CCGTGCCCGTGGTCTCACCTTCGCCATG-3′ and 5′-GGTCATTCATTGTGTCCCTGAGCTGCCTC-3′ for T1R3. The PCR condition was 30 cycles at 94°C for 30 s, 67°C for 30 s, and 72°C for 30 s for each primer pair. The PCR products from T1R1, T1R2 and T1R3 primer pairs were 238, 218 and 288 bp, respectively. The results were analyzed by using the radiation hybrid mapping service of Whitehead Institute/MIT Center (http://www.genome.wi.mit.edu).

RESULTS AND DISCUSSION

To isolate new members of the T1R family expressed in mouse taste receptor cells, degenerate primers were designed from some amino acid sequences conserved among mouse T1R and V2R (9), and RT-PCR was performed with total RNA prepared from mouse circumvallate papillae in which T1R1 and T1R2 were expressed. The amplified cDNA fragment of the expected size was subcloned, and sequencing of 12 clones revealed that 11 clones encoded T1R2, which is predominantly expressed in circumvallate papillae (9). One clone had an open reading frame encoding an amino acid sequence homologous to T1R1 and T1R2. To obtain the complete open reading frame of the novel cDNA fragment, we utilized the RACE method. A fragment of 5′-RACE had an initiation codon surrounded by a Kozak consensus sequence (16), an in-frame stop codon located 24 bp upstream of the initiation codon, and a fragment of 3′-RACE had the termination codon and poly A. The full length cDNA fragment was, then, amplified using specific primers based on the sequences of the RACE fragments, and the length of the PCR product agreed with that predicted from the total length of those cDNA fragments. The open reading frame encoded a protein of 858 amino acids with a calculated molecular mass of 94.5 kDa (Fig. 1). As expected, the data base search revealed that the highest homology was detected with mouse T1R1 and T1R2 (32 and 31% identity, respectively). Lower but still significant similarity was found with several members of GPCR subfamily 3, including metabotropic glutamate receptor (mGluR), Ca^{2+}-sensing receptor (CaSR) and V2R (17). Thus, we designated the clone as T1R3. The sequence alignment of the amino acid sequences of T1R members showed that most of cysteine residues in the extracellular domain were highly conserved (Fig. 2). It is known that a Cys-rich region with nine highly conserved cysteines proximal to the transmembrane domain is present in all of the members of GPCR subfamily 3, and thought to impose a structural constraint on the receptor protein (18). The nine cysteine residues were also conserved in T1R3, suggesting that the structure might be similar to those of GPCR subfamily 3 receptors.

Next, we examined the tissue specificity of the T1R3 expression by RT-PCR. To prevent amplification of

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                                                                                                                    CGCGGATCCGACACTCGTTTGCTGGCTTTGATGAAATTTGTTAGTGCTGGAGACTTCTACCTACC  -1

ATGCCAGCTTTGGCTATCATGGGTCTCAGCCTGGCTGCTTTCCTGGAGCTTGGGATGGGGGCCTCTTTGTGTCTGTCACAGCAATTCAAGGCACAAGGGGACTACATACTGGGCGGGCTA  120
 M  P  A  L  A  I  M  G  L  S  L  A  A  F  L  E  L  G  M  G  A  S  L  C  L  S  Q  Q  F  K  A  Q  G  D  Y  I  L  G  G  L   40

TTTCCCCTGGGCTCAACCGAGGAGGCCACTCTCAACCAGAGAACACAACCCAACAGCATCCCGTGCAACAGGTTCTCACCCCTTGGTTTGTTCCTGGCCATGGCTATGAAGATGGCTGTG  240
 F  P  L  G  S  T  E  E  A  T  L  N  Q  R  T  Q  P  N  S  I  P  C  N  R  F  S  P  L  G  L  F  L  A  M  A  M  K  M  A  V   80

GAGGAGATCAACAATGGATCTGCCTTGCTCCCTGGGCTGCGGCTGGGCTATGACCTATTTGACACATGCTCCGAGCCAGTGGTCACCATGAAATCCAGTCTCATGTTCCTGGCCAAGGTG  360
 E  E  I  N  N  G  S  A  L  L  P  G  L  R  L  G  Y  D  L  F  D  T  C  S  E  P  V  V  T  M  K  S  S  L  M  F  L  A  K  V  120

GGCAGTCAAAGCATTGCTGCCTACTGCAACTACACACAGTACCAACCCCGTGTGCTGGCTGTCATCGGCCCCCACTCATCAGAGCTTGCCCTCATTACAGGCAAGTTCTTCAGCTTCTTC  480
 G  S  Q  S  I  A  A  Y  C  N  Y  T  Q  Y  Q  P  R  V  L  A  V  I  G  P  H  S  S  E  L  A  L  I  T  G  K  F  F  S  F  F  160

CTCATGCCACAGGTCAGCTATAGTGCCAGCATGGATCGGCTAAGTGACCGGGAAACGTTTCCATCCTTCTTCCGCACAGTGCCCAGTGACCGGGTGCAGCTGCAGGCAGTTGTGACTCTG  600
 L  M  P  Q  V  S  Y  S  A  S  M  D  R  L  S  D  R  E  T  F  P  S  F  F  R  T  V  P  S  D  R  V  Q  L  Q  A  V  V  T  L  200

TTGCAGAACTTCAGCTGGAACTGGGTGGCCGCCTTAGGGAGTGATGATGACTATGGCCGGGAAGGTCTGAGCATCTTTTCTAGTCTGGCCAATGCACGAGGTATCTGCATCGCACATGAG  720
 L  Q  N  F  S  W  N  W  V  A  A  L  G  S  D  D  D  Y  G  R  E  G  L  S  I  F  S  S  L  A  N  A  R  G  I  C  I  A  H  E  240

GGCCTGGTGCCACAACATGACACTAGTGGCCAACAGTTGGGCAAGGTGCTGGATGTACTACGCCAAGTGAACCAAAGTAAAGTACAAGTGGTGGTGCTGTTTGCCTCTGCCCGTGCTGTC  840
 G  L  V  P  Q  H  D  T  S  G  Q  Q  L  G  K  V  L  D  V  L  R  Q  V  N  Q  S  K  V  Q  V  V  V  L  F  A  S  A  R  A  V  280

TACTCCCTTTTTAGTTACAGCATCCATCATGGCCTCTCACCCAAGGTATGGGTGGCCAGTGAGTCTTGGCTGACATCTGACCTGGTCATGACACTTCCCAATATTGCCCGTGTGGGCACT  960
 Y  S  L  F  S  Y  S  I  H  H  G  L  S  P  K  V  W  V  A  S  E  S  W  L  T  S  D  L  V  M  T  L  P  N  I  A  R  V  G  T  320

GTGCTTGGGTTTTTGCAGCGGGGTGCCCTACTGCCTGAATTTTCCCATTATGTGGAGACTCACCTTGCCCTGGCCGCTGACCCAGCATTCTGTGCCTCACTGAATGCGGAGTTGGATCTG 1080
 V  L  G  F  L  Q  R  G  A  L  L  P  E  F  S  H  Y  V  E  T  H  L  A  L  A  A  D  P  A  F  C  A  S  L  N  A  E  L  D  L  360

GAGGAACATGTGATGGGGCAACGCTGTCCACGGTGTGACGACATCATGCTGCAGAACCTATCATCTGGGCTGTTGCAGAACCTATCAGCTGGGCAATTGCACCACCAAATATTTGCAACC 1200
 E  E  H  V  M  G  Q  R  C  P  R  C  D  D  I  M  L  Q  N  L  S  S  G  L  L  Q  N  L  S  A  G  Q  L  H  H  Q  I  F  A  T  400

TATGCAGCTGTGTACAGTGTGGCTCAAGCCCTTCACAACACCCTACAGTGCAATGTCTCACATTGCCACGTATCAGAACATGTTCTACCCTGGCAGCTCCTGGAGAACATGTACAATATG 1320
 Y  A  A  V  Y  S  V  A  Q  A  L  H  N  T  L  Q  C  N  V  S  H  C  H  V  S  E  H  V  L  P  W  Q  L  L  E  N  M  Y  N  M  440

AGTTTCCATGCTCGAGACTTGACACTACAGTTTGATGCTGAAGGGAATGTAGACATGGAATATGACCTGAAGATGTGGGTGTGGCAGAGCCCTACACCTGTATTACATACTGTGGGCACC 1440
 S  F  H  A  R  D  L  T  L  Q  F  D  A  E  G  N  V  D  M  E  Y  D  L  K  M  W  V  W  Q  S  P  T  P  V  L  H  T  V  G  T  480

TTCAACGGCACCCTTCAGCTGCAGCAGTCTAAAATGTACTGGCCAGGCAACCAGGTGCCAGTCTCCCAGTGTTCCCGCCAGTGCAAAGATGGCCAGGTTCGCCGAGTAAAGGGCTTTCAT 1560
 F  N  G  T  L  Q  L  Q  Q  S  K  M  Y  W  P  G  N  Q  V  P  V  S  Q  C  S  R  Q  C  K  D  G  Q  V  R  R  V  K  G  F  H  520

TCCTGCTGCTATGACTGCGTGGACTGCAAGGCGGGCAGCTACCGGAAGCATCCAGATGACTTCACCTGTACTCCATGTAACCAGGACCAGTGGTCCCCAGAGAAAAGCACAGCCTGCTTA 1680
 S  C  C  Y  D  C  V  D  C  K  A  G  S  Y  R  K  H  P  D  D  F  T  C  T  P  C  N  Q  D  Q  W  S  P  E  K  S  T  A  C  L  560

CCTCGCAGGCCCAAGTTTCTGGCTTGGGGGGAGCCAGTTGTGCTGTCACTCCTCCTGCTGCTTTGCCTGGTGCTGGGTCTAGCACTGGCTGCTCTGGGGCTCTCTGTCCACCACTGGGAC 1800
 P  R  R  P  K  F  L  A  W  G  E  P  V  V  L  S  L  L  L  L  L  C  L  V  L  G  L  A  L  A  A  L  G  L  S  V  H  H  W  D  600

AGCCCTCTTGTCCAGGCCTCAGGTGGCTCACAGTTCTGCTTTGGCCTGATCTGCCTAGGCCTCTTCTGCCTCAGTGTCCTTCTGTTCCCAGGGCGGCCAAGCTCTGCCAGCTGCCTTGCA 1920
 S  P  L  V  Q  A  S  G  G  S  Q  F  C  F  G  L  I  C  L  G  L  F  C  L  S  V  L  L  F  P  G  R  P  S  S  A  S  C  L  A  640

CAACAACCAATGGCTCACCTCCCTCTCACAGGCTGCCTGAGCACACTCTTCCTGCAAGCAGCTGAGACCTTTGTGGAGTCTGAGCTGCCACTGAGCTGGGCAAACTGGCTATGCAGCTAC 2040
 Q  Q  P  M  A  H  L  P  L  T  G  C  L  S  T  L  F  L  Q  A  A  E  T  F  V  E  S  E  L  P  L  S  W  A  N  W  L  C  S  Y  680

CTTCGGGGACTCTGGGCCTGGCTAGTGGTACTGTTGGCCACTTTTGTGGAGGCAGCACTATGTGCCTGGTATTTGATCGCTTTCCCACCAGAGGTGGTGACAGACTGGTCAGTGCTGCCC 2160
 L  R  G  L  W  A  W  L  V  V  L  L  A  T  F  V  E  A  A  L  C  A  W  Y  L  I  A  F  P  P  E  V  V  T  D  W  S  V  L  P  720

ACAGAGGTACTGGAGCACTGCCACGTGCGTTCCTGGGTCAGCCTGGGCTTGGTGCACATCACCAATGCAATGTTAGCTTTCCTCTGCTTTCTGGGCACTTTCCTGGTACAGAGCCAGCCT 2280
 T  E  V  L  E  H  C  H  V  R  S  W  V  S  L  G  L  V  H  I  T  N  A  M  L  A  F  L  C  F  L  G  T  F  L  V  Q  S  Q  P  760

GGCCGCTACAACCGTGCCCGTGGTCTCACCTTCGCCATGCTAGCTTATTTCATCACCTGGGTCTCTTTTGTGCCCCTCCTGGCCAATGTGCAGGTGGCCTACCAGCCAGCTGTGCAGATG 2400
 G  R  Y  N  R  A  R  G  L  T  F  A  M  L  A  Y  F  I  T  W  V  S  F  V  P  L  L  A  N  V  Q  V  A  Y  Q  P  A  V  Q  M  800

GGTGCTATCCTAGTCTGTGCCCTGGGCATCCTGGTCACCTTCCACCTGCCCAAGTGCTATGTGCTTCTTTGGCTGCCAAAGCTCAACACCCAGGAGTTCTTCCTGGGAAGGAATGCCAAG 2520
 G  A  I  L  V  C  A  L  G  I  L  V  T  F  H  L  P  K  C  Y  V  L  L  W  L  P  K  L  N  T  Q  E  F  F  L  G  R  N  A  K  840

AAAGCAGCAGATGAGAACAGTGGCGGTGGTGAGGCAGCTCAGGGACACAATGAATGACCACTGACCCGTGACCTTCCCCTTAGGGAACCTAGCCCTACCAGAAATCTCCTAAGCCAACAA 2640
 K  A  A  D  E  N  S  G  G  G  E  A  A  Q  G  H  N  E END                                                              858

GCCCCGAATAGTACCTCAGCCTGAGACGTGAGACACTTAACTATAGACTTGGACTCCACTGACCTTAGCCTCACAGTGACCCCTTCCCCAAACCCCCAAGGCCTGCAGTGCACAAGATGG 2760
ACCCTATGAGCCCACCTATCCTTTCAAAGCAAGATTATTCTCGATCCTATTATGCCCACCTAAGGCCTGCCCAGGTGACCCACAAAAGGTTCTTTGGGACTTCATAGCCATACTTTGAAT 2880
TCAGAAATTCCCCAGGCAGACCATGGGAGACCAGAAGGTACTGCTTGCCTGAACATGCCCAGCCCTGAGCCCTCACTCAGCACCCTGTCCAGGCGTCCCAGGAATAGAAGGCTGGGCATG 3000
TATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTACGTATGTATGTATGTATCAGGACAGAACAAGACTAGACATCAGGCAGCAGGACACTCAGGAGGTAGGC 3120
AACATCCAGCCTTCTCCATCCCTAGCTGAGCCCTAGCCTGTAGGAGAGAACCAGGTCGCCGCCAGCACCTTGGACAGATCACACACAGGGTGCGGGTCAGCACCACGGCCAGCGGCCAGC 3240
CACGCGGGACCCCTGGAATCAGCTTCTAGTACCAAGGACAGAAAAGTTGCCGCAAGGCCCCTTACTGGCCAGCACCAGGGACAGAGCCACATGCCTAAGCGGCAAGGGACAAGAGCATCG 3360
TCCATCGTCCATCTGCAGGCAGGATCAGACCCGGTCAGTTGTGGACTGGCCCCCACACCTGAATCCCGGAGCAGCTCAGCTGGAGAAAAGAGAAACAAGCCACACATCAGTCCCATAAAA 3480
TTAAACGCTTTTTTTAGTGTTAAAAAAAAAAAAAAAAAAAAA                                                                          3522
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FIG. 1. Nucleotide and deduced amino acid sequences of T1R3. The nucleotide sequence data of T1R3 cDNA is in DDBJ/EMBL/GenBank database with the Accession No. AB049994.

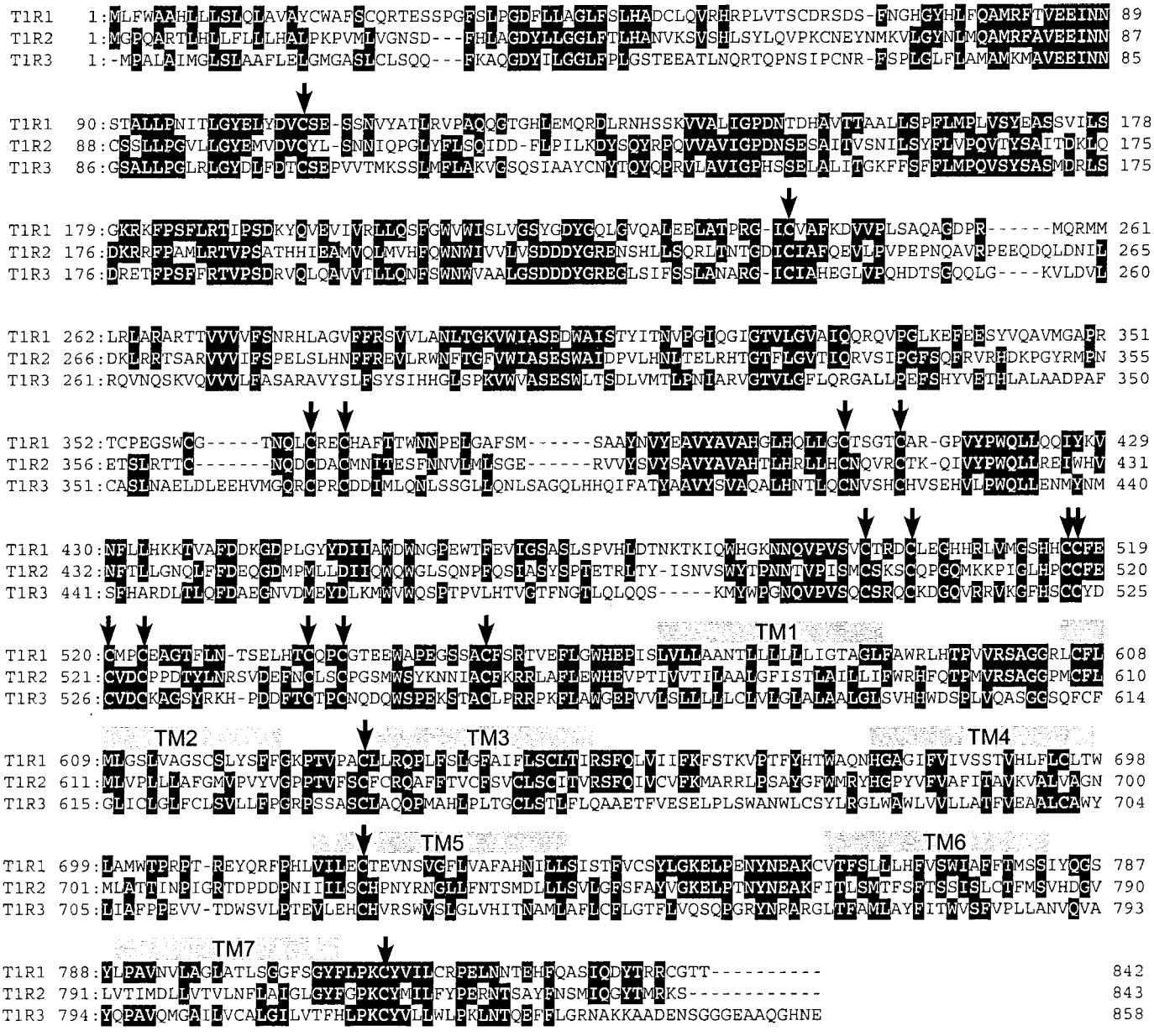

FIG. 2. Comparison of the amino acid sequence of mouse T1R1, T1R2, and T1R3. Multiple sequence alignments were carried out using the sequence analysis program CLUSTALW. Gaps introduced to maximize the homology are shown in hyphens. Residues shaded in black are identical in at least two members. Cysteine residues conserved among all members are indicated by arrows. Predicted transmembrane domains are indicated by shaded bars above the sequence.

genomic DNA (DDBJ/EMBL/GenBank Accession No. AB055708), we designed primers that span the exon-exon junction of T1R3 cDNA. As shown in Fig. 3, T1R3 was expressed in circumvallate papillae of the tongue, and also expressed in testis but not in the other tissues examined. The expression of sensory organ receptors in testis was observed in putative taste (19) and odorant receptors (20), although the function in testis is still unknown. If T1R3 functions as a taste receptor, it should be expressed in taste receptor cells. The expression pattern of T1R3 in circumvallate papillae was examined by *in situ* hybridization. As shown in Fig. 4, the expression of T1R3 was restricted to a subset of taste receptor cells, but was not detected in the other mesenchymal or epithelial cells. The sense probe did not show specific hybridization under the same conditions (data not shown). The restricted expression within taste buds was also observed for other taste receptors belonging to T1R (9) and T2R families (10), suggesting that taste receptor cells in a taste bud are separated into different groups according to function. It is also known that T1R1 is preferentially expressed

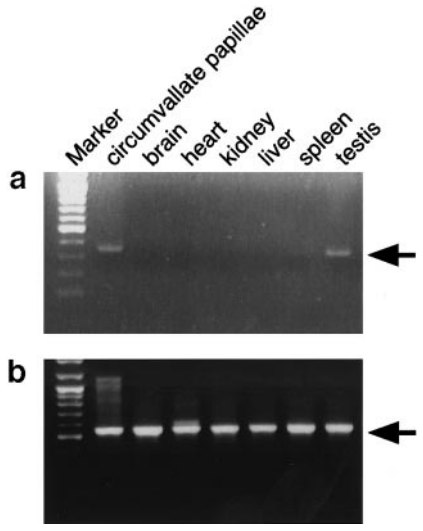

FIG. 3. Tissue distribution of mouse T1R3. Total RNA from various mouse tissues (indicated at the top) was reverse-transcribed using an oligo(dT) primer and PCR-amplified using primers specific for T1R3 (a) and for β-actin (b). The expected sizes of PCR products are indicated by arrows (a, 334 bp; b, 543 bp). Marker is 100-bp ladder.

in fungiform papillae but rarely in foliate and circumvallate papillae, and T1R2 is commonly expressed in foliate and circumvallate papillae but rarely in fungiform papillae, suggesting that the differential topographic expression of the two members in the tongue reflects topographic preferences of different tastants

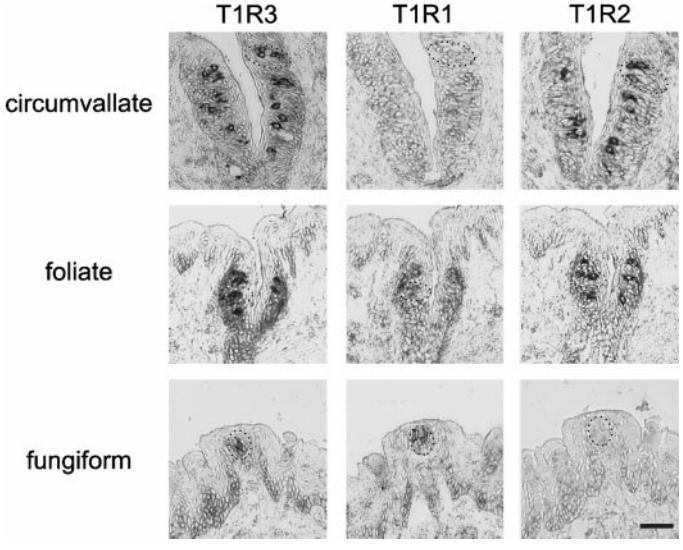

FIG. 4. *In situ* hybridization of T1R members in various taste papillae of tongue epithelium. Digoxigenin-labeled T1R3 (left column), T1R1 (center column), and T1R2 (right column) were hybridized to sections of C57BL/6NCrj mouse circumvallate (top row), foliate (middle row), and fungiform (bottom row) papillae. Digoxigenin epitope was visualized by BCIP/NBT. The dotted lines indicate the outline of a sample taste bud. Bar represents 50 μm.

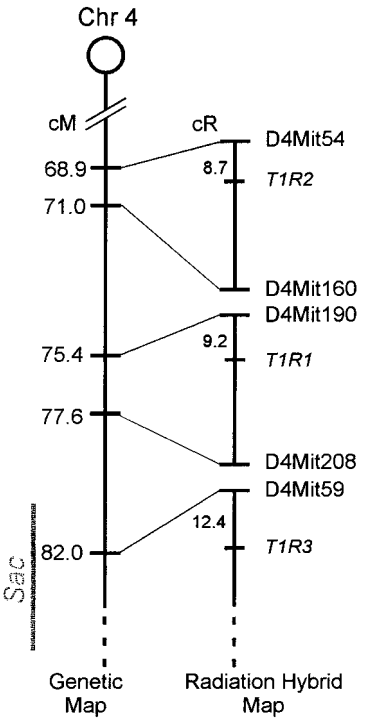

FIG. 5. Chromosomal locus mapping of T1R members. The position of T1R1, T1R2, and T1R3 and flanking markers with their respective map distance in centimorgans and centirays from Whitehead Institute/MIT Center and NCBI.

(9). To compare the topographic distribution of T1R3 with those of T1R1 and T1R2, we next examined *in situ* hybridization to sections of foliate and fungiform papillae. As a result, T1R3 was expressed in every taste bud of those papillae as strong as circumvallate papillae. Thus, the topographic distribution was clearly different from that of T1R1 and T1R2. The same results were obtained by using 3′ non-coding region of T1R3 as a probe (data not shown). However, it remains unclear whether the expression of T1R3 overlaps with those of the other members on the level of single cells.

Genetic studies in mice have determined a number of loci related to taste perception (21), which are useful to identify the genes involved in taste perception. Indeed, recent studies implicate T2R members as candidate receptors of bitter taste because the genes were mapped to loci that influence bitter perception in mice (10). To obtain a clue of the function of the T1R family, we determined the chromosomal location of T1R3 and other member genes using the mouse/hamster radiation hybrid panel (Fig. 5). The data placed T1R3 on mouse chromosome 4 between marker D4Mit59 (a site of 82 cM from the centromere; LOD score >3.0) and the distal end of the chromosome. It is well known that taste preference for sucrose and saccharin, a synthetic sweetener, differ in strains of mice and is attributed to one gene, *Sac*, located to 84.1 $\pm$ 3.4 cM on chromosome 4 (22). Although T1R1 and T1R2 were also placed on

chromosome 4, their physical map positions corresponded to locus at 68.9–71.0 cM (between D4Mit54 and D4Mit160) and 75.4–77.6 cM (between D4Mit190 and D4Mit208), respectively, and were distant from the locus of *Sac*. In addition, a recent report concluded that mutations observed in the T1R1 gene do not correlate with saccharin-sensitivity (23). As the locus of T1R3 was closely linked to the *Sac* locus, we next compared the expression pattern and sequence of T1R3 in saccharin taster (C57BL/6NCrj) and nontaster (BALB/cAnNCrj, DBA/2NCrj, 129/SVJ) strains. There was no difference in the expression pattern in each taste papilla and the ratio of T1R3-positive cells in a taste bud (data not shown). On the other hand, sequence analysis revealed that the nontaster strains carried five missense mutations (Thr55 to Ala, Ile60 to Thr, Phe61 to Leu, Arg371 to Glu, and Ile706 to Thr). As phylogenetic analyses of inbred strains of mouse indicate that C57BL/6 strain belongs to a subgroup evolutionarily distant from nontaster strains (24), it may be appropriate to consider that the mutations are specific for C57BL/6 strain. Four of the amino acid substitutions located in the extracellular region, and the region including the substitutions were corresponding to the ligand-binding region of mGluR1 (25), suggesting that the substitutions might affect the receptor-ligand interaction. *Sac* is also known to influence the afferent responses of gustatory nerves to sweeteners, suggesting that *Sac* determines peripheral sweet taste perception and thus may code for a sweet taste receptor (26). Taken together, our results suggest that T1R3 may serve as a mediator of the signaling pathway of sweet perception in mice. Although the functions of other T1R members are uncertain, it can be speculated that the mechanisms of ligand-mediated activation of the receptor might be common to all T1R members because of several conserved sequences scattered throughout the code. Thus, further experiments, such as the comparison of the expression pattern of T1R members with other gustatory signal transducers, are needed to clarify the downstream signaling components and to understand the role in taste perception.

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