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Faithful Expression of GFP from the PLCβ2 Promoter in a Functional Class of Taste Receptor Cells

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Abstract

Phospholipase C–type β2 (PLCβ2) is expressed in a subset of cells within mammalian taste buds. This enzyme is involved in the transduction of sweet, bitter, and umami stimuli and thus is believed to be a marker for gustatory sensory receptor cells. We have developed transgenic mice expressing green fluorescent protein (GFP) under the control of the PLCβ2 promoter to enable one to identify these cells and record their physiological activity in living preparations. Expression of GFP (especially in lines with more than one copy integrated) is strong enough to be detected in intact tissue preparations using epifluorescence microscopy. By immunohistochemistry, we confirmed that the overwhelming majority of cells expressing GFP are those that endogenously express PLCβ2. Expression of the GFP transgene in circumvallate papillae occurs at about the same time during development as endogenous PLCβ2 expression. When loaded with a calcium-sensitive dye *in situ*, GFP-positive taste cells produce typical Ca^{2+} responses to a taste stimulus, the bitter compound cycloheximide. These PLCβ2 promoter–GFP transgenic lines promise to be useful for studying taste transduction, sensory signal processing, and taste bud development.

Key words: GFP, mouse, PLCβ2, taste bud, taste-specific promoter, transgenic

Introduction

In mammals, the G-protein–coupled taste receptors for sweet, bitter, and umami tastes are each expressed in limited subsets of cells within taste buds of rats and mice (Chandrashekar *et al.*, 2000; Chaudhari *et al.*, 2000; Nelson *et al.*, 2001, 2002). Signal transduction downstream of taste receptors results in transient elevation of cytoplasmic Ca^{2+} concentration by release of stored Ca^{2+}. Phospholipase C (PLC) is a key mediator of Ca^{2+} release, and many taste cells express the beta-2 isoform [phospholipase C–type β2 (PLCβ2)] of this enzyme (Rossler *et al.*, 1998, 2000). *In situ* hybridization and immunocytochemical analyses show that taste cells expressing taste receptors also express PLCβ2 (Miyoshi *et al.*, 2001; Chaudhari *et al.*, 2003; Zhang *et al.*, 2003). In mice, genetic ablation of PLCβ2 results in a reduction or loss of sensitivity to these taste modalities, indicating that this enzyme is important for the transduction sweet, bitter, and umami stimuli (Zhang *et al.*, 2003; Dotson *et al.*, 2005). In sum, our current understanding of gustatory transduction is that stimulating taste receptors leads to activation of PLCβ2, generation of inositol 1,4,5-triphosphate (IP_3), and release of Ca^{2+} from intracellular stores (Ming *et al.*, 1998; Huang *et al.*, 1999; Yan *et al.*, 2001). Therefore, identifying taste cells that express PLCβ2 *in vivo* would provide a useful tool for studying taste transduction. To achieve this, we have developed transgenic mice expressing GFP under the control of the PLCβ2 promoter. Our data indicate that a 2.9-kb fragment of the PLCβ2 promoter is sufficient to confer cell-type–specific expression in taste cells.

Materials and methods

Generation of PLCβ2 promoter–GFP transgenic mice

We obtained the PLCβ2 gene and its 5′ upstream region on mouse chromosome 2 from BAC clone RP23-172B16 (accession number AL772255) from BACPAC Resources Center (Oakland, CA). The PLCβ2 promoter was isolated as an ≈8-kb *Xho*I–*Sma*I fragment that includes the presumed transcription and translation start sites within exon 1. The translated region of this fragment was removed using polymerase

chain reaction (PCR)–based mutagenesis, and the remainder was cloned into the EGFP-BasicII vector to yield the 8.0-PLC–GFP promoter–reporter construct (Figure 1A). We previously generated this vector by removing the *lacZ* gene from the pβGal-Basic vector (Clontech, Mountain View, CA) and replacing it with cDNA for enhanced green fluorescent protein (herein referred to as GFP) from pIRES-EGFP (Clontech). The 2.9-PLC–GFP construct was then produced by removing a *Kpn*I–*Kpn*I ≈5.0-kb segment from the 5′ end of the aforementioned 8.0-PLC–GFP promoter–reporter construct. Both constructs were validated by sequencing their ends. The plasmids were linearized with *Sma*I and *Sal*I to remove vector sequences, and transgenic fragments were gel purified (Figure 1A) prior to injection. DNA injection into the pronuclei of fertilized C57BL/6J × SJL/J mouse eggs and transfer into foster mothers were performed by the University of Miami Transgenic Facility. Founder mice were bred with wild-type C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) to establish the transgenic lines.

Genotyping

All experimental procedures followed the National Institutes of Health (NIH) Guidelines for the Care and Use of Animals and were approved by the University of Miami Animal Care and Use Committee. To assess the presence of transgene, we carried out genotyping PCR on tail DNA from offspring using Platinum supermix (Invitrogen, Carlsbad, CA). The number of integrated transgene copies was determined with the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using IQ SYBR Green Supermix (Bio-Rad Laboratories). Genotyping PCR primers (0.1 μM in reaction) were designed using Beacon Designer (Bio-Rad Laboratories). In conventional and real-time PCRs, primers (see locations in Figure 1A) used were as follows—for endogenous PLCβ2: (a) 5′-TGCCATGTGAGCCTAGCCTAAG-3′ and (c) 5′-GCAATAGAACAGGGTTGAGCAAAG-3′; for either transgene: (a) as above and (b) 5′-CTCCTGGACGTAGCCTTCGG-3′; to distinguish the 8.0-PLC–GFP transgene: (d) 5′-TGAGAAGGAGTAAGAAAGGGACAG-3′ and (f) 5′-TTGAAGAAGTCGTGCTGCTTCA-3′; and to distinguish the 2.9-PLC–GFP transgene: (e) 5′-GCATCAGGTGAGAAAATTATCCAC-3′ and (f) as above. Each reaction contained 100 ng of tail DNA (or water as a negative control). Optimal annealing temperatures were identified for each primer pair by employing a temperature gradient PCR in the iCycler (Bio-Rad Laboratories). After 40 cycles of amplification, the homogeneity of the PCR product was confirmed through a melting temperature paradigm. Quantitative standard DNA templates were derived from DNA sequence–validated PCR products for the transgene and endogenous gene versions of PLCβ2. Transgene copy number per genome was calculated as the ratio of transgene copies to endogenous PLCβ2 gene copies in F1 (heterozygous) mice (Figure 1C).

GFP fluorescence and immunocytochemistry

GFP expression was confirmed within whole-mounted tongue and palate using a Zeiss Axioplan epifluorescent microscope. Mouse circumvallate, foliate, fungiform, and palate tissue were fixed in 4% paraformaldehyde and cryoprotected in 30% sucrose overnight at 4°C. Frozen sections (25 μm) were prepared and blocked in 4% bovine serum albumin and 0.3% TritonX-100 in 1× phosphate-buffered saline for one and a half hours at room temperature. Sections were then incubated overnight with rabbit anti-PLCβ2, diluted 1:1000 (#SC-206, Santa Cruz Biotechnology, Santa Cruz, CA), followed by donkey anti-rabbit conjugated to Alexa 594 (1:1000) (Molecular Probes, Eugene, OR). In some cases, GFP was visualized by immunocytochemistry using mouse anti-GFP, diluted 1:1000 (#11814460001, Roche Applied Science, Columbia, NY), and donkey anti-mouse conjugated to Alexa 488 (1:1000) (Santa Cruz Biotechnology). Similar results were obtained whether GFP was viewed by its native fluorescence or by immunocytochemistry. Both these antibodies are extensively characterized and highly specific (e.g., Clapp *et al.*, 2004).

Immunostained slides were photographed on a Zeiss Axioplan epifluorescent microscope using Axiovision version 3.0 software. Approximately, 5–15 images of at least 25 vallate and foliate taste buds were used to quantify the expression patterns. Only cells with visible nuclei (inferred from the absence of PLCβ2 staining) were counted.

Calcium imaging

We obtained vallate papillae from adult transgenic mice (5288 line, ≥8 weeks old) and loaded taste cells with the fluorescent calcium indicator dye, Calcium Orange (CaO; 1 mM in H_2O; Molecular Probes) as previously described (Richter *et al.*, 2003). Briefly, CaO was injected iontophoretically (−3.5 μA square pulses, 10 min) through a glass micropipette (40-μm tip diameter) into the crypts of the vallate papilla. The tissue was then sliced at 100 μm with a Leica VT1000S vibratome (Nussloch, Germany), and slices containing vallate taste buds were mounted on a glass coverslip coated with Cell-Tak (Becton Dickinson, Franklin Lakes, NJ), placed in a recording chamber, and superfused with Tyrode's solution at 30°C at a rate of 2 ml/min. Stimuli in Tyrode's solution (in mM: 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 $NaHCO_3$, 10 4-(2-hydroxyethyl)-1-piperazineethane sulfonate, 10 glucose, and 10 sodium pyruvate, pH 7.3) were perfused for 60 s. GFP and CaO fluorescence were viewed with an Olympus (Melville, NY) Fluoview scanning laser confocal microscope using 488- or 568-nm excitation, respectively. To measure ΔCa^{2+}, CaO fluorescence signals were captured at 5-s intervals and expressed as relative fluorescence change: $\Delta F/F = (F - F_0)/F_0$, where F_0 denotes the resting fluorescence level corrected for bleaching. Using $\Delta F/F$ corrects for variations of baseline fluorescence, cell thickness, total dye concentration, and illumination (Helmchen, 2000).

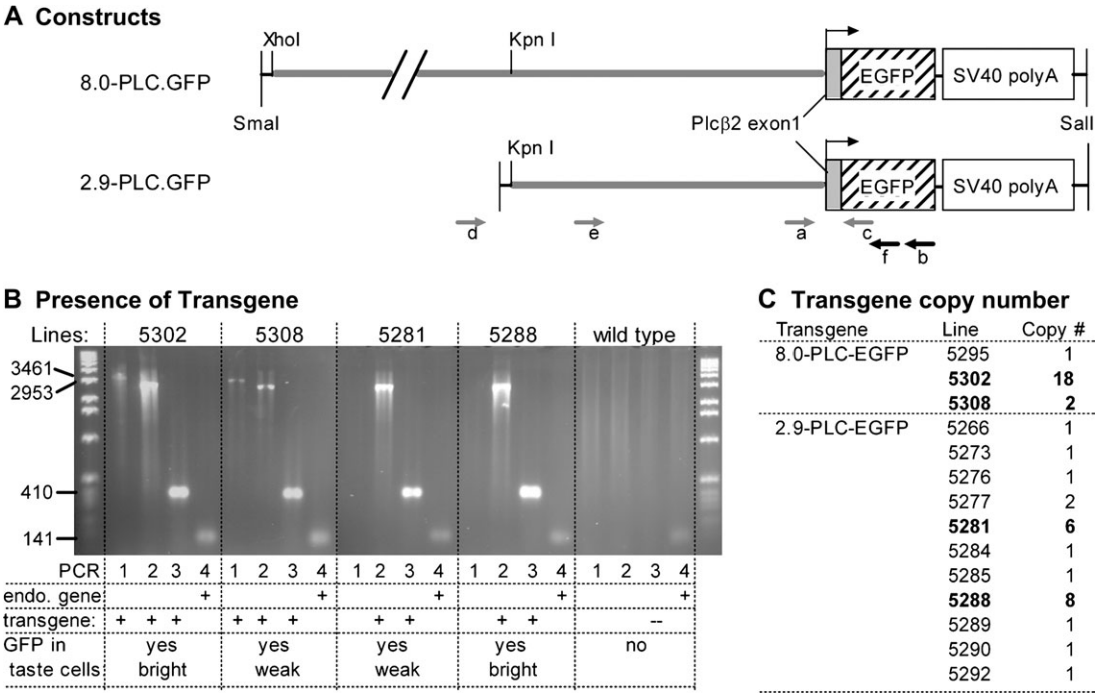


Figure 1 Reporter constructs, genotype and phenotype of transgenic mice expressing GFP, driven by the promoter for PLCβ2. **(A)** The transgenes contain either the proximal 8.0 kb (8.0-PLC–GFP) or 2.9 kb (2.9-PLC–GFP) of promoter sequence upstream of the putative transcription start site and ≈115 bp of exon 1 from the PLCβ2 gene. The GFP cDNA sequence contains the ATG translational start site preceded by a Kozak sequence. Restriction sites used for the cloning, the transcription start (bent arrow), exon 1 of PLCβ2, and the locations and names of PCR primers used in genotyping (gray arrows for PLCβ2 sequence; black arrows for GFP sequence) are all indicated. **(B)** Genotyping of four lines of transgenic mice. PCR #1: using primer pair d/f for long-distance PCR of the 8.0-kb transgene only; PCR #2: using primers e/f for long-distance PCR for either transgene; PCR #3: using primers a/b for either transgene; and PCR #4: using primers a/c for the endogenous PLCβ2 (primer c is located in exon 1, in a region lacking from the transgene). The expected sizes of all amplification products are shown on the left of the gel. **(C)** The number of copies of the transgene integrated per haploid genome was determined by real-time PCR and calculated relative to the endogeneous diploid PLCβ2 gene. Lines that showed strong GFP fluorescence in taste tissues are in bold font.

Results

Generation of PLCβ2–GFP transgenic mice

Prior to injection for producing transgenic mice, we validated two GFP reporter constructs (including 2.9 or 8.0 kb of the PLCβ2 promoter) by lipofection into taste buds (Landin *et al.*, 2005). Injected eggs produced 30 and 20 live births (for the 2.9- and 8.0-kb constructs, respectively), of which 11 and 3 were genotype positive, respectively (Table 1 and Figure 1B,C). Progeny of all the genotype-positive founder mice were evaluated for GFP expression. GFP fluorescence was observed in two lines each of the 2.9-kb and two 8.0-kb transgenics, and all of these mice have multiple copies of the transgene.

GFP expression in four taste fields

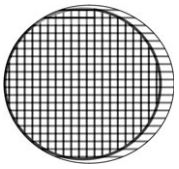
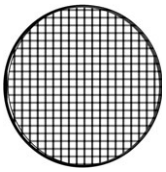
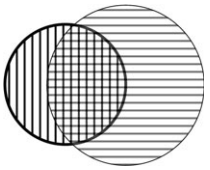
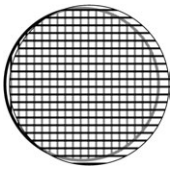
GFP expression was initially observed in freshly dissected, that is, living whole-mounted lingual and palatal tissues by epifluorescent microscopy (data not shown) to identify transgenic lines for further study. Tissues from four GFP-expressing lines were then fixed and processed for immunocytochemical detection of endogenous PLCβ2 using a well-characterized antibody. Four major taste fields were analyzed: circumvallate, foliate papillae, the anterior tongue, and the palate. By fluorescence microscopy, we analyzed the extent to which PLCβ2 immunoreactivity overlapped with GFP fluorescence (Table 2). In some instances, this analysis was repeated using an antibody against GFP in double immunocytochemistry. Qualitatively similar results were obtained with anti-GFP (not shown) as when directly viewing GFP fluorescence. In taste tissue, PLCβ2 immunoreactivity was strictly limited to a subset of cells within taste buds. To confirm specificity, we immunostained sections of vallate or foliate papillae from PLCβ2-knockout mice (Jiang *et al.*, 1997). As expected, no immunofluorescence was detected in these taste cells (Figure 2C).

We quantified overlapping expression of GFP and endogenous PLCβ2 and found that three of the transgenic lines showed GFP consistently in cells expressing PLCβ2 (Table 2; Figure 2). Mice in which GFP was a faithful reporter for PLCβ2 were derived from founders injected with either the 2.9-PLC–GFP or the 8.0-PLC–GFP construct. In all of the animals showing overlap between GFP and PLCβ2

Table 1 Production of PLCβ2–GFP transgenic mouse by DNA microinjection

Transgene construct	Number of eggs injected/transfered	Number of foster mothers	Number of successful pregnancies	Number of pups born	Number of pups genotype positive	Number of GFP-expressing transgenic lines
2.9-kb PLC–GFP	208	9	6	30	11	2
8.0-kb PLC–GFP	188	9	4	20	3	2

Table 2 Distribution of GFP and endogenous PLCβ2 among vallate and foliate taste cells in adult mice of four transgenic lines

Transgenic lines	2.9-kb PLC–GFP		8.0-kb PLC–GFP	
	5281	5288	5302	5308
Number of taste buds counted	25	90	202	67
Number of GFP-expressing cells	96	576	453	241
Number of PLCβ2-immunoreactive cells	115	570	755	263
Number of overlapping cells	96	569	280	233
Venn diagram depicting PLCβ2/GFP overlap				
	84%	99%	30%	96%

PLCβ2-immunoreactive cells and GFP-expressing cells were counted in two to six photographed fields of vallate and foliate taste papillae from each transgenic line. For each transgenic, the Venn diagram at bottom represents the overlap between PLCβ2 (horizontal hatched) and GFP (vertical hatched)-expressing cells (i.e., PLCβ2 ∩ GFP). Percentages indicate the fraction of all counted cells that express both proteins.

expression, there were no differences in the degree of overlap among the different taste fields—circumvallate, foliate, fungiform, or palate. These data indicate that the 2.9-kb fragment of the PLCβ2 promoter is sufficient to confer cell-type–specific expression in taste cells. One of the transgenic lines from the 8.0-kb promoter showed poor overlap between GFP and PLCβ2. We speculate that this may reflect a position effect of the integration site.

GFP expression in the developing circumvallate papillae

Next, we studied how the expression of the transgene was regulated during the postnatal development. We analyzed circumvallate papillae from 1-, 3-, and 10-week-old transgenic mice from the 5288 line (one of the lines derived from the 2.9-kb promoter). At 1 week, only a few cells per taste bud expressed either GFP or PLCβ2. Nevertheless, in those cells in which expression could be detected, there was a high degree of overlap between GFP and PLCβ2. We noted that GFP fluorescence was relatively less intense as compared to adults (compare Figure 4A to Figure 3A), but this was not systematically investigated. By 3 weeks, the number of cells positive for GFP and PLCβ2 had reached adult levels, and there was excellent overlap of expression (Figure 4G–I). We quantified this pattern by counting the cells and found that at all three stages, 94–99% of cells that expressed either GFP or PLCβ2 expressed both (60 of 64 cells at P7; 191 of 197 cells at P21; and 312 of 316 cells at P70). Overall, our results suggest that the expression of GFP was regulated similarly to the endogenous PLCβ2 promoter throughout the postnatal development.

GFP cells respond to tastant stimuli

PLCβ2 is believed to act downstream of the T1R and T2R receptors (Zhang *et al.*, 2003), leading to IP_3 formation and Ca^{2+} release inside taste receptor cells upon stimulation with tastants. We asked whether cells expressing GFP and PLCβ2 in the transgenic mice responded to taste stimuli, as predicted. We conducted calcium imaging experiments on living tissue preparations in which GFP cells could be identified *in situ*. Taste buds in lingual slices of vallate papillae were loaded with the indicator dye, CaO, to monitor the intracellular Ca^{2+} levels with confocal laser scanning microscopy (Richter *et al.*, 2003). We identified cells expressing "both" GFP and CaO and tested whether they responded to cycloheximide, a bitter tastant that effectively stimulates many taste cells in mice (Caicedo *et al.*, 2002). Figure 4 illustrates

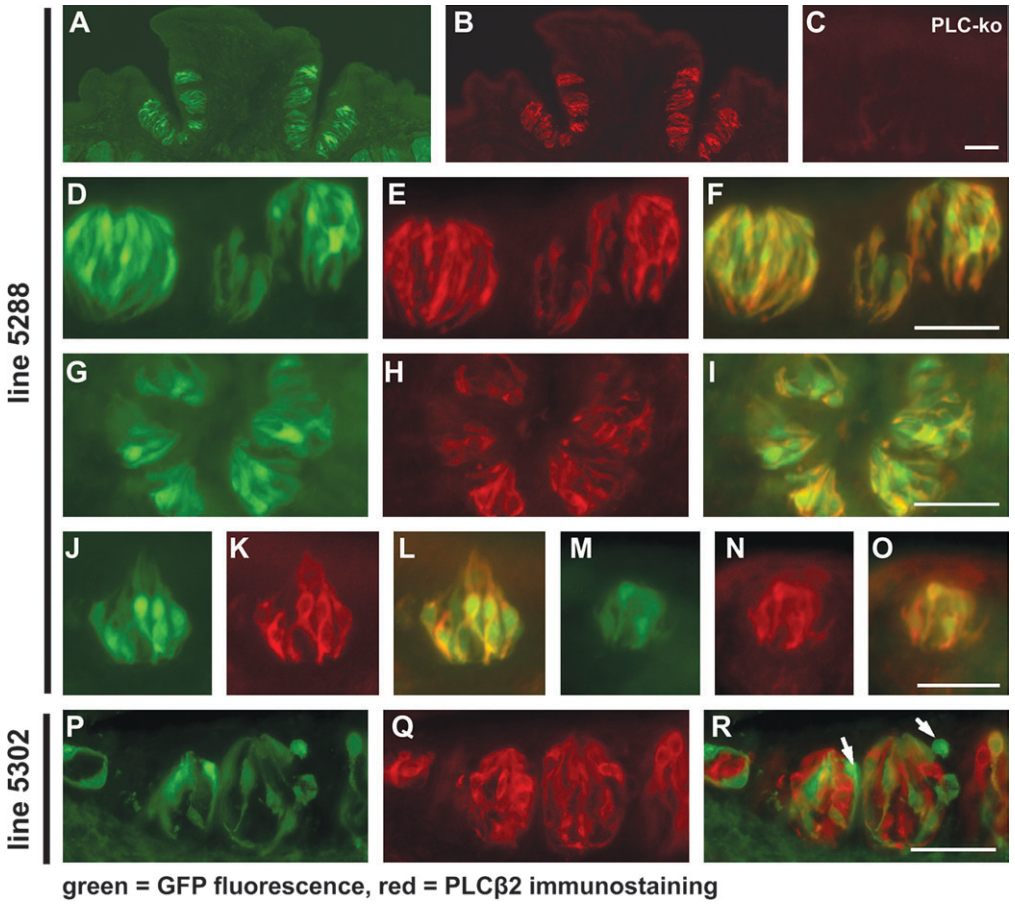


Figure 2 Appearance of GFP in taste cells from transgenic mice where expression of the fluorescent protein is driven by the PLCβ2 promoter. **(A, B, D–F)** Circumvallate papillae, **(G–I)** foliate papillae, **(J–L)** palate, and **(M–O)** fungiform papilla from adult mice carrying the 2.9-kb PLCβ2 promoter–EGFP transgene (line 5288). **(P–R)** Circumvallate papilla from an adult mouse carrying the 8.0-kb PLCβ2 promoter–EGFP transgene (line 5302). Each panel shows 25-μm sections viewed for GFP fluorescence (green: A, D, G, J, M, P), immunostaining for PLCβ2 (red: B, E, H, K, N, Q), or merged views (F, I, L, O, R). Note the excellent overlap of GFP and PLCβ2 expression (yellow–orange color in merged views) in taste cells from all fields in mice of the 5288 line. In the 5302 line (bottom panel), however, many GFP cells did not overlap with PLCβ2 cells (arrows in R). Also see Table 2 for quantification. Specificity of the anti-PLCβ2 was confirmed as lack of fluorescent signal in taste buds in PLCβ2-knockout mice **(C)**, processed in parallel with tissue from line 5288. In (A), the diffuse green fluorescence near the bottom of the micrograph is autofluorescence in skeletal muscle and was also seen in wild-type mice, even when no primary or secondary antibodies were used. Scale bars, 50 μm, apply for each row.

a typical experiment showing a GFP/CaO cell that responded to stimulation with cycloheximide (Figure 4D). The results demonstrate that GFP is a successful marker of tastant-responsive (i.e., receptor) cells in *in vitro* preparations from the transgenic mice and that GFP expression does not interfere with Ca^{2+} imaging when appropriate indicator dyes, such as CaO, are employed.

Discussion

Recent evidence suggests that taste buds are ensembles of molecularly and functionally diverse neuroepithelial cells, including gustatory receptor cells, supporting cells, stem cells, and others (Bigiani, 2001; Yee *et al.*, 2001; Medler *et al.*, 2003; Chaudhari and Roper, 2005). Yet, most cells in taste buds possess a generally similar fusiform shape and cannot be readily distinguished in living preparations. We have produced transgenic mice that faithfully express GFP in taste cells that have a key signaling element of the taste transduction cascade, PLCβ2. The transgene thus specifically identifies gustatory sensory receptor cells. Further, expression of GFP and PLCβ2 increase in parallel during postnatal development. GFP fluorescence in each of these lines is robust enough to be detected in living and fixed tissue without the need for immunodetection. GFP thus serves as an effective means to identify tastant-sensitive receptor cells for a variety of experimental approaches. Specifically, our data show that EGFP-expressing taste cells respond to tastant stimulation, as anticipated for gustatory receptor cells. These transgenic mice should prove valuable for studying transduction, development, and signal processing in taste buds and may also be useful for analyzing living interstitial cells in testis and other

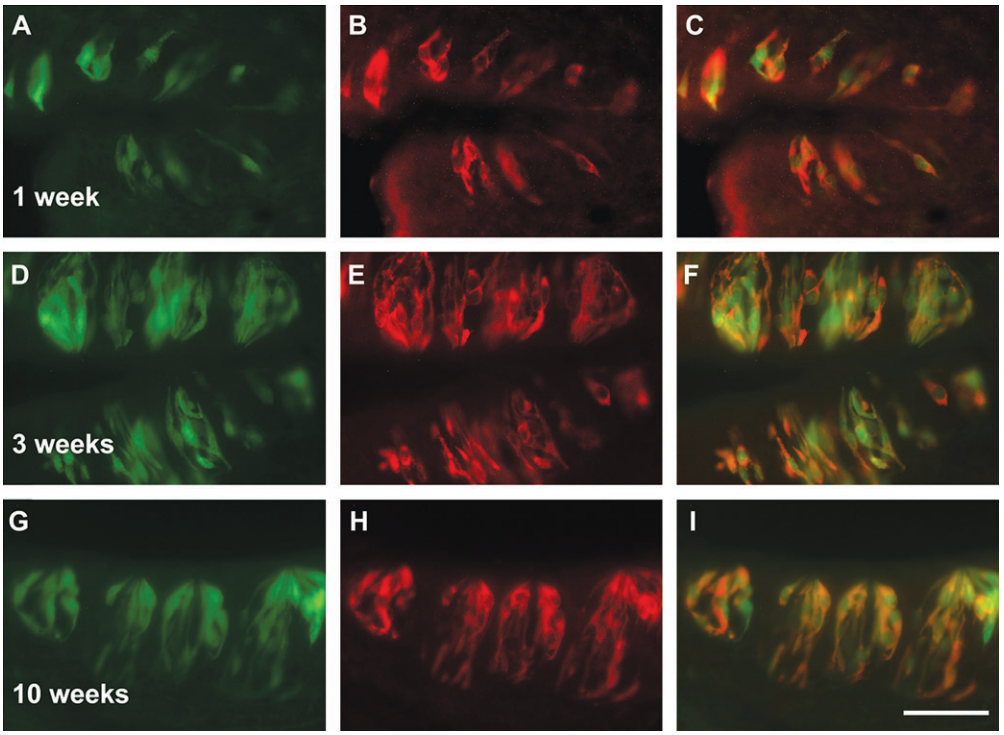


Figure 3 Pattern of GFP and endogenous PLCβ2 expression in developing circumvallate papillae of transgenic mice. Sections were taken from 1-week-old **(A–C)**, 3-week-old **(D–F)**, or 10-week-old (adult, **G–I**) mice from line 5288. Micrographs show GFP fluorescence (green: A, D, G), PLCβ2 immunostaining (red: B, E, H), or merged views (C, F, I; overlap of GFP with PLCβ2 shows as yellow–orange in almost all cells). Scale bar, 50 μm.

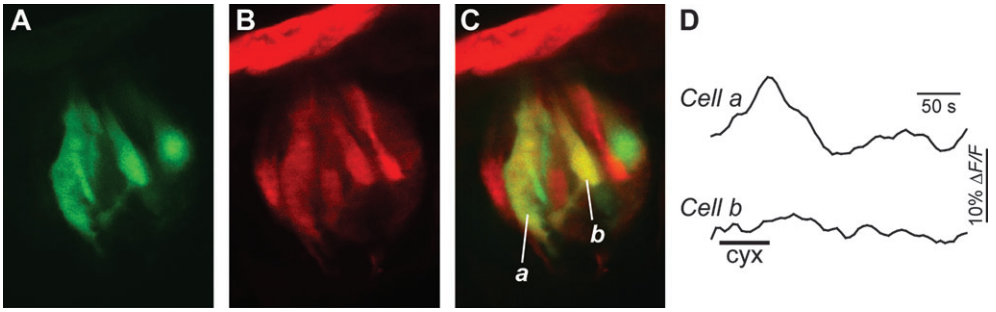


Figure 4 Cells expressing GFP respond to taste stimulation. **(A)** Micrograph of GFP fluorescence (excitation at 488 nm) in a circumvallate taste bud embedded in a living slice preparation and viewed with confocal microscopy. **(B)** Micrograph of the same taste bud excited at 568 nm to reveal the calcium indicator dye, CaO (red), that had been loaded into the taste bud for functional imaging. **(C)** Merged view of (A) and (B), showing presence of CaO in two cells that express GFP (*a*, *b*). **(D)** The preparation was stimulated with bath-applied bitter tastant at the bar (cyx, 100 μM cycloheximide; cf. Caicedo *et al.*, 2002). The traces show that only cell *a* was a bitter-sensitive taste cell (i.e., cyx stimulation evoked a Ca^{2+} response).

cell types that express PLCβ2. Previously reported transgenic mice expressing GFP in taste cells have used the promoter for gustducin (Wong *et al.*, 1999). These have proved valuable for interpreting functional responses in taste cells (Ogura *et al.*, 2002; Medler *et al.*, 2003).

We noted that of 14 genotype-positive founder mice only four, that had integrated multiple copies of the transgene, expressed GFP at a sufficiently robust level to be useful for functional studies. In summary, 2.9 kb of the PLCβ2 promoter is sufficient to confer cell-type–specific expression of the GFP transgene. Our transgenic lines appear to express GFP in a copy number–dependent fashion. Most transgenes that express in a strictly copy number–dependent, integration site–independent manner tend to include large (tens of kb) promoter segments. Instances of short promoters conferring copy number–dependent and cell-type–specific expression are less common (Dale *et al.*, 1992; Talbot *et al.*, 1994). One of our lines (5302) expresses GFP in mature taste cells but with inaccurate cell-type specificity. This suggests that the precise spatial and temporal regulation of the

transgene might be sensitive to insertional position (Grieshammer *et al.*, 1995; Umezawa *et al.*, 1997).

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References

Bigiani, A. (2001) *Mouse taste cells with glialike membrane properties.* J. Neurophysiol., 85, 1552–1560.

Caicedo, A., Kim, K.N. and **Roper, S.D.** (2002) *Individual mouse taste cells respond to multiple chemical stimuli.* J. Physiol., 544, 501–509.

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.

Chaudhari, N., Pereira, E., Landin, A.M. and **Roper, S.** (2003) *Immunodetecting a candidate umami receptor, taste-mGluR4, in taste cells.* Chem. Senses, 28, 559.

Chaudhari, N. and **Roper, S.** (2005) *Division of labor in mammalian taste buds.* Chem. Senses, 30, A129.

Clapp, T.R., Yang, R., Stoick, C.L., Kinnamon, S.C. and **Kinnamon, J.C.** (2004) *Morphologic characterization of rat taste receptor cells that express components of the phospholipase C signaling pathway.* J. Comp. Neurol., 468, 311–321.

Dale, T.C., Krnacik, M.J., Schmidhauser, C., Yang, C.L., Bissell, M.J. and **Rosen, J.M.** (1992) *High-level expression of the rat whey acidic protein gene is mediated by elements in the promoter and 3′ untranslated region.* Mol. Cell. Biol., 12, 905–914.

Dotson, C.D., Roper, S.D. and **Spector, A.C.** (2005) *PLCβ2-independent behavioral avoidance of prototypical bitter-tasting ligands.* Chem. Senses, 30, 593–600.

Grieshammer, U., McGrew, M.J. and **Rosenthal, N.** (1995) *Role of methylation in maintenance of positionally restricted transgene expression in developing muscle.* Development, 121, 2245–2253.

Helmchen, F. (2000) *Calibration of fluorescent calcium indicators.* In Yuste, R., Lanni, F. and Konnerth, A. (eds), Imaging Neurons: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Huang, L., Shanker, Y.G., Dubauskaite, J., Zheng, J.Z., Yan, W., Rosenzweig, S., Spielman, A.I., Max, M. and **Margolskee, R.F.** (1999) *Gγ13 colocalizes with gustducin in taste receptor cells and mediates IP_3 responses to bitter denatonium.* Nat. Neurosci., 2, 1055–1062.

Jiang, H., Kuang, Y., Wu, Y., Xie, W., Simon, M.I. and **Wu, D.** (1997) *Roles of phospholipase Cβ2 in chemoattractant-elicited responses.* Proc. Natl Acad. Sci. USA, 94, 7971–7975.

Landin, A.M., Kim, J.W. and **Chaudhari, N.** (2005) *Liposome-mediated transfection of mature taste cells.* J. Neurobiol., 65, 12–21.

Medler, K.F., Margolskee, R.F. and **Kinnamon, S.C.** (2003) *Electrophysiological characterization of voltage-gated currents in defined taste cell types of mice.* J. Neurosci., 23, 2608–2617.

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.

Miyoshi, M.A., Abe, K. and **Emori, Y.** (2001) *IP_3 receptor type 3 and PLCβ2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells.* Chem. Senses, 26, 259–265.

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.

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.

Ogura, T., Margolskee, R.F. and **Kinnamon, S.C.** (2002) *Taste receptor cell responses to the bitter stimulus denatonium involve Ca^{2+} influx via store-operated channels.* J. Neurophysiol., 87, 3152–3155.

Richter, T.A., Caicedo, A. and **Roper, S.D.** (2003) *Sour taste stimuli evoke Ca2+ and pH responses in mouse taste cells.* J. Physiol., 547, 475–483.

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

Rossler, P., Kroner, C., Freitag, J., Noe, J. and **Breer, H.** (1998) *Identification of a phospholipase Cβ subtype in rat taste cells.* Eur. J. Cell Biol., 77, 253–261.

Talbot, D., Descombes, P. and **Schibler, U.** (1994) *The 5′ flanking region of the rat LAP (C/EBP beta) gene can direct high-level, position-independent, copy number-dependent expression in multiple tissues in transgenic mice.* Nucleic Acids Res., 22, 756–766.

Umezawa, A., Yamamoto, H., Rhodes, K., Klemsz, M.J., Maki, R.A. and **Oshima, R.G.** (1997) *Methylation of an ETS site in the intron enhancer of the keratin 18 gene participates in tissue-specific repression.* Mol. Cell. Biol., 17, 4885–4894.

Wong, G.T., Ruiz-Avila, L. and **Margolskee, R.F.** (1999) *Directing gene expression to gustducin-positive taste receptor cells.* J. Neurosci., 19, 5802–5809.

Yan, W., Sunavala, G., Rosenzweig, S., Dasso, M., Brand, J.G. and **Spielman, A.I.** (2001) *Bitter taste transduced by PLCβ2-dependent rise in IP_3 and α-gustducin-dependent fall in cyclic nucleotides.* Am. J. Physiol. Cell Physiol., 280, C742–C751.

Yee, C.L., Yang, R., Bottger, B., Finger, T.E. and **Kinnamon, J.C.** (2001) *"Type III" cells of rat taste buds: immunohistochemical and ultrastructural studies of neuron-specific enolase, protein gene product 9.5, and serotonin.* J. Comp. Neurol., 440, 97–108.

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

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