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A Novel Family of Mammalian Taste Receptors

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Summary

In mammals, taste perception is a major mode of sensory input. We have identified a novel family of 40–80 human and rodent G protein–coupled receptors expressed in subsets of taste receptor cells of the tongue and palate epithelia. These candidate taste receptors (T2Rs) are organized in the genome in clusters and are genetically linked to loci that influence bitter perception in mice and humans. Notably, a single taste receptor cell expresses a large repertoire of T2Rs, suggesting that each cell may be capable of recognizing multiple tastants. T2Rs are exclusively expressed in taste receptor cells that contain the G protein α subunit gustducin, implying that they function as gustducin-linked receptors. In the accompanying paper, we demonstrate that T2Rs couple to gustducin in vitro, and respond to bitter tastants in a functional expression assay.

Introduction

Mammals taste many compounds but are believed to distinguish between only five basic taste modalities: sweet, bitter, sour, salty, and umami (the taste of monosodium glutamate). Although the discriminatory power of taste appears modest, it provides animals with valuable sensory information for the evaluation of food. The sense of taste evokes responses that range from innate behavioral actions such as aversion and attraction to food sources, to the pleasure of food consumption.

Mammalian taste receptor cells are clustered in taste buds, which are distributed on the surface of the tongue and palate. Each taste modality is thought to be mediated by distinct transduction pathways expressed in subsets of receptor cells (Kinnamon and Cummings, 1992; Lindemann, 1996; Stewart et al., 1997). Electrophysiological studies suggest that sour and salty tastants modulate taste receptor cell function by direct effects on specialized membrane channels (Heck et al., 1984; Brand et al., 1985; Avenet and Lindemann, 1988; Kinnamon et al., 1988; Gilbertson et al., 1992). In contrast, sweet, bitter, and umami taste transduction are believed to be mediated by G protein–coupled receptor (GPCR) signaling pathways (Striem et al., 1989; Wong et al., 1996; Chaudhari et al., 2000). These cell surface receptors interact with tastants and initiate signaling cascades that culminate in neurotransmitter release. Afferent nerve fibers from cranial nerve ganglia then relay the signals via the thalamus to cortical taste centers, where information is processed and integrated.

How does the brain interpret chemosensory information? Some of the most valuable insights into chemosensory coding have been derived from studies of olfactory reception in mice, worms, and flies. In mammals, individual olfactory neurons express only 1 of ~1000 different olfactory receptors, and all neurons expressing a common receptor project to the same set of glomeruli (reviewed by Mombaerts et al., 1996). Interestingly, a single olfactory receptor recognizes multiple odorants, and an odorant is recognized by multiple receptors (Malnic et al., 1999). Thus, mammals utilize combinatorial codes of glomeruli activation to respond to a wide diversity of odorants, and do so with exquisite discriminatory power (see for example Rubin and Katz, 1999). Worms also have hundreds of different receptors, but have only a few chemosensory neurons each expressing a large repertoire of receptor molecules (Troemel et al., 1995). Therefore, the system preserves the ability to respond to a wide diversity of odorants, but sacrifices discriminatory power. This simpler coding paradigm makes sense in an organism that needs to respond differentially to attractive and repulsive stimuli, but not between signals within each of these two modalities.

In contrast to the olfactory system, our understanding of taste coding and information processing is very limited, even at the basic cellular level. For example, it is not known whether individual taste receptor cells are tuned to specific or to many stimuli, or whether functionally similar cells are innervated by common fibers. Furthermore, while it is well established that taste buds from the different papillae in the tongue and palate epithelium exhibit specific taste sensitivities (Frank et al., 1983; Nejad, 1986; Frank, 1991), we do not understand how such differences are encoded in the organization and composition of the various taste buds (see for example Hoon et al., 1999).

We have been interested in basic questions of taste signal detection and information coding, and have focused primarily on sweet and bitter transduction. What are the receptors for sweet and bitter pathways? How is tastant specificity and taste discrimination accomplished? What is the topographic organization of sweet and bitter responding cells in the various taste buds and papillae? And, how is the information transmitted and encoded in the afferent nerves (i.e., are there specifically tuned lines)? Answering these questions would be aided by the isolation of genes involved in taste signaling, ideally taste receptors, that can be used to mark the cells, define the corresponding signaling pathways and

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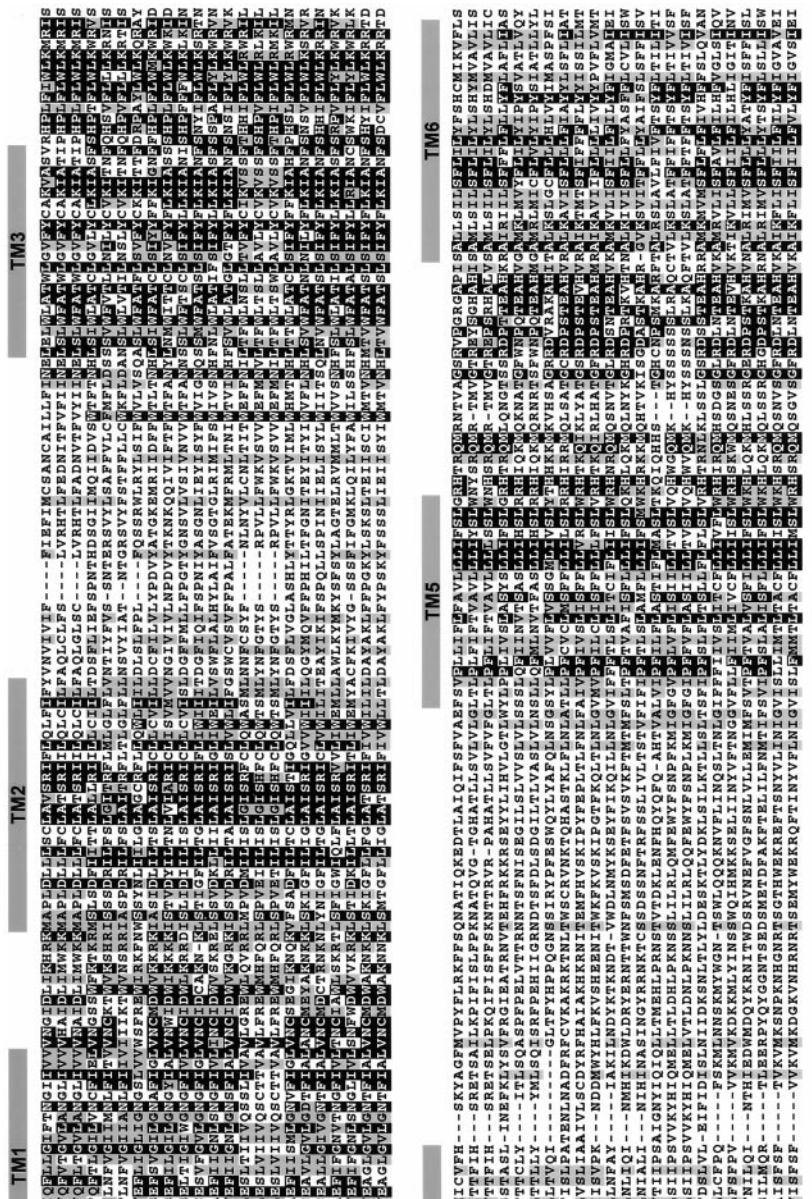

Figure 1. T2Rs Define a Novel GPCR Gene Family

Predicted amino acid sequences of representative human, rat, and mouse T2R genes (h, r, and mT2Rs) were aligned using ClustalW. Residues shaded in black are identical in at least half of the aligned sequences; conservative substitutions are highlighted in gray. Predicted transmembrane segments are indicated by bars above the sequence.

receptor specificity, generate topographic maps, and trace the respective neuronal connectivity circuits.

Recently, we isolated two novel GPCRs, initially called TR1 and TR2 (Hoon et al., 1999) and now referred to as T1R1 and T1R2, that are expressed in distinct subsets of taste receptor cells. While these may be receptors for sweet, bitter, or umami tastants, we reasoned that two receptors are too few to sample the chemically

diverse universe of sweet and bitter substances. In this and the accompanying paper (Chandrashekar et al., 2000 [this issue of *Cell*]), we report the isolation and characterization of a novel family of human and rodent taste receptors.

Results and Discussion

Identification of Novel Candidate Taste Receptors

To date, there are just a few G protein–coupled signaling molecules that have been implicated in taste transduction. T1R1 and T1R2 are putative taste receptors expressed in subsets of taste receptor cells of the tongue and palate epithelia (Hoon et al., 1999). In situ hybridization experiments showed that T1Rs are expressed in ~30% of the cells in the various taste buds. Gustducin is a G protein α subunit that is also found in a similar fraction of taste receptor cells of all taste buds (McLaughlin et al., 1992). Yet, for the most part, T1Rs are not coexpressed with gustducin, implying that there is an additional set of G protein–coupled receptors that must be expressed in gustducin-positive cells (Hoon et al., 1999). Genetic and biochemical evidence suggests that gustducin mediates some bitter responses (Wong et al., 1996; Ming et al., 1998). Thus, to try to identify receptors expressed in gustducin-positive cells, we searched for GPCRs in genomic intervals linked to bitter taste perception.

Recent genetic linkage studies in humans identified a locus at 5p15 that is associated with the ability to respond to the bitter substance 6-n-propyl-2-thiouracil (PROP; Reed et al., 1999). We reasoned that differences in PROP sensitivity may reflect functional differences in a bitter taste receptor, and searched DNA sequence databases for genes encoding candidate transmembrane proteins at this location. Analysis of open reading frames in 450 kb of DNA spanning six sequenced human genomic BAC clones from this interval identified a novel GPCR (T2R-1) at 5p15.2. T2R-1 has seven putative transmembrane segments as well as several conserved residues often present in GPCRs (Probst et al., 1992; Figure 1). Computer searches using T2R-1, and reiterated with T2R-1-related sequences, revealed 19 additional human receptors (12 full-length and 7 pseudogenes; see Figure 1). These novel receptors, referred to as T2Rs, define a novel family of GPCRs distantly related to V1R vomeronasal receptors (Dulac and Axel, 1995) and opsins (Figures 1 and 2). In contrast to T1Rs, which belong to the superfamily of GPCRs characterized by a large N-terminal domain (Hoon et al., 1999), the T2Rs have only a short extracellular N terminus. Individual members of the T2R family exhibit 30%–70% amino acid identity, and most share highly conserved sequence motifs in the first three and last transmembrane segments, and also in the second cytoplasmic loop (Figure 1, shaded boxes). The most divergent regions between T2Rs are the extracellular segments, extending partway into the transmembrane helices. We presume that the high degree of variability between T2Rs reflects the need to recognize many structurally diverse ligands. Like many other GPCR genes, T2Rs do not contain introns that interrupt coding regions.

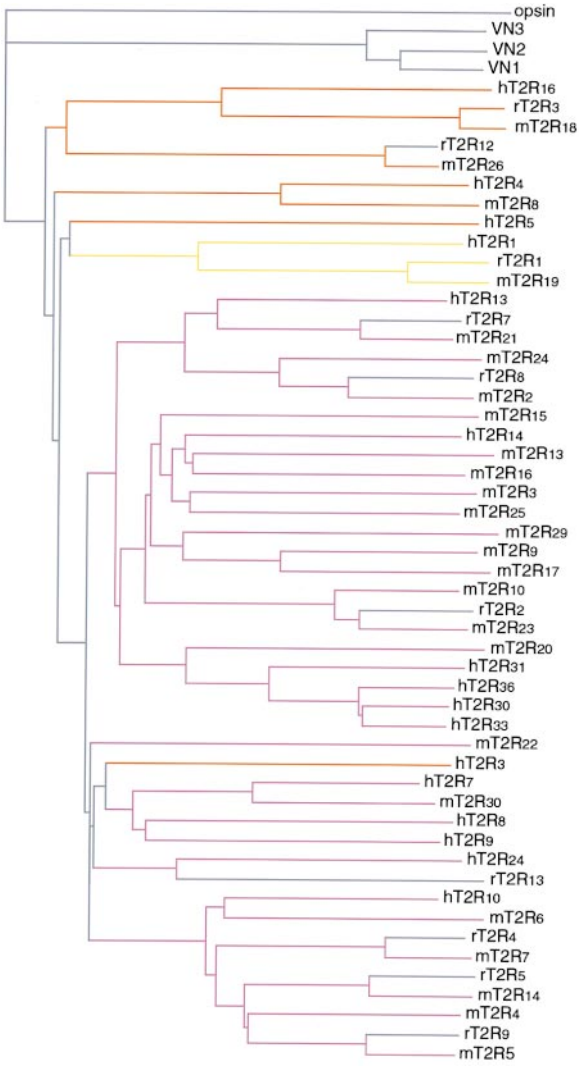

Figure 2. T2Rs Are a Structurally Diverse Family of Receptors Distantly Related to V1R Pheromone Receptors and Opsins

Sequence relationships between full-length human, mouse, and rat T2Rs, opsin, and V1R vomeronasal receptors (VN1–3) are displayed as a cladogram. The roots linking T2Rs are color-coded according to the chromosomal location of the various genes (see Figure 3). The identity between potentially orthologous rat and mouse T2Rs ranges from 74% for mT2R-2/rT2R-8 to 92% for mT2R-18/rT2R-3; identities between the three potentially orthologous pairs of human and mouse T2Rs are 67% for hT2R-4/mT2R-8, 51% for hT2R-16/mT2R-18, and 46% for hT2R-1/mT2R-19.

Organization of Human T2R Genes

The identified human T2R genes are localized on three chromosomes and are often organized as head-to-tail arrays (Figure 3). For example, four receptor genes are clustered within a single PAC clone from 7q31 and nine in a BAC clone from 12p13. There may be more T2Rs in these arrays, for example, several additional T2Rs are contained within partially sequenced BAC clones that overlap the 9 gene T2R cluster. Within an array, the similarity of receptors is highly variable, including both relatively related (e.g., T2R-13, T2R-14, and T2R-15), and highly divergent receptors (e.g., T2R-3 and T2R-4; Figures 2 and 3). This type of organization is mirrored

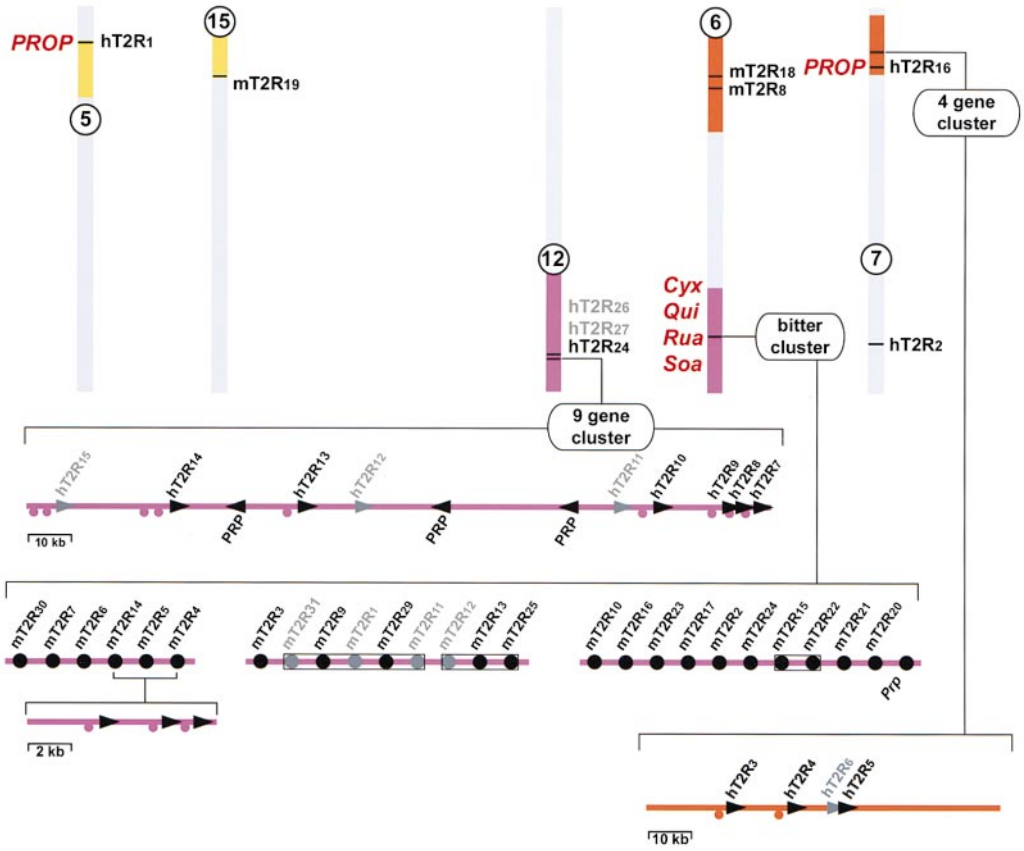

Figure 3. T2R Genes Map to Loci that Influence Bitter Taste

Schematic representation of human (chromosomes 5, 12, and 7) and mouse (15 and 6) chromosomes with homologous intervals color-coded; loci implicated in bitter perception are labeled red; T2R pseudogenes are gray. Also shown are expansions of the human 9 T2R gene cluster (accession number AC006518), human 4 T2R gene cluster (accession number AC004979), and three BAC contigs from the mouse chromosome 6 bitter cluster showing the order of some of the mT2R genes. Arrowheads indicate the direction of transcription. *PRP* refers to salivary proline-rich-protein genes (accession numbers M13058, K03202, and S79048). Offset colored dots represent a quasi-palindromic 18 bp sequence (e.g., ATTTGCATGGTTGCAAAT for hT2R-13) found in the 5′ upstream sequences of most T2R coding sequences. In general, this sequence is found 150–600 nt upstream of the putative initiator methionine. The mouse BAC contigs and mT2R genes within boxes are unordered and the relative orientation of the mT2R-4, -5, -14 cluster within the BAC contig is unknown.

in the mouse (see below) and resembles the genomic organization that has been observed for olfactory receptor genes in humans, mice, flies, and worms (Troemel et al., 1995; Sullivan et al., 1996; Rouquier et al., 1998; Clyne et al., 1999; Vosshall et al., 1999).

To get estimates of the size of this gene family, we examined various genomic resources. Analysis of the Genome Sequence Survey database (gss) yielded 12 partial T2R sequences. Because this database represents an essentially random sampling of ~14% of the human genome, there may be ~90 T2R genes in the human genome. Similar searches of the finished (nr) and unfinished high-throughput human genomic sequence databases (htgs) produced 36 full-length and 15 partial T2R sequences. These databases contain ~50% of the genome sequence, also pointing to ~100 T2R genes in the genome. Recognizing that this analysis may be inaccurate due to the quality of the available databases, and the clustered, nonrandom distribution of T2Rs in the human genome, we estimate that the T2R family consists of between 80 and 120 members. However, more than 1/3 of the full-length human T2Rs are pseudogenes; thus, the final number of functional human receptors may be significantly smaller (i.e., 40–80). This is similar to what has been observed for human olfactory receptors, where many of the genes appear to be pseudogenes (Rouquier et al., 1998).

T2R Genes Are Linked to Loci Involved in Bitter Taste

The genetics of sweet and bitter tasting has been extensively studied in mice, where a number of loci influencing responses to sweet and bitter tastants have been mapped by behavioral taste-choice assays (Warren and Lewis, 1970; Fuller, 1974). The distal end of mouse chromosome 6 contains a cluster of bitter genes that includes *Soa* (for sucrose octaacetate; Capeless et al., 1992), *Rua* (raffinose undecaacetate; Lush, 1986), *Cyx* (cycloheximide; Lush and Holland, 1988), and *Qui* (quinine; Lush, 1984). Recombination studies indicated that these four loci are closely linked to each other, and to *Prp* (salivary proline rich protein; Figure 3; Azen et al., 1986). Notably, the human 9 gene T2R cluster contains three interspersed *PRP* genes, and maps to an interval that is homologous with the mouse chromosome 6 bitter

cluster, thus raising the possibility that T2Rs are bitter taste receptors.

To define the relationship between the mouse chromosome 6 bitter cluster and T2Rs, we isolated a large number of mouse T2R genes and examined their genomic organization and physical and genetic map locations. We used human T2Rs to screen mouse genomic libraries and isolated 61 BAC clones containing 28 mouse T2Rs. The mouse and human receptors display significant amino acid sequence divergence (see Figures 1 and 2), but share the sequence motifs common to members of this novel family of receptors. Radiation hybrid and recombinant inbred strain mapping studies showed that these mouse genes are clustered at only a few genomic locations (Figure 3). Remarkably, each genomic interval containing mouse T2Rs is homologous to one containing its closest human counterpart: mT2R-8 and hT2R-4, mT2R-18 and hT2R-16, and mT2R-19 and hT2R-1. Of these three sets of potentially orthologous pairs of human/mouse receptors, both the human *T2R-1* and *T2R-16* genes map to locations implicated in human bitter perception (Conneally et al., 1976; Reed et al., 1999). The remaining 25 mT2Rs all map to the distal end of chromosome 6 and are represented by three BAC contigs spanning at least 400 kb.

Since *Prp* and the bitter-cluster also map to the distal end of mouse chromosome 6, we examined whether they localize within this array of T2Rs. Analysis of a DBA/2 × C57BL/6 recombinant inbred panel revealed that receptors within all three BAC contigs cosegregate with *Prp* and the bitter cluster (data not shown). We also isolated the mouse *Prp* gene (accession number M23236, containing *D6Mit13*) and showed that it lies within the large chromosome 6 T2R cluster (Figure 3). These results demonstrate that T2Rs are intimately linked to loci implicated in bitter perception, and substantiate the postulate that T2Rs may function as taste receptors.

T2Rs Are Expressed in Taste Receptor Cells

If T2Rs function as taste receptors, they should be expressed in taste receptor cells. The lingual epithelium contains taste buds in three types of papillae: circumvallate papillae at the very back of the tongue, foliate papillae at the posterior lateral edge of the tongue, and fungiform papillae dispersed throughout the front half of the tongue surface. Other parts of the oral cavity also have taste buds; these are particularly prominent in the palate epithelium in an area known as the geschmackstreifen and in the epiglottis (Figure 4). To examine the patterns of expression of T2Rs, we performed in situ hybridizations to sections of various taste papillae. To ensure that we tested probes expressed in taste tissue, we screened a rat circumvallate cDNA library. We isolated 14 rat T2Rs cDNAs, each of which is an ortholog of a mouse genomic clone (Figure 2).

T2Rs are selectively expressed in subsets of taste receptor cells of the tongue and palate epithelium. Figures 5a–5e show representative sections of rat circumvallate papilla taste buds hybridized with antisense cRNA probes to five different T2Rs. Each receptor hybridizes to an average of two cells per taste bud per section. Since our sections contain 1/5–1/3 the depth of a taste bud, this reflects a total of 6–10 positive cells/taste bud/probe (or about 15% of the cells in a taste bud). Examination of serial sections demonstrated that all of the taste buds of the circumvallate papilla contain cells that are positive for each of these probes. Thus far, we have observed comparable results with 11 rat T2Rs, and in mouse sections hybridized with 17 different mT2R probes (data not shown).

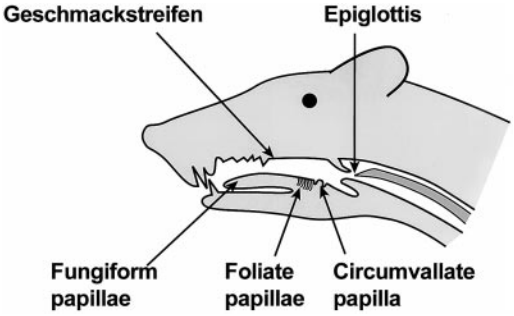

Figure 4. Functional Anatomy of the Rodent Oral Cavity

The diagram shows a drawing of a rodent head highlighting regions containing taste buds. We thank E. A. for modeling for this diagram.

Similar studies in foliate, geschmackstreifen, and epiglottis taste buds demonstrated that each receptor probe also labels ~15% of the cells in every taste bud (Figures 5f–5h). In contrast, T2Rs are rarely expressed in fungiform papillae. We examined hundreds of fungiform taste buds using 11 different T2R probes and found that less than 10% of all fungiform papillae contain T2R-expressing cells. Interestingly, the few fungiform taste buds that do express T2Rs regularly contain multiple positive cells (see Figure 5i). In fact, the number of positive cells in these papillae is not significantly different from that seen in taste buds from other regions of the oral cavity. Furthermore, fungiform papillae that contain T2R-expressing cells generally appear clustered. This unexpected finding may provide an important clue about the logic of taste coding. It is known that single fibers of the chorda tympani nerve innervate multiple cells in a fungiform taste bud, and that the same fiber often projects to neighboring papillae (Miller, 1974). Perhaps the nonrandom distribution of T2R-positive taste receptor cells and taste buds in fungiform papillae reflect a map of connectivity between similar cells.

Individual Receptor Cells Express Multiple T2R Receptors

We have shown that any one T2R is expressed in ~15% of the cells of circumvallate, foliate, and palate taste buds. Given that there are over 30 T2Rs in the rodent genome, a taste cell must express more than one receptor. But how many receptors are expressed in any cell and what fraction of taste receptor cells express T2Rs? We compared the number of circumvallate cells labeled with various mixes of 2, 5, or 10 receptors with those labeled with the corresponding individual probes (Figure 6). By counting positive cells in multiple serial sections, we determined that the number of taste cells labeled with the mixed probes (~20%) was only slightly larger than that labeled by any individual receptor (~15%;

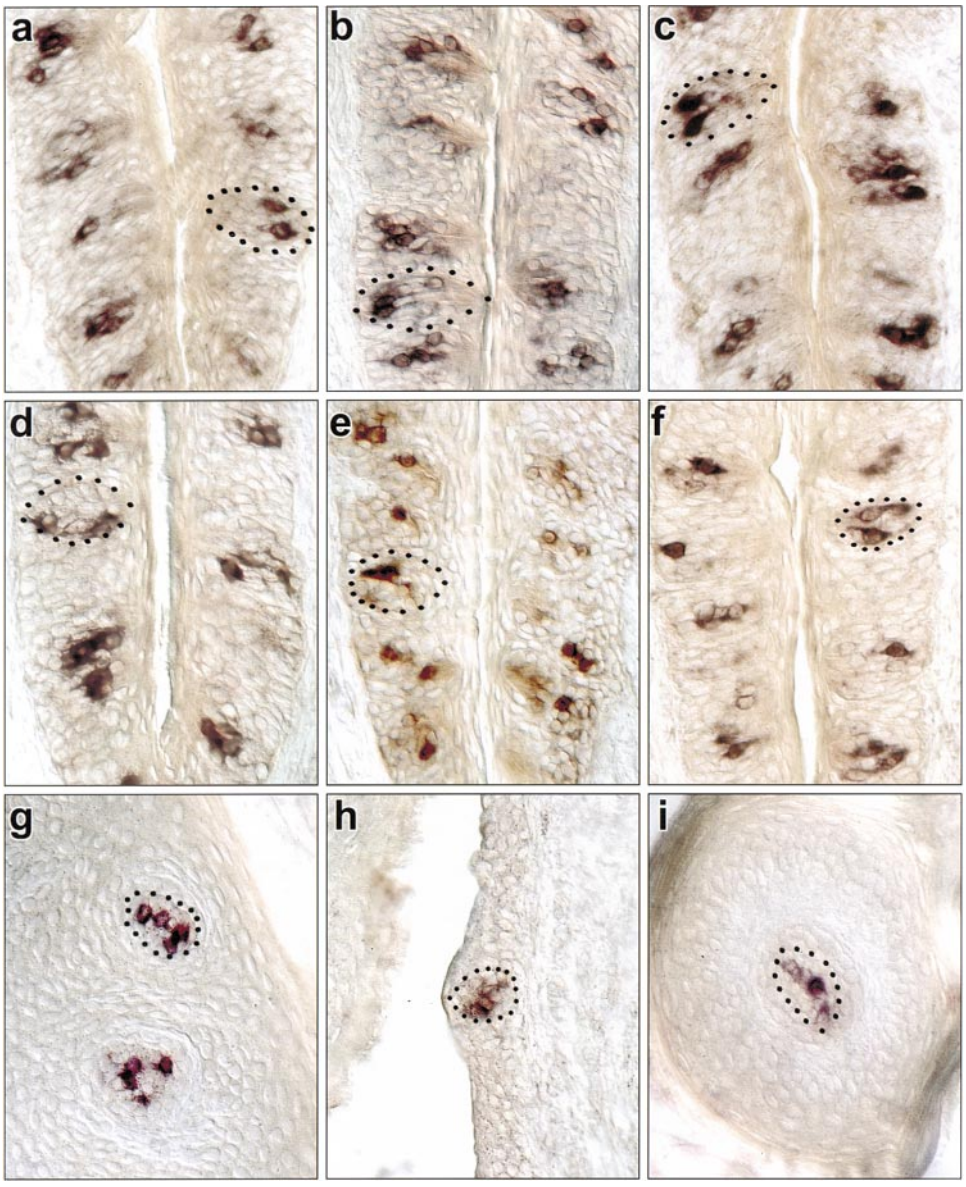

Figure 5. Expression of T2Rs in Subsets of Taste Receptor Cells

In situ hybridizations with T2R digoxigenin-labeled antisense RNA probes demonstrated that members of this family of receptors are expressed in subsets of taste receptor cells. All rat circumvallate taste buds contain cells expressing T2Rs: (a) rT2R-7, (b) rT2R-8, (c) rT2R-3, (d) rT2R-2, (e) rT2R-4. T2Rs are also expressed in all taste buds in the foliate papillae, geschmackstreifen and epiglottis: (f) foliate with rT2R-7, (g) geschmackstreifen with rT2R-3, and (h) epiglottis with rT2R-7. In contrast, less than 10% of all fungiform papillae contain T2R-expressing cells. However, the few fungiform taste buds that express T2Rs regularly contain multiple positive cells (i) (distribution of T2R-positive cells in sections of 400 fungiform taste buds: 3.5% one labeled cell, 5.25% two labeled cells, and 0.75% three or more labeled cells per section). The dotted lines indicate the outline of a sample taste bud.

compare Figures 5 and 6). Not surprisingly, the signal intensity was significantly enhanced in the mixed probe hybridizations. Similar results were observed in taste buds from other regions of the oral cavity including the fungiform papillae. To directly demonstrate coexpression we performed two-color double-label in situ hybridization experiments using a collection of differentially labeled cRNA probes. As expected, the majority of cells expressed multiple receptors (Figure 6d).

Our data on the expression patterns of T2Rs provide important insight into the organization of the taste system. First, as initially demonstrated with the T1Rs, there are marked topographic differences in the expression patterns of candidate signaling molecules in the various taste buds and papillae. Second, the complexity of the receptor repertoire is significantly larger than previously thought. Third, each cell expresses multiple receptors. Moreover, the demonstration that different mixtures of 2 or 5 probes detected as many positive cells as the mix of 10 suggests that each positive cell expresses nearly the full complement of T2Rs. If we assume that each receptor signals via the same pathway, and that the patterns of receptor expression delineate the logic of taste coding, these results indicate that there would

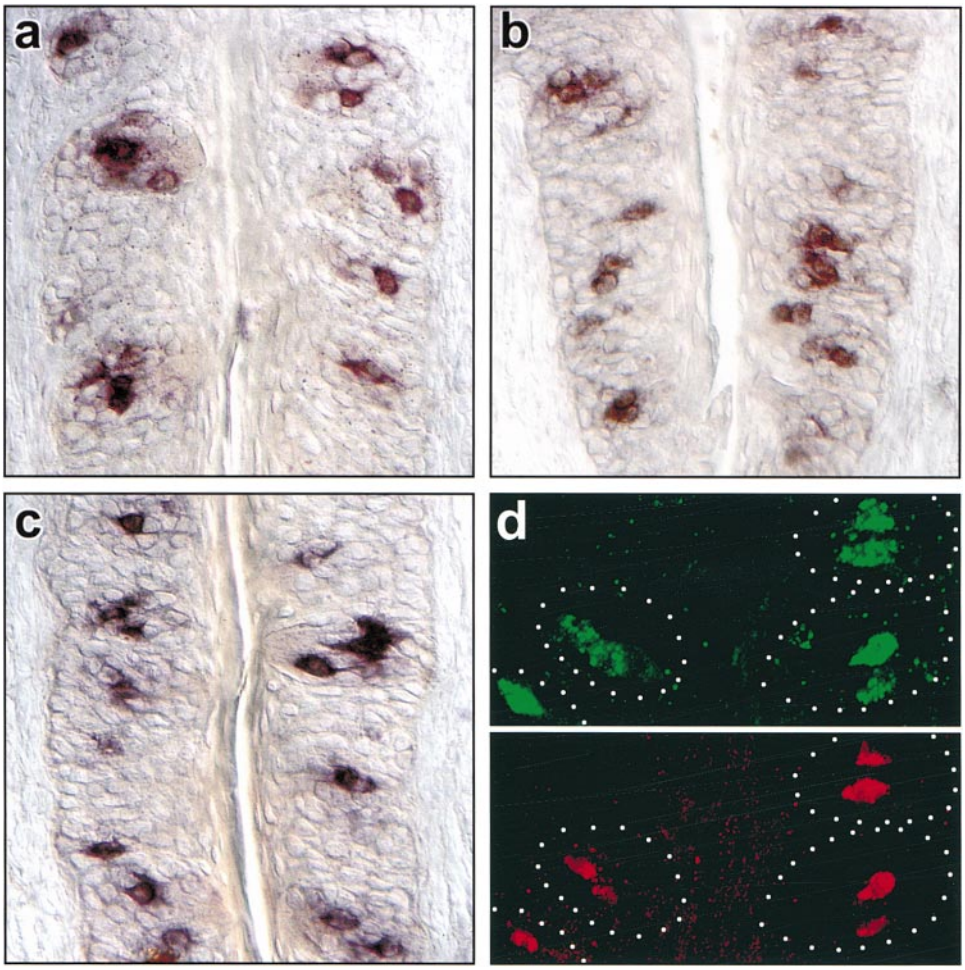

Figure 6. Many T2Rs Are Coexpressed in the Same Taste Receptor Cell

Mixtures of (a) 2, (b) 5, or (c) 10 T2R probes hybridized to only very few more cells than detected by any individual probe in circumvallate taste buds (see Figure 5 and Experimental Procedures for list of probes). Similar results were obtained in taste buds from other regions of the oral cavity. Double-label fluorescent in situ hybridizations (d) directly demonstrated coexpression of T2R-3 (green) and T2R-7 (red) in the same taste receptor cells; other mixtures of receptors produced equivalent results. The dotted lines outline the approximate area of sectioned taste buds.

be limited functional discrimination between T2R-positive cells.

T2R Genes Are Selectively Expressed in Gustducin-Expressing Cells

Previously, we have shown that T1Rs are expressed in ~30% of taste receptor cells. In situ hybridizations with differentially labeled T1R and T2R probes showed that there is no overlap in the expression of these two classes of receptors (Figure 7d). Gustducin is also expressed in a large subset of taste receptor cells, but for the most part is not coexpressed with T1Rs (Hoon et al., 1999). To determine if T2Rs are expressed in gustducin cells, we performed in situ hybridizations using differentially labeled T2Rs and gustducin riboprobes. Figure 7 demonstrates that T2Rs are exclusively expressed in gustducin-positive cells of the tongue and palate taste buds.

Do all gustducin-positive cells express T2Rs? Approximately 1/3 of the gustducin cells in the circumvallate, foliate, and palate taste buds did not label with a mix of 10 T2R probes (Figure 7). These cells may express other, perhaps more distantly related receptors, or could be at a different developmental stage. In fungiform taste buds the situation is quite different. Since only 10% of fungiform taste buds contain T2R-positive cells, the great majority of gustducin-positive cells in the front of the tongue do not coexpress members of the T2R family of receptors. While it is formally possible that fungiform receptor cells express T2Rs at levels below our limits of detection, we do not believe this to be the case. First, even when we used mixed probes and extended developing times, we did not detect additional positive cells. Second, PCR amplification reactions using T2R-specific primers on fungiform taste buds did not reveal a population of rarely expressed T2Rs. Third, the few fungiform taste buds that express T2Rs are positive for the full repertoire of probes, suggesting that all receptors are also expressed in the front of the tongue, but in a much smaller subset of taste buds. Therefore, there is likely to be an additional set of receptors expressed

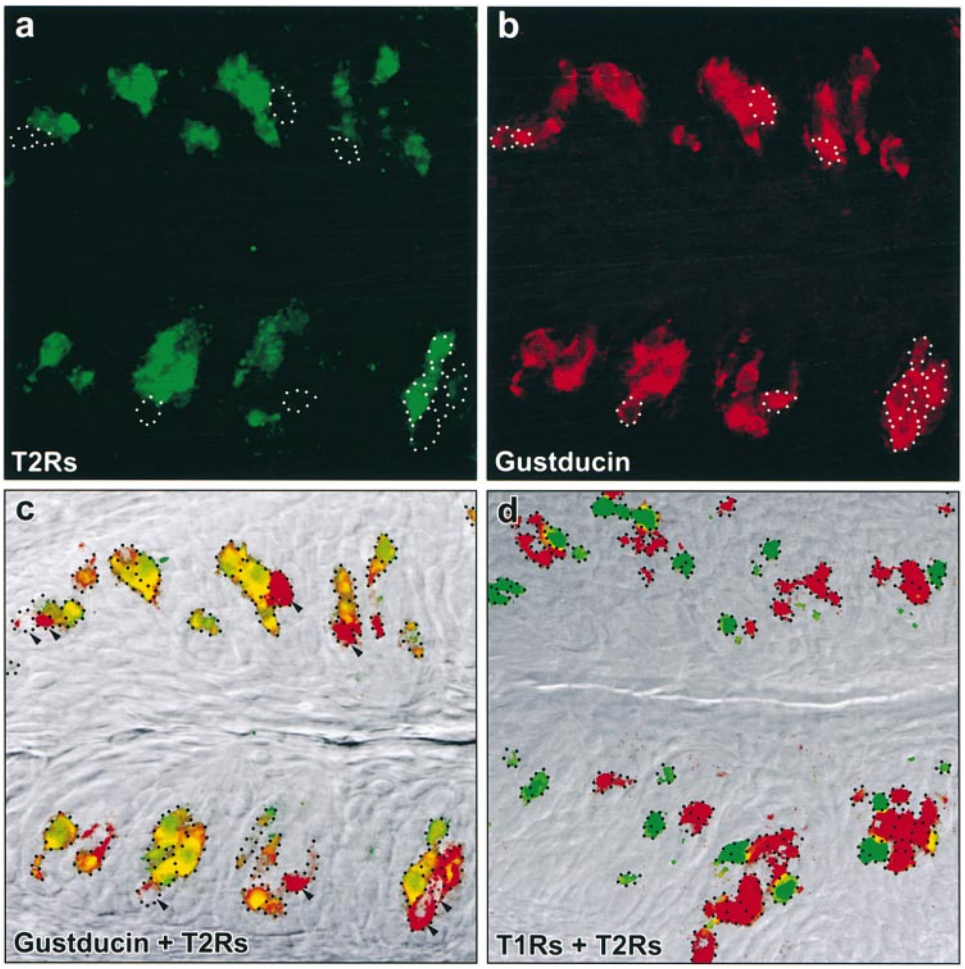

Figure 7. T2Rs Are Expressed in Taste Receptor Cells that Contain Gustducin

Double-label fluorescent in situ hybridizations were used to examine the expression of T2Rs with gustducin and T1Rs. (a) T2Rs are expressed in the same cells as (b) gustducin, as shown in (c) where the two-channel fluorescent image (1 μm optical section of a rat cicumvallate papilla) is overlaid on a difference interference contrast image. The dotted lines outline the approximate area of labeled taste receptor cells; arrows indicate gustducin-expressing cells that do not contain T2Rs. In contrast, (d) shows that T1Rs (red) are expressed in different subset of taste receptor cells from T2Rs (green).

in the gustducin-positive cells of fungiform papillae (see concluding remarks). Interestingly, gustducin is expressed outside taste receptor cells in isolated cells in the gastrointestinal tract (Hofer et al., 1996; Hofer and Drenckhahn, 1998), trachea, pharynx, nasal respiratory epithelium, ducts of salivary glands, and vomeronasal organ (data not shown). Some of these cells also express a small subset T2Rs, further supporting the idea that T2Rs are gustducin-linked receptors (data not shown). It will be interesting to determine whether these cells play a role in chemoreception.

It has been proposed that gustducin is involved in bitter and sweet transduction since gustducin knockout mice show decreased sensitivity to some sweet and bitter tastants (Wong et al., 1996). In addition, gustducin can be activated in vitro by stimulating taste membranes with bitter compounds, likely through the activation of bitter receptors (Ming et al., 1998). While our studies do not directly address the function of gustducin, the demonstration that T2Rs are expressed selectively in gustducin-positive cells is consistent with the proposal that they function as gustducin-linked taste receptors (see Chandrashekar et al., 2000).

Concluding Remarks

In this paper, we describe the identification of a novel family of GPCRs, T2Rs, selectively expressed in taste receptor cells of the tongue and palate epithelium. T2R receptors map to loci that have been reported to influence bitter taste perception in humans and mice, suggesting they function as bitter receptors. Numerous genetic and psychophysical studies point to different receptors for various types of bitter compounds (McBurney et al., 1972; Lush and Holland, 1988). However, perception of bitter compounds appears uniform to a human subject. Our finding that each taste receptor cell expresses a large number of T2Rs is consistent with the observation that mammals are capable of recognizing a wide range of bitter substances, but not distinguishing between them. In contrast, the distinct expression patterns of T1Rs and T2Rs suggest that these receptor families may encode different modalities.

How might coexpression of T2Rs be controlled? One possibility is that the clustering of genes allows coordinate regulation by the use of shared regulatory sequences. Another is that these genes have separate, but similar controlling elements. Notably, we found a common sequence motif (see Figure 3) present upstream of the initiator methionine in the majority of the human T2R genes. It should be possible to genetically manipulate this sequence and define its impact on the expression of T2Rs. This could be best accomplished in the mT2R-4, mT2R-5, and mT2R-14 cluster, where all three genes are organized as a head-to-tail array within a single 6 kb DNA fragment (Figure 3).

T2Rs are expressed exclusively in gustducin-positive cells, suggesting that these are gustducin-linked receptors. However, not all gustducin-positive cells express T2Rs, consistent with the proposal of multiple functions for gustducin (Wong et al., 1996). This is best illustrated in fungiform papillae, where only a minor fraction of gustducin-positive cells express T2Rs. In the accompanying paper (Chandrashekar et al., 2000), we directly demonstrate that T2Rs couple to gustducin and function as bitter receptors. The identification of candidate bitter taste receptors opens novel avenues into our understanding of taste biology, and provides a rational strategy for isolating bitter antagonists and modulating mammalian taste perception.

Experimental Procedures

Molecular Cloning of Taste Receptors

Human T2R-1 was discovered as an open reading frame with limited homology to GPCRs in a BAC (accession number AC003015) from 5p15.2. Additional T2Rs (numbered in order of discovery) were identified by reiterated sequence searches of DNA sequence databases. Full-length hT2Rs were isolated by PCR amplification of genomic DNA, and used to probe a rat circumvallate cDNA library (Hoon et al., 1999) and mouse BAC filter arrays (Genome Systems) at low stringency (50°C–55°C wash in 1× SSC). Southern hybridization experiments were used to identify a nonredundant set of positive BACs and to order overlapping BACs. Mouse T2Rs were mapped using a mouse/hamster radiation hybrid panel (Research Genetics) and by examining the strain distribution pattern of single nucleotide polymorphisms in a panel of C57BL/6J × DBA/2J recombinant inbred lines (Jackson Laboratory).

In Situ Hybridization

Tissue was obtained from adult rats and mice. No sex-specific differences of expression patterns were observed, therefore male and female animals were used interchangeably. For foliate sections, no differences in expression pattern were observed between the papillae. Fresh frozen sections (16 μm/section) were attached to silanized slides and prepared for in situ hybridization as described previously (Hoon et al., 1999). All in situ hybridizations were carried out at high stringency (hybridization, 5× SSC, 50% formamide, 65°C–72°C; washing, 0.2× SSC, 72°C). For single-label detection, signals were developed using alkaline-phosphatase conjugated antibodies to digoxigenin and standard chromogenic substrates (Boehringer Mannheim). Where possible, probes contained extensive 3′-nontranslated sequence to minimize potential cross-hybridization between T2Rs. The probes did not cross-hybridize at the stringency used for in situ hybridization. Control hybridizations with sense probes produced no specific signals in any of the taste papillae, while hybridization with a cDNA encoding a Gαi subunit demonstrated uniform labeling in all taste cells of all taste buds (data not shown). In all cases we examined at least 50 taste buds derived from a minimum of 3 animals. Quantitative studies were based on examination of 16 μm serial sections through various papillae. Cells were counted based on the position of their nucleus as previously described (Boughter et al., 1997). Experiments shown in Figure 6 used the following probes: (a) T2R-3 and T2R-7; (b) T2R-4, T2R-5, T2R-6, T2R-8, and T2R-12; (c) T2R-1, T2R-2, T2R-3, T2R-4, T2R-5, T2R-6, T2R-7, T2R-8, T2R-10, and T2R-12. Identical results were obtained with four additional combinations of two and nine additional combinations of five receptors (data not shown). Northern analysis and in situ hybridization demonstrated that T2Rs are not widely expressed outside taste tissue (data not shown). For double-label fluorescent detection, probes were labeled either with fluorescein or with digoxigenin. At least 50 taste buds from three different animals were analyzed. An alkaline-phosphatase conjugated anti-fluorescein antibody (Amersham) and a horseradish-peroxidase conjugated anti-digoxigenin antibody were used in combination with fast-red and tyramide fluorogenic substrates (Boehringer Mannheim and New England Nuclear). Confocal images were obtained with a Leica TSC confocal microscope using an argon-krypton laser; 1 μm optical sections were recorded to ensure that any overlapping signal originated from single cells.

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