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Identification of Taste-Tissue-Specific cDNA Clones from a Subtraction cDNA Library of Rat Circumvallate and Foliate Papillae[1]

Misaki Asano-Miyoshi,*,†,‡,[2] Yuko Kusakabe,† Keiko Abe,† and Yasufumi Emori‡

Bio-oriented Technology Research Advancement Institution, 1-40-2 Nisshin-cho, Oomiya, Saitama 331-8537; †Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657; and ‡Department of Biophysics and Biochemistry, Division of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033

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To identify genes specifically expressed in taste tissues, we constructed a subtraction cDNA library of epithelium of rat circumvallate and foliate papillae and carried out differential screening of this library. Dot blot analysis showed 46 out of 88 clones obtained by this method to be expressed in the epithelium of papillae. The cDNA inserts in these clones were sequenced and analyzed for similarity to entries the GenBank database. About 54.3% of the clones were known sequences, including the sequences of ebnerin, cytokeratin 18, and Na^+,K^+-ATPase, that were shown by *in situ* hybridization to be expressed in the circumvallate papillae. About 41.3% of the papillae-specific clones had no significant similarity to known sequences and are candidates for novel taste bud-specific marker genes.

Key words: gustatory papillae, subtraction cDNA library, taste bud.

All vertebrates have the sensory organs specialized for gustatory chemoreception referred to as taste buds (*1, 2*). In mammals, large numbers of taste buds occur in three types of gustatory papillae on the dorsal surface of the tongue, the circumvallate papillae, foliate papillae, and fungiform papillae, as well as throughout the oral cavity (*1, 3*). Taste buds have discrete ovoid structures composed of about 50 cells of varying morphologic types surrounded by basement membrane. The cells in taste buds are reported to originate from the local epithelium (*1, 4*) and to turn over every 10 days (*1, 5, 6*). At least four cell types, types I to IV, are found in the taste buds of vertebrates. Nerve fibers are observed to penetrate and form synaptic contacts with several elongated cells designated as type III at the base of the taste bud (*1, 2, 7*). Although type III cells have been suggested to be taste receptor cells according to the observations described above, some controversial points remain concerning the classification, lineage, and function of the cells in taste buds. A taste bud is open to the oral environment through a taste pore. Taste transduction begins at the taste pore with taste receptor cells, and these cells transmit the signals of taste stimuli to the primary sensory nerve fibers. However, it is not known which molecules are involved in the primary recognition of various tastants, which intracellular signaling pathways are used in the individual taste receptor cell, or which molecules play roles in transmitting the patterns of activity from the receptor cells to the sensory axons.

To understand the molecular regulation of these processes of gustatory transduction, relevant subsets of genes that are specifically expressed in taste tissues must be identified, cloned, and studied in detail. Experiments involving the cloning and analysis of gustducin (*8-10*), multiple G-protein-coupled receptors (*11-13*), and a cyclic nucleotide-activated channel, CNGgust (*14*), have contributed greatly to our understanding of the mechanism of cell signaling in taste transduction in the past several years. In addition, several immunological markers have been found to be related to the morphological phenotypes of the cells, exemplified by sodium channels (*15*), NCAM (*16*), neuron-specific enolase (*17*), calbindin (*18*), keratins (*19, 20*), and the antigens of histo-blood groups (*21*). However, the cellular relationships among these genes and immunological markers are not known, and further information concerning the molecular biology of the individual cells and the organization of the taste bud would be useful for understanding taste signaling at the molecular level. Recently, Hoon and Ryba (*22*) reported the construction and sequence analysis of a taste bud-enriched cDNA library using hydroxyapatite chromatography. However, it seems to be difficult to achieve sufficient enrichment of taste bud-specific clones by the usual methods, partly because of the small number of taste cells.

Here, we constructed a subtracted cDNA library by a modification of the protocol for PCR-based cDNA subtraction (*23*) that includes an additional subtraction step using biotin-labeled driver cDNA. Differential screening of this library by dot blot analysis showed 4.6% of the clones to be expressed in the papillae. Included among them were known genes such as ebnerin, cytokeratin 18, and the Na^+,K^+-ATPase β subunit. On the other hand, about 41.3% of the papillae-specific clones showed no significant similarity to known sequences.

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[2] To whom correspondence should be addressed. Misaki Asano-Miyoshi, c/o Dr. Emori, Department of Biophysics and Biochemistry, Division of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033. Tel: +81-3-3812-2111, Fax: +81-3-5684-2394, E-mail: amiyoshi@hongo.ecc.u-tokyo.ac.jp

EXPERIMENTAL PROCEDURES

Construction of a cDNA Library—Adult rat tongue (Wister) epithelium was separated from the muscle tissue as follows. Collagenase (type I; Sigma) at 2 mg/ml in mouse T Ringer solution containing 3 mM HEPES, pH 7.0, 150 mM NaCl, 5 mM KCl, 4.5 mM $CaCl_2$, 7.8 mM glucose, and 2 mM $MgCl_2$ was injected between the dorsal epithelium and muscle layers, and the tongue was incubated for 1 h at room temperature in the Ringer solution. The epithelium was then removed from the muscle tissue with forceps and the region of papillae was dissected with Weckel's scissors from the surrounding epithelium.

Total RNA was prepared from the tongue epithelia from 24 rats by acid-phenol extraction using ISOGEN (Nippon Gene). Poly(A)$^+$ RNA was purified on an oligo(dT)-cellulose column.

PCR-based subtraction was first performed using a PCR-select cDNA subtraction kit (Clontech) following the manufacturer's instructions. In brief, double stranded cDNA was synthesized from 2 μg of poly(A)$^+$ RNA extracted from the epithelium of circumvallate papillae and foliate papillae (tester) and from the surrounding epithelium containing no taste buds (driver). Tester and driver cDNAs were digested with *Rsa*I. Then, a portion of one-third of the tester cDNAs was ligated with the adaptor I:

5′-CTAATACGACTCACTATAGGGCTGAGCGGCCGCCCGGGCAGGT-3′
3′-GGCCCGTCCA-5′

and another one-third was ligated with the adaptor II:

5′-GTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGT-3′
3′-CGGCTCCA-5′.

In order to subtract the cDNA population present in both the tester and driver samples, each of the adaptor-ligated tester cDNAs was first hybridized in 1×hybridization buffer (Clontech) at 68°C for 17 h with excess amounts of driver cDNAs, about 30 times as much as tester cDNAs, and 10 ng of two *Rsa*I fragments derived from 28S rRNA, which were very efficiently amplified through the following PCR steps. Then, the two samples from first hybridization and freshly denatured driver cDNAs, about 15 times as much as tester cDNAs, were mixed together and hybridized in 1×hybridization buffer at 68°C overnight. Residual single-stranded cDNAs were specifically amplified by PCR with Ex taq (Takara) with a primer corresponding to the common sequence of the 5′ end of the adaptors, 5′-CTAATACGCTCACTATAGGGC-3′ (primer 1). The PCR was performed 30 cycles using the following temperature profile: denaturing at 94°C for 30 s, annealing primers at 50°C for 30 s, extending the primers at 72°C for 1.5 min. Next, they were amplified again with nested primers corresponding the 3′ side of each adaptor, 5′-TCGAGCGGCCGCCCGGGCAGGT-3′ (primer a) and 5′-AGCGTGGTCGCGGCCAGGT-3′ (primer b). The PCR was performed for 15 cycles using the following temperature profile: denaturing at 94°C for 30 s, annealing primers at 68°C for 30 s, extending the primers at 72°C for 1.5 min.

Next, we added an additional subtraction step involving biotin-avidin precipitation of hybrid complexes to elevate the efficiency of subtraction. We amplified the *Rsa*I-digested and adaptor-ligated driver cDNAs with the 5′-biotinylated primers, 5′-CCGCCCGGGCAGGTAC-3′ (primer c) and 5′-GTCGCGGCCGAGGTAC-3′ (primer d), corresponding to the 3′ ends of the nested primers, a and b. The PCR was performed for 25 cycles using the following temperature profile: denaturing at 94°C for 30 s, annealing primers at 60°C for 30 s, extending the primers at 72°C for 1.5 min. The subtracted cDNAs were again hybridized in 1×hybridization buffer (Clontech) at 68°C overnight with the amplified driver cDNAs, about 10 times as much as the subtracted cDNAs, and hybrid complexes were precipitated with the streptavidin-agarose beads (Sigma) for 30 min at room temperature in TNE buffer containing 10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA. Residual single-stranded cDNAs containing tester-specific cDNAs were specifically amplified by PCR with primers, 5′-TCGAGCGGCCGCCCGG-3′ (primer e) and 5′-AGCGTGGTCGCGGCCG-3′ (primer f), corresponding approximately to the 5′ regions of primers a and b. The PCR was performed for 20 cycles using the following temperature profile: denaturing at 94°C for 30 s, annealing primers at 65°C for 30 s, extending the primers at 72°C for 1.5 min. At this time, driver cDNAs amplified with biotinylated primers c and d were not amplified with primers e and f under the conditions used here. Finally, the subtracted cDNAs obtained were ligated to pUC18 plasmid vector at the *Sma*I site and introduced into *Escherichia coli* JM83.

Differential Screening—White colonies were picked up, propagated in duplicate on the plates, and transferred onto nylon filters (Hybond N; Amersham). Each filter was prehybridized for 4 h at 65°C as described previously, and hybridized overnight at 65°C with [α-^{32}P]dCTP-labeled probes (*24*). One filter was hybridized with the subtracted cDNAs as a probe, and the other with the driver cDNAs amplified by PCR with the primers a and b. Finally, the filters were washed in 0.2×SSC containing 0.1% SDS at 65°C.

Dot Blot Hybridization—Dot blot hybridization was performed with Gene Images random prime labeling and detection system (Amersham) following the manufacturer's instructions. Ten nanograms of plasmid DNAs were denatured for 3 min at 95°C and transferred onto two nylon membranes (Hybond N). The membranes were then hybridized with fluorescein-dUTP-labeled cDNA probe at 60°C in hybridization buffer containing 5×SSC, 0.1% SDS, 5% dextran sulphate, and 1:20 dilution liquid blocking reagent. The probes were synthesized from tester and driver cDNAs prior to *Rsa*I digestion and adaptor ligation. Finally, the filters were washed in 0.5×SSC containing 0.1% SDS, blocked in 10% blocking solution in buffer A (100 mM Tris-HCl, pH 9.5, and 300 mM NaCl) at 60°C, and incubated with a 1:5,000 dilution of alkaline phosphatase-conjugated anti-fluorescein antibody for 1 h in buffer A containing 0.5% BSA. The filters were washed in buffer A and the CDP-star detection reagent was applied. The signals were detected on X-ray film.

RT-PCR and In Situ Hybridization—The longer cDNA fragments of ebnerin, cytokeratin 18, and Na^+,K^+-ATPase were cloned by RT-PCR with poly(A)$^+$ RNA extracted from tongue epithelium, and used for *in situ* hybridization experiments. The sequences of the primers were 5′-AGCAAACGATGTCTCCTCAC-3′ and 5′-AAAGTCAGTATAAAAGGGGG-3′ for ebnerin (gene accession No. U32681), 5′-CAAGATCATCGAAGACCTGA-3′ and 5′-TCTGACTGTACGTCTCAGCT-3′ for cytokeratin 18 (gene accession

No. X81448), and 5′-AATCTTTCCCACTAGCCATT-3′ and 5′-GACACAAGACCAACAAGAGC-3′ for Na^+,K^+-ATPase (gene accession No. J02701). Each fragment was subcloned into SK(−) pBluescript vector and digoxygenin-UTP-labeled RNA probes were synthesized by T3 or T7 RNA polymerase.

In situ hybridization was performed as follows (*25*). The circumvallate papillae of the tongue of a 5-week-old rat (Fisher) was removed, frozen in O.C.T. compound, and sectioned into 10 μm slices. The sections were post-fixed for 10 min in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.0, washed three times in PBS for 5 min, acetylated in 0.1 M triethanolamine (pH 8.0) containing 0.25% acetic anhydride for 10 min, washed three times in PBS for 5 min, and prehybridized in prehybridization solution containing 50% formamide and 5×SSC for 1 h at room temperature. Then, 0.1 ml of hybridization buffer containing 50% formamide, 5×SSC, 5×Denhardt's solution, 500 μg/ml sonicated, denatured herring sperm DNA, 250 μg/ml torula tRNA, 1 mM DTT, and 10 ng of probe was added to the slide. The slide was covered with a siliconized coverslip and hybridized overnight at 72°C. The coverslips were removed by soaking in 5×SSC at 72°C, and the slides were washed two times in 0.2×SSC for 30 min at 72°C. The sections were washed in TBST for 5 min, blocked in 0.5% blocking reagent (Boehringer) in TBS for 1 h at room temperature, and incubated in the same solution containing a 1:200 dilution of anti-digoxygenin Fab fragments conjugated with alkaline phosphatase for 1 h. Then the slides were washed three times in TBST for 15 min and once in the alkaline phosphatase buffer containing 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, and 50 mM $MgCl_2$ for 5 min. The color reaction was performed by adding the color developing solution containing nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in alkaline phosphatase buffer onto the slide.

RESULTS

Construction of a Taste Tissue-Specific Library—cDNAs synthesized from the epithelia of rat circumvallate papillae and foliate papillae poly(A)$^+$ RNAs were first subtracted from cDNAs derived from poly(A)$^+$ RNAs of the surrounding epithelium essentially according to the instructions of a PCR-select cDNA subtraction kit (Clontech). To examine the efficiency of subtraction, we performed differential screening on 1,217 colonies with [α-^{32}P]dCTP-labeled probes synthesized from the uncloned subtracted cDNAs and driver cDNAs amplified by PCR with primers corresponding to the adaptors (see "MATERIALS AND METHODS"). However, we failed to obtain clones that hybridized only with the subtracted cDNA probe. Although 23 clones gave more intense signals with the subtracted cDNA probe than with the driver cDNA probe (data not shown), the subtraction appeared to be insufficient. Therefore, we added a secondary subtraction step involving the precipitation of the avidin-biotin complex to elevate the efficiency of subtraction (see "MATERIALS AND METHODS"). As a result, the second differential screening yielded 26 out of 1,000 clones that hybridized only with the subtracted cDNA probe and 62 clones that gave more intense signals with the subtracted cDNA probe than with the driver cDNA probe (data not shown).

To confirm the differential expression of these clones, we carried out dot blot analysis on their plasmid DNAs using tester and driver cDNAs as probes (Fig. 1). A few clones showed no difference in signal intensity between the two blots, and finally, 42 clones showed little or no difference in signal intensity between the two probes. In the end, 46 out of 88 clones (52.3%) were confirmed to show more intense signals with the tester cDNA probe than with the driver cDNA probe.

Analysis of the Nucleotide Sequences of Clones from the Subtracted Library—The cDNA inserts of the 88 clones (46 clones, tester>driver; 42 clones, tester≒driver) were sequenced and analyzed for similarity at the NCBI by the

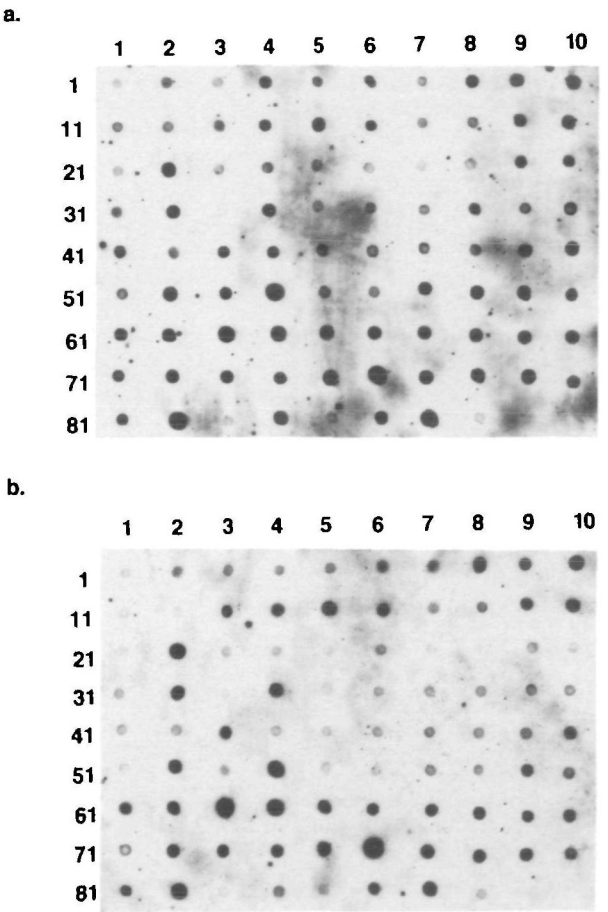

Fig. 1. **Dot blot analysis of the clones hybridized with the differential probes.** Plasmid DNAs of the 88 clones picked up as a result of differential screening (see text) were dot-blotted onto nylon membranes in duplicate in numerical order. They were then hybridized with two different probes, the tester cDNA probe (a) and the driver cDNA probe (b). Clones that showed more intense signals with the tester cDNA probe than with the driver cDNA probe were clones 2, 4, 5, 6, 9, 11, 12, 17, 18, 21, 23, 24, 25, 27, 28, 29, 30, 31, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 51, 53, 56, 57, 58, 60, 69, 71, 72, 79, 80, 84, 86, and 87. The novel nucleotide sequences among them will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession numbers as follows: clone 2, AB016903; 5, AB016923; 11, AB016921; 25, AB016920; 27, AB016904; 30, AB016905; 31, AB016906; 35, AB016907; 36, AB016908; 37, AB016909; 38, AB016910; 40, AB016922; 41, AB016911; 48, AB016912; 49, AB016913; 51, AB016914; 53, AB016915; 56, AB016916; 58, AB016917; 60, AB016918; 80, AB016919.

BLAST Homology Search Program (*26*) using the GenBank databases. The results are summarized in Table I. Among the 46 differentially expressed genes, 11 clones were found to be identical to known rat genes and 7 showed similarities to known mammalian genes that were greater than 60%. The clones showing high similarities to known genes are listed in Table IIa. They include ebnerin (clone No. 44) (*27*) and cytokeratin 18 (clone No. 72) (*28*), which have previously been reported to be expressed in the circumvallate papillae. Seven clones showed significant similarities greater than 80% to expressed sequence tags (ESTs) (Table IIIa). Two clones contained some repetitive sequences registered in the database. The remaining 19 clones showed no significant similarity to entries in the database and include cDNAs from potentially novel taste bud-specific genes. Among the 42 clones not specific for tester cDNAs, 4 were fragments of 28S rRNA, and the others included genes abundant in the epithelium, such as radixin (No. 78) and keratin 6 (No. 82).

TABLE I. **Summary of the sequences and clones contained in the subtracted library.**

	No. clones (%) tester > driver	No. clones (%) tester ≒ driver	Total (%)
Known genes			
Exact match	11 (23.9%)	7 (16.7%)	18 (20.5%)
With high homology	7 (15.2%)	11 (26.2%)	18 (20.5%)
ESTs	7 (15.2%)	7 (16.7%)	14 (15.9%)
Repetitive sequences	2 (4.3%)	6 (14.3%)	8 (9.1%)
No data base match	19 (41.3%)	11 (26.2%)	30 (34.0%)
Total	46 (52.3%)	42 (47.8%)	88

TABLE II. **List of clones showing high similarity to known genes.** Clones that have similarities greater than 60% to known genes in the GenBank database are listed in numerical order with the accession numbers of the database entries. (a) List of the papillae-specific clones showing signals that were more intense with the tester cDNA probe than with the driver cDNA probe (see text). (b) List of non-specific clones. The clones without listed similarities are rat genes showing 100% homology, except clone No. 45, which showed 70% similarity to rat VCAM-1.

a. tester > driver

Clone	Gene (organism)	Accession	Homology
6	c-Ha-Ras	L06433	
9	Ebnerin	U32681	
12	eIF4γ (human)	D12686	73%
17	eIF5	L11651	
18	eIF5	L11651	
21	KIAA0069 (human)	D31885	92%
23	eph A1 (human)	M18391	78%
24	eIF3p110 (human)	U46025	91%
28	Na^+,K^+-ATPase β subunit	J02701	
29	Plakoglobin	U58858	
44	Ebnerin	U32681	
45	VCAM-1	L22355	70%
47	TIG-1 (human)	U27185	70%
57	Plakoglobin	U58858	
71	Common salivary protein	U00964	
72	Cytokeratin18 (human)	M26326	84%
84	Calpactin II	G20325	
86	Heparin binding growth associated molecule	M55601	

b. tester ≒ driver

Clone	Gene (organism)	Accession	Homology
7	KIAA0137 (human)	D50927	74%
14	ATPase subunit 6	M27315	
15	PYK2 (human)	U33284	62%
16	son-P (human)	X63754	95%
19	Coprophirinogen oxydase (mouse)	D16333	88%
20	BTG-1	L26268	78%
22	28S rRNA	V01270	
62	Cytokeratin 16 (human)	S79867	85%
63	28S rRNA	V01270	
64	28S rRNA	V01270	
66	f8a (mouse)	M83118	92%
70	SRp30c (human)	U30825	88%
74	TopoisomeraseII	Z46372	
76	28S rRNA	V01270	
78	Radixin (mouse)	X60672	93%
82	Cytokeratin 6	D78059	
83	Surfeit4 protein (mouse)	M63114	96%
85	TIS II regulated protein (mouse)	M58564	93%

In Situ Hybridization Analysis of the Clones—To investigate the expression of the tester-specific genes in the epithelium of papillae at the cellular level, we carried out *in situ* hybridization analysis using as probes several genes cloned from the library. However, the subtracted library was constructed from cDNAs digested with *Rsa*I giving an average clone length of about 160 basepairs (data not shown), too short to be sensitive enough for *in situ* hybridization experiments. Thus, as an initial step to examine the expression profiles of tester-specific cDNA clones, we selected three genes, ebnerin, a protein secreted from von Ebner's gland, cytokeratin 18, a major structural protein, and the β subunit of Na^+,K^+-ATPase, a sodium pump, as probes because their nucleotide sequences were available from the database and longer fragments could be easily obtained by RT-PCR. As described in "MATERIALS AND METHODS," three RT-PCR fragments of 630, 443, and 1,090 basepairs, corresponding to ebnerin, cytokeratin 18, and Na^+,K^+-ATPase, respectively, were obtained and used for *in situ* hybridization analysis.

As shown in Fig. 2A, ebnerin is highly expressed in von Ebner's glands, whose ducts open into the trough at the base

TABLE III. **List of clones with limited sequence homology to GenBank EST entries.** Clonal sequences showing no significant matches with sequences in the GenBank were compared with GenBank ESTs. Clones that showed some significant similarity to ESTs in the database are listed in numerical order with the accession numbers and the organism and tissues. Match quality is presented as the ratio of the number of identical base pairs in the stretches identified with the highest match. (a) List of papillae-specific clones. (b) List of non-specific clones.

a. tester > driver

Clone	Accession	Organism, tissue	%ID
4	W38904	human, parathyroid tumor	92% (92/100)
39	AA530683	mouse, heart	93% (122/130)
42	N38836	human	94% (50/53)
46	AA505824	human	93% (78/83)
69	AA664508	human, brain	94% (253/270)
79	H32433	rat	96% (165/171)
87	AA596585	mouse, T cell	95% (125/132)

b. tester ≒ driver

Clone	Accession	Organism, tissue	%ID
1	H00099	rat, small intestine	95% (45/47)
13	AA629579	human, lung carcinoma	81% (61/83)
26	AA108471	mouse	95% (165/174)
34	AA217340	mouse, lymph node	100% (56/56)
61	AA612103	mouse, skin	88% (96/109)
65	AA511166	mouse, mammary gland	93% (73/78)
77	AA022286	human	83% (98/117)

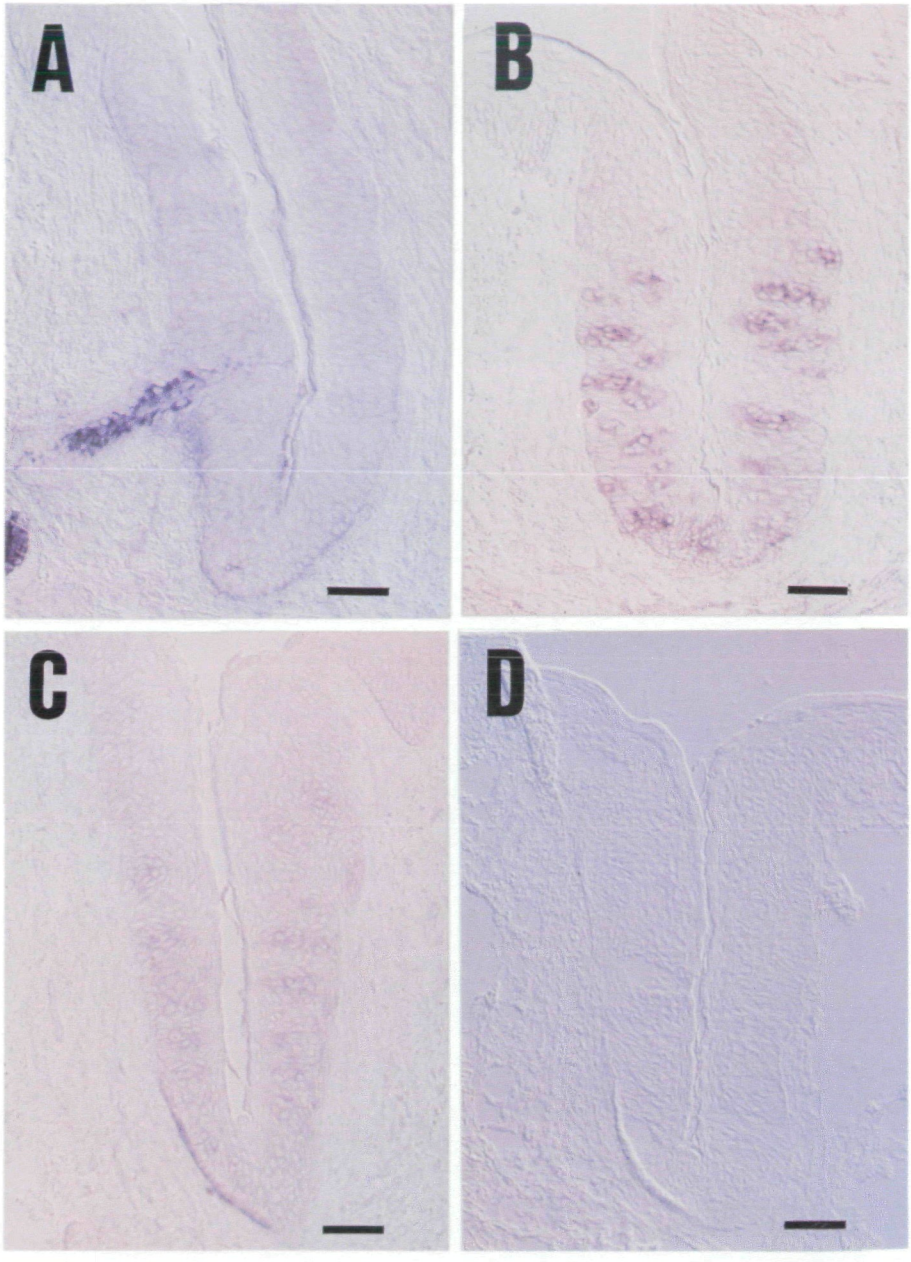

Fig. 2. Examples of *in situ* hybridization analysis with cloned papillae-specific cDNAs. *In situ* hybridization experiments on the circumvallate papillae with antisense RNA probes to ebnerin (A), cytokeratin 18 (B), and the β subunit of Na^+,K^+-ATPase (C) were performed. Panel D is the result of hybridization with sense probe to the β subunit of Na^+,K^+-ATPase as a control. Bars represent 50 μm.

of the papillae. Cytokeratin 18 is specifically expressed in taste bud cells in the papillae (Fig. 2B), although some signals were detected in the ducts of von Ebner's gland (data not shown). The expression of the β subunit of Na^+,K^+-ATPase (clone No. 28) (*29*) was also detected in taste buds (Fig. 2C) as well as the muscular tissues underneath the papillae (data not shown). Thus, the tester-specific cDNA clones obtained here appear to be useful as molecular markers in taste tissues at the cellular level, at least for *in situ* hybridization and dot blot analyses.

DISCUSSION

The difficulty in carrying out biochemical and molecular biological studies of taste transduction compared with olfactory or light transduction lies in the small number of taste receptor cells. The limited number of molecular markers available adds to the difficulty. Therefore, we developed a modified PCR-based subtraction method to construct a taste bud-specific cDNA library from which to isolate cDNA probes specific for taste tissues. Differential screening of the library using tester and driver cDNAs as hybridization probes yielded 46 clones whose signals with the tester cDNA probe were more intense than with the driver cDNA probe, accounting for 4.6% of the clones in the library (Fig. 1, Table I). Clones not specific for the tester were thought to be fragments of abundantly expressed cDNAs contained in both the tester and driver cDNAs. These might have failed to be subtracted thoroughly and been amplified by PCR. Although some of these genes were also cloned after differential screening and eliminated by dot blot experiments, the overall enrichment of taste tissue-specific clones was successful compared with the usual methods reported previously (*22*).

Sequence analysis of the clones revealed about 54.3% to show significant similarity to known genes. Two genes known to be expressed in circumvallate papillae were also cloned, ebnerin (*27*) and cytokeratin 18 (*20*). Although ebnerin is a secreted protein expressed in von Ebner's gland and not the product of a taste-specific gene, the ducts of von Ebner's gland open into the papillae (Fig. 2). Therefore, it is reasonable that ebnerin would be cloned in this subtracted library. Cytokeratin 18 is one of cytokeratins, which are major structural proteins of the vertebrate epidermis and its appendages. At least 18 types of cytokeratins have been found to be expressed in skin in complex patterns (*30*). Taste buds are reported to contain materials that immunoreact with antibodies against cytokeratins 7, 8, 19, and 18. Among them, cytokeratin 18 is considered to be expressed in fully differentiated taste cell (*31*). Our *in situ* hybridization experiments also showed cytokeratin 18 to be expressed precisely in elongated cells in taste buds (Fig. 2B), consistent with the immunohistochemical results. Although other cytokeratins, cytokeratins 16 (clone No. 62) (*32*) and 6 (clone No. 82) (*33*), were cloned in our screening, these clones turned out not to be specific for the tester cDNA probe by dot blot analysis.

Several kinds of molecules related to cell adhesion were also cloned, including plakoglobin (clone No. 29 and 57) and a VCAM-1-like cDNA (clone No. 45). These may have some function in taste bud maintenance by contributing to cell-to-cell adhesion. The isolation of eukaryotic initiation factors 3 (clone No. 24), 4 (clone No. 12), and 5 (clone No. 17 and 18) might reflect the high translational activity of taste tissues, possibly connected with differential gene expression in taste cells. A fragment of the β subunit of Na^+,K^+-ATPase, a type of sodium pump, was also included among the clones. This pump is known to participate in maintaining the unequal distribution of sodium and potassium ions across the plasma membrane (*34*) and is widely distributed in several tissues including kidney, brain, and muscle (*35*). Our *in situ* hybridization results showing that the β subunit of Na^+,K^+-ATPase is expressed especially in taste buds suggest that it performs a specific function in the pathway of taste transduction.

On the other hand, 41.3% of the clones obtained showed no significant similarity to known genes, a finding that seems most intriguing because these clones might include novel and representative clones of genes not yet identified. We are now starting to investigate these clones.

Recently, Hoom and Ryba (*22*) have also analyzed the sequences of 410 clones from their own subtracted library of circumvallate papillae and reported that they included Pax-1, esp1, Notch, and many other genes. Secreted proteins, structural proteins, adhesion molecules, and eukaryotic initiation factors were included in both their and our libraries, although the number of identical clones found in both libraries was limited, probably because of the difference in the methods of library construction and the small number of clones analyzed at nucleotide sequence level.

In summary, we have shown that taste-specific markers could be successfully enriched in this subtracted cDNA library by our modified method. Further screening and analysis of these clones would appear to be useful for identifying molecules that play important roles in gustatory transduction.

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