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Analysis of a Human Fungiform Papillae cDNA Library and Identification of Taste-related Genes

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Abstract

Various genes related to early events in human gustation have recently been discovered, yet a thorough understanding of taste transduction is hampered by gaps in our knowledge of the signaling chain. As a first step toward gaining additional insight, the expression specificity of genes in human taste tissue needs to be determined. To this end, a fungiform papillae cDNA library has been generated and analyzed. For validation of the library, taste-related gene probes were used to detect known molecules. Subsequently, DNA sequence analysis was performed to identify further candidates. Of 987 clones sequenced, clustering results in 288 contigs. Comparison of these contigs with genomic databases reveals that 207 contigs (71.9%) match known genes, 16 (5.6%) match hypothetical genes, eight (2.8%) match repetitive sequences and 57 (19.8%) have no or low similarity to annotated genes. The results indicate that despite a high level of redundancy, this human fungiform cDNA library contains specific taste markers and is valuable for investigation of both known and novel taste-related genes.

Key words: cDNA library, gene expression, human fungiform papillae, signal transduction, taste marker, tastomics

Introduction

The sense of taste allows organisms to detect nutritionally important or potentially harmful compounds. The initial step in taste perception is the detection of chemical stimuli by taste cells, modified epithelial cells grouped in taste buds. These are found in fungiform papillae on the anterior part of the tongue and within vallate and foliate papillae on the posterior part. Taste buds are composed of 50–100 cells, only a few of which (five to eight) may actually be exposed to the oral environment at any given time, thus likely to be active receptor cells (Kinnamon *et al.*, 1985). Approximately 80% of all taste buds are located within the average eight to ten circumvallate papillae and the two foliate regions. The total number of lingual taste buds varies widely among individuals, with an average of 4500 to 5000 (Witt *et al.*, 2003). In man, individual fungiform papillae contain from zero to as many as 18 or 20 taste buds, with an average of three or four per papilla (Miller, 1995).

Taste receptor cells mediate the distinction among the five basic taste modalities sour, salty, bitter, sweet and *umami*. Sour taste may be mediated by a number of integrating mechanisms including channels belonging to the acid sensing ion channel (ASIC) family (Ugawa *et al.*, 1998), hyperpolarization-activated channels (Stevens *et al.*, 2001) and changes in intracellular pH (Lyall *et al.*, 2001). Salty taste is thought to be transduced via specific cation channels such as the amiloride-sensitive epithelial sodium channel, ENaC (DeSimone *et al.*, 1981; Schiffman *et al.*, 1983; Heck *et al.*, 1984; Brand *et al.*, 1985) or because amiloride sensitivity of salt taste is not always pronounced in humans, possibly through another channel (Breslin and Tharp, 2001; Lindemann, 2001). ENaCs are generally composed of three

homologous subunits α, β and γ (Canessa *et al.*, 1994). All three subunits have been localized in taste cells from fungiform, foliate and vallate papillae, but the expression of the β and γ subunits is higher in the fungiform papillae (anterior tongue) (Kretz *et al.*, 1999; Lin *et al.*, 1999). A fourth δ subunit can replace the α subunit (Waldmann *et al.*, 1995) and this subunit has recently been detected in human taste cells by a single-cell PCR/sequencing approach (Huque *et al.*, 2002). In addition to their possible role in salty taste, ENaCs have been co-localized with glutamate receptors in rat fungiform taste cells, suggesting a basis for cellular integration of the independent pathways, as might be required for imparting an *umami* taste by monosodium glutamate (Lin and Kinnamon, 1999).

New insights into the transduction mechanisms of bitter, sweet and *umami* tastes have been gained recently through the cloning and characterization of two GPCR families: one (the TAS2Rs) containing bitter taste receptors (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000), the other (the TAS1Rs) comprising an amino acid and a sweet taste receptor (Bachmanov *et al.*, 2001; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001, 2002; Sainz *et al.*, 2001; Li *et al.*, 2002). One of the G proteins associated with bitter and sweet tastes is gustducin, a G protein closely related to the transducins and therefore likely to be an inhibitor of phosphodiesterases (Wong *et al.*, 1996; Ming *et al.*, 1998; Yan *et al.*, 2001).

Different candidate taste receptors have been proposed for *umami* taste. It has been suggested that a taste-specific truncated metabotropic glutamate receptor, taste-mGluR4, may act as an *umami* (L-glutamate) receptor (Chaudhari *et al.*, 2000). Alternatively, or perhaps in addition to taste-mGluR4, there is evidence that a heterodimer of TAS1R1 and TAS1R3 acts as a glutamate taste receptor. The human dimer is specific for L-glutamate and is sensitive to certain 5′ ribonucleotides, whereas the equivalent dimer from rodent sequence is more broadly tuned for several L-amino acids. TAS1R3 also forms a heterodimer with TAS1R2 to recognize various natural and synthetic sweeteners (Kitagawa *et al.*, 2001; Li *et al.*, 2001, 2002; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001; Nelson *et al.*, 2002).

The chemical diversity of taste stimuli, both among the modalities and within certain modalities, necessitates multiple transduction mechanisms (Kinnamon, 1988; Spielman *et al.*, 1992; Gilbertson *et al.*, 2000). Moreover, various gustatory signal transduction proteins have been discovered in non-taste tissue such as the gastrointestinal tract raising questions of similarities and differences between sensory and non-sensory taste systems (Hofer *et al.*, 1996; Wu *et al.*, 2002; le Coutre, 2003). By gaining a more complete understanding of the sensory (oral) taste system, mechanisms of the non-sensory taste system can be more readily approached. And while several taste-specific pathways have been partially elucidated, a number of key players remain elusive.

Oral taste tissues are thought to express specific genes (Hoon and Ryba, 1997; Asano-Miyoshi *et al.*, 1998) and determination of these can lead to a more complete understanding of gustatory mechanisms. As of now, there are no analyses of a cDNA library from human taste tissue. Clearly such a library would be useful for the discovery of novel human taste-related genes. Therefore, a fungiform papillae cDNA library was created from excised human papillae of healthy adult volunteers. PCR probes of this library revealed several known gustatory genes. In addition, 987 clones were sequence analyzed, assembled into contigs and annotated by comparison to known sequence databases. Various potentially important genes are presented here in a qualitative and quantitative manner.

Materials and methods

Criteria for selection of sample donors

Healthy, young to middle age (21–45 years) adults of both genders were screened as potential donors of lingual fungiform papillae. Subjects read, understood and signed a consent form approved by Schulman Associates Institutional Review Board Inc., Cincinnati, Ohio. Ethical standards as set forth by the Helsinki Declaration of 1973, as revised in 1983, were adhered to. Subject exclusion criteria on initial screening included: those reporting a medical history or present systemic, chronic disease; those reporting the regular use of prescription medications; those reporting dry mouth or oral disease; and those reporting no experience with lidocaine anesthesia in a dental setting. Subjects meeting these criteria were invited to participate in the protocol. Exclusion criteria at the time of biopsy included a heart rate >100 or <60; systolic blood pressure >145 or <100; diastolic blood pressure >90 or <60; and the oral surgeon's opinion that the subject was not appropriate for the study. The oral cavity was inspected for general tissue health, with attention to indicators of xerostomia. All data collected were recorded by subject identity code and maintained confidential.

Biopsy procedure

The general human fungiform papilla biopsy procedure has been outlined previously (Spielman and Brand, 1995). Briefly, the procedure involves anesthetizing a small (1 cm^2) area of the dorsal surface of the anterior tongue, with lidocaine (2%) containing epinephrine (1:100 000) into one site just below the dorsal surface of the anterior portion of the tongue. The site of anesthesia is distal to the collection site to avoid interference by the lidocaine with subsequent studies. The dorsal half of six to eight fungiform papillae is removed from that anesthetized area using curved spring microscissors. Immediately (5–10 s) after removal, each papilla is placed into liquid N_2 and stored. A total of 154 papillae (200 mg) was collected from 22 volunteers (seven males, 15 females). Removed fungiform papillae are ~0.15–0.25 mm^3

each in volume. Using this procedure, very little non-papillar epithelial tissue is removed. New papillae form in this exact area after 3–5 weeks and regain functioning taste buds (Spielman and Brand, unpublished observations).

Library construction and screening

A cDNA library was generated at ResGen Inc. following the standard protocol using micro-quantity mRNA SUPER-Script technology (Invitrogen). The cDNA sequences were directionally inserted (3′*Not*I/5′*Sal*I) into the pCMV-SPORT 6.0 vector and transformed into *Escherichia coli* DH10B to produce a library containing 6.2×10^7 cfu (2.22×10^6 cfu/ml) with an average insert size of 2.1 kb and all inserts having >200 bp.

Library screening was performed by filter hybridization and PCR amplification for the following genes: BNaC2, ENaCα, ENaCβ, ENaCδ, ENaCγ, phosphodiesterase (PDE) 1B, PDE4B, phospholipase Cβ2 (PLCβ2), G protein β3-subunit (Gβ3), G protein β4-subunit (Gβ4), G protein γ13-subunit (Gγ13), α-gustducin, mGluR3, mGluR4, mGluR5, mGluR6, mGluR7, mGluR8, TAS1R1, TAS1R3, TAS2R10. Hybridization probes were PCR labeled with digoxigenin (DIG-11-UTP) using a kit and standard protocol of Roche Molecular Biochemicals. Probes from members of the same gene family (e.g. the T1Rs, the Gβs) were pooled. The hybridization and washing conditions were of moderate stringency (overnight hybridizations at 55°C; washings at 55°C 30 min in 0.2 × SSC). Detection was either chromogenic or chemiluminescent.

For PCR screening a strategy of successive library dilutions beginning with a titer of 10^5 clones/ml and followed by PCR amplification on pooled plasmid extracts was employed to screen for individual clones.

Sequencing and bioinformatic analysis

Individual clones from the plated library were randomly selected and cultured in twelve 96-well plates. Plasmid templates for sequencing were extracted from each clone. Sequencing was performed using Applied Biosystems ABI PRISM® Big Dye™ Terminators kits and SP6 primers.

Individual sequences were processed using Lasergene SeqMan sequence assembler (DNASTAR, Madison, WI) to remove remaining vector, polyA/polyT tails, low quality or low complexity sequences, adapter and bacterial sequences. Using BLASTx and BLASTn contigs were compared to Swiss-Prot and Ensembl sequence databases, respectively (Altschul *et al.*, 1990).

Results and discussion

PCR analysis

The genes which were identified by PCR represent both known taste-related and putative taste-related ones (Table 1). Twelve of these were sequence confirmed and five were cloned. These five were mGluR8, PLCβ2, taste receptors TAS1R1 and TAS1R3, and gustducin.

Table 1 Twenty-one known and putative taste-related signal transduction genes were identified by PCR to validate the fungiform papillae cDNA library

Description	Sequence confirmed	NCBI accession No.
BNaC2	+	NM_020039
ENaCα	+	NM_001038
ENaCβ	+	NM_000336
ENaCδ	–	NM_002978
ENaCγ	+	NM_001039
PDE1B	–	NM_000924
PDE4B	+	NM_002600
PLCβ2	+	NM_004573
Gβ3	+	NM_002075
Gβ4	+	NM_021629
Gγ13	–	NM_016541
α-gustducin	+	XM_294370
mGluR3	–	NM_000840
mGluR4	–	NM_000841
mGluR5	–	NM_000842
mGluR6	–	NM_000843
mGluR7	–	NM_000844
mGluR8	+	NM_000845
TAS1R1	+	NM_177539
TAS1R3	+	BK000152
TAS2R10	–	NM_023921

+PCR-positive gene further confirmed by sequencing.
–PCR-positive gene for which sequencing was not performed.

A series of epithelial sodium channel subunits have been probed for: ENaCα, β and γ subunits were confirmed, as well as BNaC2, another member of the DEG/ENaC superfamily. Given that α-gustducin is present in human taste cells (Takami *et al.*, 1994) it was probed for and located in the cDNA library. The G protein subunit Gβ3 is expressed in taste receptor cells, couples with Gγ13, and its expression overlaps that of gustducin (Huang *et al.*, 1999; Rossler *et al.*, 2000). Gβ4 was probed for because it is present in taste tissue and can form stable dimers with Gγ13 that can stimulate phospholipase Cβ2 (Rosskopf *et al.*, 2003). Phosphodiesterase 1B and 4B were also observed. Taste tissue contains high levels of several types of phosphodiesterases with PDE1A being primarily responsive to gustducin in taste cells (Ruiz-Avila *et al.*, 1995). Metabotropic glutamate receptors mGluR3 through mGluR8 were detected and it is not yet known if the found mGluR4 is a homologue of the truncated

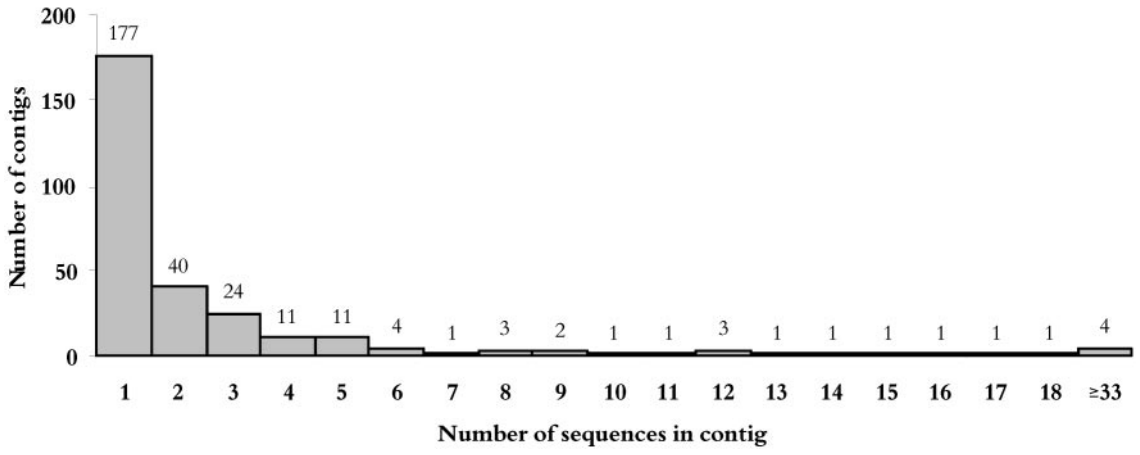

Figure 1 Frequency distribution showing the number of sequences per contig in the fungiform papillae cDNA library (sequence frequency bold). The 13 most abundant transcripts are: keratin type II cytoskeletal 6A (cytokeratin 6A) (**134**); keratin type I cytoskeletal 13 (cytokeratin 13) (**87**); keratin type I cytoskeletal 16 (cytokeratin 16) (**66**); mitochondrial 2-oxoglutarate/malate carrier protein (**33**); human esophagus-specific gene c1orf10 (**18**); keratin type II cytoskeletal 1 (cytokeratin 1) (**17**); keratin type II cytoskeletal 4 (cytokeratin 4) (**16**); olfactomedin 2 (**15**); connexin 26 (**14**); keratin type I cytoskeletal 10 (cytokeratin 10) (**12**); RH type C glycoprotein (**12**); connexin 43 (**11**); and eukaryotic initiation factor 4A-I (**10**). The contig with 13 sequences and one of the contigs with 12 sequences are not listed because they have no or low similarity to annotated genes.

taste-mGluR4. Finally, three taste receptor genes were detected, TAS1R1, TAS1R3 and TAS2R10.

Because of the relatively low stringency conditions for colony hybridization as well as the nature of the pooled PCR probes, a number of clones not directly targeted by the primers were detected and sequenced. Among these are various well-known and abundant genes and also the gene for synembryn (Swiss-Prot Q86WD3). This protein could conceivably play a role in gustatory signaling. Synembryn is an ortholog of the *C. elegans* Ric-8 protein. It has recently been characterized as a heterotrimeric Gα protein guanine nucleotide exchange factor in human brain tissue (Tall *et al.*, 2003) and in the *C. elegans* nervous system (Miller *et al.*, 2000). Additional localization studies are required to dissect its function in fungiform papillae, but its presence in taste tissue might reflect the neuronal aspects of taste receptor cells, wherein it may regulate Gα protein function and thus influence GPCR signaling.

Table 2 Keratin genes identified in the fungiform papillae cDNA library, representing 35.3% of the sequences

Keratin type	Number of sequences	Accession No.	Source
Type II cytoskeletal 6A (cytokeratin 6A)	134	P02538	Swiss-Prot
Type I cytoskeletal 13 (cytokeratin 13)	87	P13646	Swiss-Prot
Type I cytoskeletal 16 (cytokeratin 16)	66	P08779	Swiss-Prot
Type II cytoskeletal 1 (cytokeratin 1)	17	P04264	Swiss-Prot
Type II cytoskeletal 4 (cytokeratin 4)	16	P19013	Swiss-Prot
Type I cytoskeletal 10 (cytokeratin 10)	12	P13645	Swiss-Prot
Type II cytoskeletal 5 (cytokeratin 5)	9	P13647	Swiss-Prot
Type I cuticular HA6	6	O76013	Swiss-Prot
Type I cytoskeletal 20 (cytokeratin 20)	1	P35900	Swiss-Prot

Bioinformatic analysis

To survey the genes expressed in human fungiform papillae, the library was analysed by sequencing 987 randomly selected clones and by comparing them to the Swiss-Prot and Ensembl databases. Assembly of the sequences of these clones yielded 288 different contigs. Of these contigs 223 gave hits with annotation in the selected databases, including 207 (71.9% of contigs) with high homology to known genes and 16 (5.6%) matching hypothetical genes. Eight (2.8%) matched repetitive sequences and 57 (19.8%) had no or low similarity (defined here as having hits with <50% identity and/or *e* value $>10^{-4}$) to annotated genes.

The redundancy of sequences in a non-normalized cDNA library is dependent upon the representation of mRNA transcripts at the time of sampling. Therefore, the annotation of the contigs generated from such libraries allows a good picture of the abundance of transcripts in the cell. As shown in the distribution in Figure 1, 47.6% of the sequences (470) are represented in 15 contigs.

Cytokeratin proteins

Among 288 contigs, nine were found to be identical to known human keratins (Table 2), representing a total of 348 sequences (35.3%). Of the keratin clones reported in Table 2, keratin 20 has previously been reported to be in taste bud cells of human fungiform papillae (Zhang and Oakley, 1996). Cytokeratins are important for establishing the spindle-like shape of gustatory cells when they elongate during morphogenesis (Ganchrow, 2000). A high prevalence of keratins was expected, given the epithelial nature of the

tissue. Moreover, despite neuronal characteristics, taste buds likely come from the same progenitor population as the lingual epithelium (Stone *et al.*, 1995). In addition, taste buds are surrounded by a highly keratinized stratified squamous epithelium (Witt *et al.*, 2003).

Epithelial tissue proteins

Message for proteins typical of epithelial tissue was readily detected. Examples include: epithelial membrane protein-1 (Swiss-Prot P54849); kallikrein 10 (Swiss-Prot O43240); lympho-epithelial Kazal-type related inhibitor (Swiss-Prot Q9NQ38); 14-3-3 protein σ (epithelial cell marker protein 1; Swiss-Prot P31947); kallikrein 7 (Swiss-Prot P49862; also known as *stratum corneum* chymotryptic enzyme) that catalyzes the degradation of intercellular cohesive structures in the cornified layer of the skin; WD-repeat protein 1 (actin interacting protein 1; Swiss-Prot O75083); huntingtin-interacting protein 1 (Swiss-Prot O00291) that may play a role in the cell filament networks; transglutaminase K (Swiss-Prot P22735), responsible for cross-linking epidermal proteins during formation of the stratum corneum; protocadherin Fat 2 (Swiss-Prot Q9NYQ8), a member of the 'nonclassic cadherins', a family of calcium-dependent cell–cell adhesion molecules in the cadherin superfamily (Wolverton and Lalande, 2001); and collagen XVII (RefSeq NM_000494), an epidermal protein with a critical function in epithelial cell adhesion (Franzke *et al.*, 2002). All these hits are represented by only one sequence, with the exception of the epithelial membrane protein-1, represented by five sequences.

Cytoskeletal proteins

Genes coding for cytoskeletal proteins in the library are represented by seven contigs: tubulin β-5 chain (Swiss-Prot P05218); tubulin β-4 chain (Swiss-Prot Q13509); destrin (Swiss-Prot P18282); tubulin-specific chaperone C (Swiss-Prot Q15814); and *h*2-calponin (Swiss-Prot Q99439) that may regulate cytokinesis by inhibiting the activity of the actin cytoskeleton (Hossain *et al.*, 2003). Also detected were actin-like protein 3 (Swiss-Prot P32391) and band 4.1-like protein 3 (Swiss-Prot Q9Y2J2). The members of the 'band 4.1′ superfamily are thought to play crucial roles in the regulation of cytoskeletal plasma membrane interaction just beneath plasma membranes.

Cell adhesion proteins

The major representatives of this class (Table 3) in the library are the gap junction proteins connexin 26 (Swiss-Prot P29033) and connexin 43 (Swiss-Prot P17302), with a total of 25 sequences (2.5%). Also found were, for example, plakoglobins (homologous of β-catenin), which are proteins linking cadherin adhesion receptors at cell–cell adhesion sites to the actin cytoskeleton; desmosomal glycoprotein 1 (Swiss-Prot Q02413); DGCR2/IDD (Swiss-Prot P98153), a putative adhesion receptor that could be involved in cell–cell or cell–matrix interactions required for normal cell differentiation and migration; and stromal interaction molecule 1 (Swiss-Prot Q13586), a possible cell surface transmembrane adhesion glycoprotein implicated in stromal-haematopoietic cell interactions. Being epithelial cells by lineage, cells of the taste buds display desmosomes and gap junctions. There is evidence that cells within a taste bud are electrically coupled via these junctions (Bigiani and Roper, 1995). This coupling may play a functional role in taste transduction by allowing the synchronous spread of excitation of one cell to its neighbors, possibly modulating the signal.

Table 3 Genes related to cell adhesion, cell–cell interactions and extracellular matrix identified in the fungiform papillae cDNA library, representing 3.4% of the sequences

Name	Number of sequences	Accession No.	Source
Connexin 26	14	P29033	Swiss-Prot
Connexin 43	11	P17302	Swiss-Prot
Junction plakoglobin	2	P14923	Swiss-Prot
Desmoplakin	1	P15924	Swiss-Prot
Plakophilin 3	1	Q9Y446	Swiss-Prot
Desmosomal glycoprotein 1	1	Q02413	Swiss-Prot
Integral membrane protein DGCR2/IDD	1	P98153	Swiss-Prot
Stromal interaction molecule 1	1	Q13586	Swiss-Prot
Extracellular matrix protein 1	1	Q16610	Swiss-Prot
Basigin	1	P35613	Swiss-Prot

Mitochondrial genes

Genes from the mitochondrial genome (Table 4) are found with high frequency (nine contigs with 5.0% of the sequences). Taste cells are heavily invested with mitochondria, necessary for the cell to undergo and recover from excitation (Murray, 1973).

Sorting, trafficking and protein fate

Various proteins were identified that are involved in cell trafficking, such as coatomer γ-2 subunit (Swiss-Prot Q9UBF2), a cytosolic protein complex that reversibly associates with Golgi non-clathrin-coated vesicles, which further mediate protein transport from the ER to the *trans* Golgi network. In mammals, the coatomer can only be recruited by membranes associated to ADP-ribosylation factors (ARFs), small GTP-binding proteins from which one representative (ARF1, Swiss-Prot P32889) has been found in the library.

We also identified FHOS (Swiss-Prot Q9Y613), a member of the formin homology (FH) family of proteins expressed at high levels in splenic cells. FH proteins are known to link cellular signaling pathways to the actin cytoskeleton. Other proteins involved in trafficking include LTBP-4 (latent

Table 4 Mitochondrial genes identified in the fungiform papillae cDNA library, representing 5.0% of the sequences

Name	Number of sequences	Accession No.	Source
Mitochondrial 2-oxoglutarate/malate carrier protein	33	Q02978	Swiss-Prot
Phosphate carrier protein, mitochondrial	5	Q00325	Swiss-Prot
Mitochondrion cytochrome c oxidase subunit I	5	Q9B2U7	TrEMBL
NADH-ubiquinone oxidoreductase chain 4L	2	P03901	Swiss-Prot
PMP34 (34 kDa peroxisomal membrane protein)	1	O43808	Swiss-Prot
Mitochondrion NADH dehydrogenase subunit 1	1	Q9B2U3	TrEMBL
Mitochondrial 60s ribosomal protein L49	1	Q13405	Swiss-Prot
Acyl-CoA dehydrogenase, very-long-chain specific, mitochondrial	1	P49748	Swiss-Prot
Mitochondrial processing peptidase beta subunit	1	O75439	Swiss-Prot

transforming growth factor-β-binding protein-4; RefSeq NM_003573) and SRP9 (signal recognition particle 9 kDa protein; Swiss-Prot P49458), a signal recognition particle assembly with a role in targeting secretory proteins to the rough endoplasmic reticulum membrane. Genes involved in protein fate include: ubiquitin C (RefSeq NM_021009); ubiquitin-protein ligase E2-17 kDa 3 (Swiss-Prot P47986); ubiquitin carboxyl-terminal hydrolase 7 (Swiss-Prot Q93009); cathepsin D (Swiss-Prot P07339), an acid protease active in intracellular protein breakdown; the heat shock protein 40 homologue (Swiss-Prot Q9UDY4); and the heat shock 70 kDa protein 1 (Swiss-Prot P08107) that, together with other chaperones, prevents existing proteins from aggregation and mediates the folding of newly translated polypeptides.

Gene expression and regulation proteins

Thirty-six contigs (6.9% of the sequences) identified proteins involved in gene expression and regulation (Table 5). Examples include eukaryotic translation initiation factor 2 subunit 2 (Swiss-Prot P20042), or eukaryotic initiation factor 4A-I (Swiss-Prot P04765). In addition, proteins involved in development have been identified: one hit being similar to development- and differentiation-enhancing factor 2 (RefSeq NM_017707); and another to the ephrin type-B receptor 3 (developmental kinase 5; Swiss-Prot P54753). The identification of eukaryotic initiation factors might reflect the high translational activity of taste tissues, possibly connected with differential gene expression in taste cells (Asano-Miyoshi *et al.*, 1998).

Neuronal markers

Some contigs have homology to genes specific to cells with neuronal characteristics: synaptic vesicle membrane protein VAT-1 homologue (Swiss-Prot Q99536, one sequence); proactivator polypeptide (contains saposin A, B, C and D; Swiss-Prot P07602, eight sequences); phosphatidylethanolamine-binding protein (Swiss-Prot P30086, three sequences); brain Acyl-CoA hydrolase (Swiss-Prot O00154, one sequence); neuroendocrine differentiation factor (RefSeq NM_016079, one sequence). The presence of these neuronal markers may be due to the partly neuronal nature of taste cells (Gilbertson *et al.*, 2000); it also may be due to the presence of message in nerve fibers innervating the taste buds and other areas of the fungiform papillae.

Signal transduction proteins

A large number of genes (22 contigs) in the library are involved in cell signaling (Table 6), including several members of the G protein superfamily, such as the Ras-related small GTP-binding proteins Rab-1B, Rab-5A (Swiss-Prot Q9H0U4 and P20339) and RAG C (RefSeq NM_022157); ADP-ribosylation factor protein 1 ARF1 (Swiss-Prot P32889); the guanine nucleotide-binding proteins 1 and 2 (Swiss-Prot P32455 and P32456); and G protein β1-subunit (Swiss-Prot P04901). Other signal transduction transcripts were identified, including: calmodulin (Swiss-Prot P02593); calcium/calmodulin-dependent protein kinase type I (Swiss-Prot Q14012); tyrosine-protein kinase JAK1 (Swiss-Prot P23458); and cytosolic phospholipase A2β (Swiss-Prot O95712). One contig corresponding to a known olfactory gene was detected: the noelin precursor, olfactomedin 2 (Swiss-Prot O95897). Olfactomedin-related proteins were originally identified as a component of the mucus layer surrounding the chemosensory dendrites of olfactory neurons and are thought to play a role in neuronal growth and differentiation (Yokoe and Anholt, 1993; Kulkarni *et al.*, 2000). Additional cell signaling elements include: karyopherin α-2 (Swiss-Prot P52292), a member of a protein family that plays a central role in nucleocytoplasmic transport; tumor protein D52 (N8 protein; Swiss-Prot P55327), that belongs to a family of signaling molecules which may play a role in calcium-mediated signal transduction and cell proliferation; and cytoplasmic protein NCK1 (Swiss-Prot P16333), an adapter protein which associates with tyrosine-phosphorylated growth factor receptors or

Table 5 Proteins involved in gene regulation/expression and nuclear proteins identified in the fungiform papillae cDNA library, representing 6.9% of the sequences

Name	Number of sequences	Accession No.	Source
Eukaryotic initiation factor 4A-I	10	P04765	Swiss-Prot
Eukaryotic translation initiation factor 2 subunit 2	5	P20042	Swiss-Prot
Eukaryotic translation initiation factor 4B	1	P23588	Swiss-Prot
Eukaryotic translation initiation factor 3 subunit 10	1	Q14152	Swiss-Prot
Elongation factor 1-alpha 1	5	P04720	Swiss-Prot
Elongation factor 1-gamma	3	P26641	Swiss-Prot
RING finger protein 4	3	P78317	Swiss-Prot
PC2 glutamine/Q-rich-associated protein	3	Q96RN5	Swiss-Prot
Transcription factor MAFB	3	Q9Y5Q3	Swiss-Prot
TFIIH basal transcription factor complex p34 subunit	1	Q13889	Swiss-Prot
TFIIH basal transcription factor complex p62 subunit	1	P32780	Swiss-Prot
Histone H1.0	2	P07305	Swiss-Prot
RNA-binding protein 12	2	Q9NTZ6	Swiss-Prot
Regulator of nonsense transcripts 1	2	Q92900	Swiss-Prot
Tripartite motif protein TRIM29, isoform alpha	1	NM_012101	RefSeq
Cytokine-like nuclear factor n-pac	2	Q9BXK2	Swiss-Prot
60S ribosomal protein L4	2	P36578	Swiss-Prot
Transcription initiation factor IIF, beta subunit	1	P13984	Swiss-Prot
Probable ATP-dependent RNA helicase p47	1	Q13838	Swiss-Prot
Helicase SMARCAD1	1	Q9H4L7	Swiss-Prot
ATP-dependent RNA helicase DDX19	1	Q9UMR2	Swiss-Prot
RNA-binding protein S1, serine-rich domain	1	NM_006711	RefSeq
Double-stranded RNA-binding protein Staufen homolog	1	O95793	Swiss-Prot
PDZ-LIM protein mystique	1	NM_021630	RefSeq
Basic leucine-zipper protein BZAP45	1	NM_014670	RefSeq
Histone H2A.l	1	P02261	Swiss-Prot
Polyadenylate-binding protein 1	1	P11940	Swiss-Prot
Similar to CG11334 gene product	1	Q9BV20	Swiss-Prot
Zinc finger protein 16	1	P17020	Swiss-Prot
Similar to nucleolin	1	BC009855	TrEMBL
Target of MYB 1	1	NM_005488	RefSeq
Zinc finger protein 38 homolog	1	Q9Y5A6	Swiss-Prot
SPT3-associated factor 42	1	NM_053053	RefSeq
Heterogeneous nuclear ribonucleoprotein A1	1	P09651	Swiss-Prot
TCOF1 gene product (treacle)	1	Q13428	Swiss-Prot
Nuclear pore complex protein Nup160	3	Q12769	Swiss-Prot

Table 6 Signal transduction genes identified in the fungiform papillae cDNA library, representing 4.9% of the sequences

Name	Number of sequences	Accession No.	Source
Ras-related protein Rab-5A	4	P20339	Swiss-Prot
Ras-related protein Rab-1B	3	Q9H0U4	Swiss-Prot
RAG C protein	3	NM_022157	RefSeq
ADP-ribosylation factor protein 1 ARF1	1	P32889	Swiss-Prot
Guanine nucleotide-binding protein 1	4	P32455	Swiss-Prot
Guanine nucleotide-binding protein 2	1	P32456	Swiss-Prot
Gβ1	1	P04901	Swiss-Prot
Olfactomedin 2	15	O95897	Swiss-Prot
Tyrosine-protein kinase JAK1	2	P23458	Swiss-Prot
BNIP-Salpha	2	NM_138278	RefSeq
Calmodulin	1	P02593	Swiss-Prot
Mothers against decapentaplegic homolog 7 (SMAD 7)	1	O15105	Swiss-Prot
Karyopherin α-2	1	P52292	Swiss-Prot
Tumor protein D52 (N8 protein)	1	P55327	Swiss-Prot
Dickkopf related protein-4	1	Q9UBT3	Swiss-Prot
Cytoplasmic protein NCK1	1	P16333	Swiss-Prot
Tumor necrosis factor receptor superfamily member 19	1	Q9NS68	Swiss-Prot
Cytosolic phospholipase A2β	1	O95712	TrEMBL
ADP-ribosylation factor 1	1	P32889	Swiss-Prot
Calcium/calmodulin-dependent protein kinase type I	1	Q14012	Swiss-Prot
FH1/FH2 domains-containing protein	1	Q9Y613	Swiss-Prot
Latent transforming growth factor-β-binding protein-4	1	NM_003573	RefSeq

their cellular substrates. As the primary function of the taste cell is signaling, such a high percentage of signaling genes may reflect the relative abundance of taste-related genes in the library.

Proteases and proteases inhibitors

A series of 11 proteases and seven protease inhibitors have been found. The proteases are legumain (Swiss-Prot Q99538); glutamate carboxypeptidase (Swiss-Prot Q96KP4); calpain 1 catalytic subunit (Swiss-Prot P07384); airway trypsin-like protease (Swiss-Prot O60235); kallikrein 6 (Swiss-Prot Q92876); ubiquitin carboxyl-terminal hydrolase 7 (Swiss-Prot Q93009); basigin (Swiss-Prot P35613); carboxypeptidase C (Swiss-Prot P10619); kallikrein 10 (Swiss-Prot O43240); mitochondrial processing peptidase β-subunit (Swiss-Prot O75439); and ADAMTS-2 (Swiss-Prot O95450). The protease inhibitors identified are osteogenin (Swiss-Prot P12645); cystatin B (Swiss-Prot P04080); Kunitz-type protease inhibitor 2 (Swiss-Prot O43291); hurpin (Swiss-Prot Q9UIV8); serine protease inhibitor Kazal-type 5 (Swiss-Prot Q9NQ38); Kunitz-type protease inhibitor 1 (Swiss-Prot O43278); and calpain inhibitor (Swiss-Prot P20810). Calpains have been detected in taste bud cells in the taste bud-dense barbell epithelium of channel catfish (Ookura *et al.*, 1997). Their role in taste has not been extensively investigated but they could be involved, as they are in other cells, in signal transduction, in proteolysis of substrates involved in cytoskeletal remodeling and in apoptosis.

Channels or ATPases

Four contigs representing seven sequences gave hits corresponding to channels or ATPases: the non-gastric H^+/K^+ ATPase α subunit (Swiss-Prot P54707); Na^+/K^+-dependent ATPase β-3 subunit (Swiss-Prot P54709); potassium channel modulatory factor (Swiss-Prot Q9BWK2); and aquaporin 3 (Swiss-Prot Q92482). ATPase activity is particularly high in taste cells of most mammals, including humans (Ambro *et al.*, 2002) and its presence has long been used as a marker for functional taste buds (Zalewski, 1969).

Housekeeping genes

Various other housekeeping genes were identified, such as β-actin (Swiss-Prot P02570), lamin A/C (Swiss-Prot P02545) and myosin-Ic (Swiss-Prot Q12965). The lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Myosins are actin-based motor molecules with ATPase activity. Also identified were some genes corresponding to membrane proteins, such as translocon-associated protein γ subunit (Swiss-Prot Q9UNL2). Two peroxisomal genes were found: PMP34 (34 kDa peroxisomal membrane protein; Swiss-Prot O43808); and peroxisomal 2,4-dienoyl-CoA reductase (RefSeq NM_020664). Three contigs (eight sequences) represent lysosomal proteins: lysosome-associated membrane glycoprotein 1 (Swiss-Prot P11279); glycosylasparaginase (Swiss-Prot P20933); and cathepsin A (lysosomal protective protein precursor) (Swiss-Prot P10619).

Some genes related to HLA/MHC or blood groups were present: MHC class I antigen E (Swiss-Prot P13747); HLA-B associated transcript-1 (probable ATP-dependent RNA helicase p47; Swiss-Prot Q13838); and RH type C glycoprotein (RefSeq NM_016321). Blood group antigens have been localized to fungiform, foliate and vallate papillae. The blood group antigens are expressed in developing olfactory neurons and may play roles in cell–cell recognition, adhesion and other interactions important in the developing nervous system. They could have similar but unknown functions in both the taste and the olfactory systems, where the receptors are continually renewed and new synapses between the receptors and their neural targets continually form (Smith *et al.*, 1994).

Moreover, a gene characteristic of the oral cavity has been identified: tuftelin 1 (Swiss-Prot Q9NNX1), involved in the mineralization and structural organization of enamel. Tuftelin is secreted at a very early stage of enamel formation, concentrated at the dentin–enamel junction and tightly bound to the surface of the growing crystallites.

Interestingly, one G protein-coupled receptor that has not been characterized to date was identified in the library, represented by two sequences: the *Homo sapiens* G protein-coupled receptor 108 (GPR108; RefSeq XM_290854). This GPCR has 51% identity with the human lung seven transmembrane receptor 1 (LUSTR1) and a 80% identity to the mouse lung seven transmembrane receptor 2 (LUSTR2); LUSTR1 and LUSTR2 have been recently cloned from mouse and human subtracted primary pulmonary hypertensive cDNA libraries (Edgar and Polak, 2002). The human GPR108 encodes a 543 amino acid protein predicted to have seven transmembrane helices (Hofmann and Stoffel, 1993). Should this new GPCR be localized within taste receptor cells, it is possible that it may be a novel taste receptor protein.

Conclusions

Clones generated in this library contain taste-specific entities and may contain new taste-specific cDNA species. However, because the source tissue for the construction of the cDNA library contained only a small percentage (<5%) of taste receptor cells, with the higher percentage being epithelial and connective tissues, this library shows a high redundancy indicated by a high prevalence of transcripts for keratins and other epithelial proteins. Due largely to this redundancy, taste-specific genes were difficult to locate by sequencing. The presence of taste-specific genes was, however, verified by PCR amplification (Table 1). Given the known molecular characteristics of the signaling process in taste, using GPCR-activated second messenger transduction and stimulus-gated ion channel receptors, the identification of several papilla-specific proteins by hybridization and bioinformatics is suggestive of additional processes in taste.

To improve the efficiency of identifying new taste-specific genes, it would be of interest to construct a normalized library in order to gain specificity by lowering the number of redundant 'housekeeping' genes. Alternatively, a library generated exclusively from taste receptor cells would be of great interest because of its intrinsic specialization.

Notes

All the tables in this paper are available on the *Chemical Senses* website (http://chemse.oupjournals.org/) with clickable links for the accession numbers.

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References

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J. and **Zuker, C.S.** (2000) *A novel family of mammalian taste receptors*. Cell, 100, 693–702.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and **Lipman, D.J.** (1990) *Basic local alignment search tool*. J. Mol. Biol., 215, 403–410.

Ambro, B.T., Pribitkin, E.A., Wysocki, L., Brand, J.G. and **Keane, W.M.** (2002) *Evidence that taste buds are not present on the human adult uvula*. Ear Nose Throat J., 81, 456–457.

Asano-Miyoshi, M., Kusakabe, Y., Abe, K. and **Emori, Y.** (1998) *Identification of taste-tissue-specific cDNA clones from a subtraction cDNA library of rat circumvallate and foliate papillae*. J. Biochem. (Tokyo), 124, 927–933.

Bachmanov, A.A., Li, X., Reed, D.R., Ohmen, J.D., Li, S., Chen, Z., Tordoff, M.G., de Jong, P.J., Wu, C., West, D.B., Chatterjee, A., Ross, D.A. and **Beauchamp, G.K.** (2001) *Positional cloning of the mouse saccharin preference (Sac) locus*. Chem. Senses, 26, 925–933.

Bigiani, **A.** and **Roper, S.D.** (1995) *Estimation of the junctional resistance between electrically coupled receptor cells in* Necturus *taste buds*. J. Gen. Physiol., 106, 705–725.

Brand, J.G., Teeter, J.H. and **Silver, W.L.** (1985) *Inhibition by amiloride of chorda tympani responses evoked by monovalent salts*. Brain Res., 334, 207–214.

Breslin, **P.A.** and **Tharp, C.D.** (2001) *Reduction of saltiness and bitterness after a chlorhexidine rinse*. Chem. Senses, 26, 105–116.

Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D. and **Rossier, B.C.** (1994) *Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits*. Nature, 367, 463–467.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S. and **Ryba, N.J.** (2000) *T2Rs function as bitter taste receptors*. Cell, 100, 703–711.

Chaudhari, N., Landin, A.M. and **Roper, S.D.** (2000) *A metabotropic glutamate receptor variant functions as a taste receptor*. Nat. Neurosci., 3, 113–119.

DeSimone, J.A., Heck, G.L. and **DeSimone, S.K.** (1981) *Active ion transport in dog tongue: a possible role in taste*. Science, 214, 1039–1041.

Edgar, **A.J.** and **Polak, J.M.** (2002) *Novel putative G protein-coupled receptors cloned from lung*. J. Anat., 200, 202.

Franzke, C.W., Tasanen, K., Schacke, H., Zhou, Z., Tryggvason, K., Mauch, C., Zigrino, P., Sunnarborg, S., Lee, D.C., Fahrenholz, F. and **Bruckner-Tuderman, L.** (2002) *Transmembrane collagen XVII, an epithelial adhesion protein, is shed from the cell surface by ADAMs*. EMBO J., 21, 5026–5035.

Ganchrow, **J.R.** (2000) *Taste cell function. Structural and biochemical implications*. Physiol Behav., 69, 29–40.

Gilbertson, T.A., Damak, S. and **Margolskee, R.F.** (2000) *The molecular physiology of taste transduction*. Curr. Opin. Neurobiol., 10, 519–527.

Heck, G.L., Mierson, S. and **DeSimone, J.A.** (1984) *Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway*. Science, 223, 403–405.

Hofer, D., Puschel, B. and **Drenckhahn, D.** (1996) *Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin*. Proc. Natl Acad. Sci. USA, 93, 6631–6634.

Hofmann, **K.** and **Stoffel, W.** (1993) *Tmbase—a database of membrane spanning proteins segments*. Biol. Chem. Hoppe-Seyler, 374, 166.

Hoon, **M.A.** and **Ryba, N.J.** (1997) *Analysis and comparison of partial sequences of clones from a taste-bud-enriched cDNA library*. J. Dent. Res., 76, 831–838.

Hossain, M.M., Hwang, D.Y., Huang, Q.Q., Sasaki, Y. and **Jin, J.P.** (2003) *Developmentally regulated expression of calponin isoforms and the effect of h2-calponin on cell proliferation*. Am. J. Physiol. Cell Physiol., 284, C156–C167.

Huang, L., Shanker, Y.G., Dubauskaite, J., Zheng, J.Z., Yan, W., Rosenzweig, S., Spielman, A.I., Max, M. and **Margolskee, R.F.** (1999) *Ggamma13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium*. Nat. Neurosci., 2, 1055–1062.

Huque, T., Wysocki, L., Bayley, D.L., Breslin, P.A.S., Spielman A. and **Brand, J.G.** (2002) Expression of epithelial sodium channels (ENaCs) in Human Fungiform Papillae. Association for Chemoreception Sciences XXIVth Meeting, Sarasota, FL.

Kinnamon, **S.C.** (1988) *Taste transduction: a diversity of mechanisms*. Trends Neurosci., 11, 491–496.

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

Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y. and **Hino, A.** (2001) *Molecular genetic identification of a candidate receptor gene for sweet taste*. Biochem. Biophys. Res. Commun., 283, 236–242.

Kretz, O., Barbry, P., Bock, R. and **Lindemann, B.** (1999) *Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat*. J. Histochem. Cytochem., 47, 51–64.

Kulkarni, N.H., Karavanich, C.A., Atchley, W.R. and **Anholt, R.R.** (2000) *Characterization and differential expression of a human gene family of olfactomedin-related proteins*. Genet. Res., 76, 41–50.

le Coutre, **J.** (2003) *Taste: the metabolic sense*. Food Technol., 57, 34–39.

Li, X., **Inoue, M., Reed, D.R., Huque, T., Puchalski, R.B., Tordoff, M.G., Ninomiya, Y., Beauchamp, G.K.** and **Bachmanov, A.A.** (2001) *High-resolution genetic mapping of the saccharin preference locus (Sac) and the putative sweet taste receptor (T1R1) gene (Gpr70) to mouse distal Chromosome 4*. Mamm. Genome, 12, 13–16

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M. and **Adler, E.** (2002) *Human receptors for sweet and* umami *taste*. Proc. Natl Acad. Sci. USA, 99, 4692–4696.

Lin, **W.** and **Kinnamon, S.C.** (1999) *Co-localization of epithelial sodium channels and glutamate receptors in single taste cells*. Biol. Signals Recept., 8, 360–365.

Lin, W., Finger, T.E., Rossier, B.C. and **Kinnamon, S.C.** (1999) *Epithelial Na^+ channel subunits in rat taste cells: localization and regulation by aldosterone*. J. Comp. Neurol., 405, 406–420.

Lindemann, **B.** (2001) *Receptors and transduction in taste*. Nature, 413, 219–225.

Lyall, V., Alam, R.I., Phan, D.Q., Ereso, G.L., Phan, T.H., Malik, S.A., Montrose, M.H., Chu, S., Heck, G.L., Feldman, G.M. and **DeSimone, J.A.** (2001) *Decrease in rat taste receptor cell intracellular pH is the proximate stimulus in sour taste transduction*. Am. J. Physiol. Cell Physiol., 281, C1005–C1013.

Matsunami, H., Montmayeur, J.P. and **Buck, L.B.** (2000) *A family of candidate taste receptors in human and mouse*. Nature, 404, 601–604.

Max, M., Shanker, Y.G., Huang, L., Rong, M., Liu, Z., Campagne, F., Weinstein, H., Damak, S. and **Margolskee, R.F.** (2001) *Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac*. Nature Genet., 28, 58–63.

Miller, **I.J.** (1995) *Anatomy of the peripheral taste system.* In Doty, R.L. (ed.), Handbook of Olfaction and Gustation. Marcel Dekker, New York, pp. 521–547.

Miller, K.G., Emerson, M.D., McManus, J.R. and **Rand, J.B.** (2000) *RIC-8 (synembryn): a novel conserved protein that is required for G(q)alpha signaling in the* C. elegans *nervous system*. Neuron, 27, 289–299.

Ming, D., Ruiz-Avila, L. and **Margolskee, R.F.** (1998) *Characterization and solubilization of bitter-responsive receptors that couple to gustducin*. Proc. Natl Acad. Sci. USA, 95, 8933–8938.

Montmayeur, J.P., Liberles, S.D., Matsunami, H. and **Buck, L.B.** (2001) *A candidate taste receptor gene near a sweet taste locus*. Nat. Neurosci., 4, 492–498.

Murray, **R.G.** (1973) *The ultrastructure of taste buds.* In Friedmann, I. (ed.), The Ultrastructure of Sensory Organs. Elsevier, New York, pp. 1–81.

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

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

Ookura, T., Koyama, E., Puchalski, R.B., Teeter, J.H., Bayley, D.L. and **Brand, J.G.** (1997) *Molecular cloning of calpain from taste epithelium of the channel catfish,* Ictalurus punctatus. Chem. Senses, 22, 764–765.

Rosskopf, D., Nikula, C., Manthey, I., Joisten, M., Frey, U., Kohnen, S. and **Siffert, W.** (2003) *The human G protein beta4 subunit: gene structure, expression, Ggamma and effector interaction*. FEBS Lett., 544, 27–32.

Rossler, P., Boekhoff, I., Tareilus, E., Beck, S., Breer, H. and **Freitag, J.** (2000) *G protein betagamma complexes in circumvallate taste cells involved in bitter transduction*. Chem. Senses, 25, 413–421.

Ruiz-Avila, L., McLaughlin, S.K., Wildman, D., McKinnon, P.J., Robichon, A., Spickofsky, N. and **Margolskee, R.F.** (1995) *Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells*. Nature, 376, 80–85.

Sainz, E., Korley, J.N., Battey, J.F. and **Sullivan, S.L.** (2001) *Identification of a novel member of the T1R family of putative taste receptors*. J. Neurochem., 77, 896–903.

Schiffman, S.S., Lockhead, E. and **Maes, F.W.** (1983) *Amiloride reduces the taste intensity of Na+ and Li+ salts and sweeteners*. Proc. Natl Acad. Sci. USA, 80, 6136–6140.

Smith, D.V., Klevitsky, R., Akeson, R.A. and **Shipley, M.T.** (1994) *Taste bud expression of human blood group antigens*. J. Comp. Neurol., 343, 130–142.

Spielman, **A.I.** and **Brand, J.G.** (1995) *Collection of taste tissue from mammals.* In Spielman, A.I. and Brand, J.G. (eds), Experimental Cell Biology of Taste and Olfaction. CRC Press, Boca Raton, FL, pp. 25–32.

Spielman, A.I., Huque, T., Whitney, G. and **Brand, J.G.** (1992) *The diversity of bitter taste signal transduction mechanisms*. Soc. Gen. Physiol Ser., 47, 307–324.

Stevens, **D.R., Seifert, R., Bufe, B., Muller, F., Kremmer, E., Gauss, R., Meyerhof, W., Kaupp, U.B.** and **Lindemann, B.** (2001) *Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli.* Nature, 413, 631–635.

Stone, L.M., Finger, T.E., Tam, P.P. and **Tan, S.S.** (1995) *Taste receptor cells arise from local epithelium, not neurogenic ectoderm*. Proc. Natl Acad. Sci. USA, 92, 1916–1920.

Takami, S., Getchell, T.V., McLaughlin, S.K., Margolskee, R.F. and **Getchell, M.L.** (1994) *Human taste cells express the G protein alpha-gustducin and neuron-specific enolase*. Brain Res. Mol. Brain Res., 22, 193–203.

Tall, G.G., Krumins, A.M. and **Gilman, A.G.** (2003) *Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor*. J. Biol. Chem., 278, 8356–8362.

Ugawa, S., Minami, Y., Guo, W., Saishin, Y., Takatsuji, K., Yamamoto, T., Tohyama, M. and **Shimada, S.** (1998) *Receptor that leaves a sour taste in the mouth*. Nature, 395, 555–556.

Waldmann, R., Champigny, G., Bassilana, F., Voilley, N. and **Lazdunski, M.** (1995) *Molecular cloning and functional expression of a novel amiloride-sensitive Na^+ channel*. J. Biol. Chem., 270, 27411–27414.

Witt, M., Reutter, K. and **Miller, I.J.** (2003) *Morphology of the peripheral taste system.* In Doty, R.L. (ed.), Handbook of Olfaction and Gusation. Marcel Dekker, New York, pp. 651–677.

Wolverton, **T.** and **Lalande, M.** (2001) *Identification and characterization of three members of a novel subclass of protocadherins*. Genomics, 76, 66–72.

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

Wu, S.V., Rozengurt, N., Yang, M., Young, S.H., Sinnett-Smith, J. and **Rozengurt, E.** (2002) *Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells*. Proc. Natl Acad. Sci. USA, 99, 2392–2397.

Yan, W., Sunavala, G., Rosenzweig, S., Dasso, M., Brand, J.G. and **Spielman, A.I.** (2001) *Bitter taste transduced by PLC-beta(2)-dependent rise in IP(3) and alpha-gustducin-dependent fall in cyclic nucleotides*. Am. J. Physiol. Cell Physiol., 280, C742–C751.

Yokoe, **H.** and **Anholt, R.R.** (1993) *Molecular cloning of olfactomedin, an extracellular matrix protein specific to olfactory neuroepithelium*. Proc. Natl Acad. Sci. USA, 90, 4655–4659.

Zalewski, **A.A.** (1969) *Regeneration of taste buds after reinnervation by peripheral or central sensory fibers of vagal ganglia*. Exp. Neurol., 25, 429–437.

Zhang, **C.** and **Oakley, B.** (1996) *The distribution and origin of keratin 20-containing taste buds in rat and human*. Differentiation, 61, 121–127.

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