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A Strong Nerve Dependence of *Sonic hedgehog* Expression in Basal Cells in Mouse Taste Bud and an Autonomous Transcriptional Control of Genes in Differentiated Taste Cells

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Abstract

The nerve-dependency of gene expression in mouse taste bud was examined through an analysis of changes in gene expression in and around the taste buds in circumvallate papillae after surgery of cranial nerve IXth (glossopharyngeal nerve). The number of cells expressing *T1r3*, *gustducin*, *Mash1* and *Nkx2.2* gradually decreased after denervation. However, the expression intensity of these genes was barely influenced by denervation, and strong expression was observed at 6 days after denervation. In contrast, the basal cell-specific *Sonic hedgehog* (*Shh*) expression in the taste buds was decreased markedly at 6 h after denervation. In the regeneration process of taste buds, *Shh* expression was observed during a very early phase before taste bud formation. These results indicate the autonomous transcriptional control of genes in differentiated taste cells and the strong nerve-dependency of *Shh* expression in basal cells. Furthermore, in order to reveal the mitotic activity of *Shh*-expressing cells in taste buds, the BrdU-labeling experiments were performed using a combination of BrdU-immunohistochemistry and *in situ* hybridization. BrdU-signal was very rarely observed in *Shh*-expressing cells immediately after BrdU injection, and the signals were noted mainly in *Ptc*-expressing cells. BrdU signals rapidly increased in *Shh*-expressing cells in following 12 h and began to decrease after 2 days post-injection. These results suggest that most *Shh*-expressing cells are not mitotically active, but that *Shh*-expressing cells may be in the early transient developmental state of taste cells in taste buds.

Key words: denervation, glossopharyngeal nerve, *gustducin*, *Mash1*, *T1r3*

Introduction

In mammals, the renewal of taste cells is a continuous process in taste buds. Denervation results in the disappearance of taste buds, and the regeneration of taste buds follows reinnervation, indicating that taste buds are trophically maintained by the taste nerve (Hosley *et al.*, 1987; Smith *et al.*, 1994). Therefore, it is assumed that nerve-dependent cell proliferation, differentiation and cell death are in balance to maintain the structure of the taste buds, which makes it important to elucidate the character of neuronal control of gene expression therein. Mammalian taste bud cells have an average lifespan of 10 days, and are continuously differentiated from epithelial precursor cells in a proliferative population in the basal region under the control of the taste nerve (Beidler and Smallman, 1965; Farbman, 1980; Delay *et al.*, 1986; Stone *et al.*, 1995). Recently, information regarding the gene expression in taste buds has been increased (Kim *et al.*, 2003). In mouse circumvallate papillae, sweet taste receptor, *T1r2*/*T1r3*, and bitter taste receptors, *T2rs*, were reported to be expressed in separate taste cell populations, and taste-specific G-protein, *gustducin*, was selectively expressed in the cells expressing *T2rs* (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001). In addition, neuronal differentiation transcription factors such as *Mash1* and *Nkx2.2* are expressed in a non-overlapping population of cells expressing taste receptor genes (Kusakabe *et al.*, 2002; Miura *et al.*, 2003). These transcription factors are speculated to be expressed in premature taste cells based on the segregated expression patterns in adult mouse and preceding expression timing during development. It has also been reported that

NeuroD, another bHLH transcription factor for neuronal differentiation, is expressed in mature taste cells, including *gustducin*-expressing cells (Suzuki *et al.*, 2002), raising the possibility that sequential expression of *Mash1* and *NeuroD* might be important for taste cell differentiation, as in olfactory neurons (Cau *et al.*, 1997). The various types of gene expression in taste buds are assumed to be maintained in a taste nerve-dependent manner. The specificity of taste nerve response to taste stimuli suggests the correlation between the gene expression in taste cells and taste neurons (Ninomiya, 1998; Yasumatsu *et al.*, 2003). However, at present, the nerve dependency of gene expression in taste buds is unclear, although immunohistochemical studies of *NeuroD* and *NCAM* have demonstrated that the protein products can be detected after denervation (Suzuki *et al.*, 2002; Smith *et al.*, 1994). We have previously reported that, in circumvallate papillae in mice, the expression of *Ptc* around taste buds and *Shh* in the basal cells of taste buds disappears at 4 days after denervation, when taste bud structure and the *cytokeratin8*-mRNA in taste buds are still detectable (Miura *et al.*, 2001). This report provided an example of dominant regulation of gene expression by a taste nerve in the basal region of taste buds in comparison with gene expression, such as that of *cytokeatin8*, in fusiform cells in taste buds. However, it remains unclear whether gene expression of the taste reception-related genes such as *T1r3* and *gustducin* and transcription factors, which are speculated to express before taste reception-related genes in taste buds, also depend on the taste nerve, as in the case of *Shh* in the basal region of taste buds. The experimental period of 4 days after denervation was too long to discuss in detail the nerve dependency of gene expression, and the relationship of the timing of loss of expressions between *Shh* and *Ptc* was also unclear.

Two mechanisms can be assumed to explain the control of gene expression by taste nerve: first, each gene transcription in the taste buds is almost directly regulated by a taste neuron in differentiated taste cells, and denervation would therefore cause a rapid loss of expression; second, expression of each gene is maintained in an autonomous manner after taste cell differentiation, and denervation would have almost no effect on gene expression except in terms of loss associated with cell death. In the present report, to clarify the nerve dependency of gene expression in mouse taste buds, we used *in situ* hybridization to examine the changes in these genes in and around the taste buds of circumvallate papillae (i) after denervation and (ii) at the beginning of regeneration of taste buds. The mitotic activity of basal cells expressing *Shh* was also examined.

Materials and methods

Experimental animals and surgical procedures

The adult animals used in this study were 10- to 12-week-old C57BL/6N mice. These animals were initially purchased from Charles River Japan (Yokohama, Japan), and were bred at the National Food Research Institute. Adult mice were anesthetized with pentobarbital sodium (40–50 mg/kg, i.p.; Nembutal; Abbott Laboratories, Abbott Park, IL), and the bilateral glossopharyngeal (IXth) nerves were transected or crushed in the neck under the digastric muscle. Nerve crush was performed as described previously (Yasumatsu *et al.*, 2003). We followed the guidelines of our institute for the care and use of experimental animals.

In situ hybridization

Mouse tongues were dissected, placed in embedding compound (OCT; Sakura Finetech, Los Angeles, CA), and rapidly frozen in a liquid nitrogen bath. Tissues were sectioned at 5 μm, and single- and double-color *in situ* hybridization was performed as previously described (Miura *et al.*, 2003). Antisense RNA probes were transcribed *in vitro* with digoxigenin- or fluorescent-labeled UTP from a linearized plasmid containing one of the following cDNA inserts: *Shh* (Kitamura *et al.*, 1997), *Ptc* (1318–2362: Genbank MMU46155), *Mash1* (10012: Genbank U68534–783: Genbank M65603, 1276 bp), *Nkx2.2* (338–2006: Genbank NM010919), *T1r3* (Kitagawa *et al.*, 2001) and *gustducin* (Kawai *et al.*, 2000). The sections were hybridized with RNA probes in hybridization buffer (50% formamide, 5 × SSC, 5 × Denhardt's solution, 500 μg/ml salmon sperm DNA, 250 μg/ml tRNA and 1 mM dithiothreitol) at 65°C and washed with 0.2 × SSC at 65°C. The sections were then incubated with alkaline phosphate (AP)-conjugated anti-digoxigenin antibody (Roche Diagnostics GmbH, Mannheim, Germany) for single-color detection or with AP-conjugated anti-fluorescent antibody and peroxidase (POD)-conjugated anti-digoxigenin antibody (Roche Diagnostics GmbH) for double-color detection. For single-color detection, a color reaction was preformed with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitoroblue tetrazolium (NBT) or with 2-hydroxy-3-naphthoic acid-2'-phenylanilidephosphate (HNPP)/Fast Red (Roche Diagnostics GmbH). For double-color detection, after treatment with Tyramid-biotin (Perkin-Elmer Life Science, Boston, MA) and washing, the sections were incubated with Streptavidin-Alexa 488 (Molecular Probes, Eugene, OR), washed, and then treated with HNPP/Fast Red alkaline phosphatase substrate (Roche Diagnostics).

BrdU labeling and the detection after *in situ* hybridization

Adult mice were injected intraperitoneally with bromodeoxyuridine (BrdU: 50 mg/kg body wt) (Roche Diagnostics). Mouse tongues were dissected and treated in the same manner as that used for *in situ* hybridization at 1, 3 and 12 h and 1, 2, 3 and 4 days after BrdU injection. After the hybridization with digoxigenin-labeled *Shh* or *Ptc* cRNA probe, sections were incubated with an AP-conjugated anti-digoxigenin antibody, washed and incubated with HNPP/Fast Red alkaline phosphatase substrate (Roche Diagnostics). The sections were then washed and incubated with anti-

BrdU monoclonal antibody according to the instructions for use of the BrdU Labeling and Detection Kit II (Roche Diagnostics). After washing, the sections were incubated with an Alexa 488-anti mouse IgG (Molecular Probes) and washed. Double-color fluorescent images were merged with light field images using Photoshop (Adobe Systems, San Jose, CA). Signals in every fourth section were analyzed in order to avoid any double counting of the gene-expressing cells. The ratio of BrdU-positive cells was analyzed in *Shh*-expressing cells at various time periods after BrdU injection, and was plotted. The position of BrdU signals was analyzed in comparison with the site of *Shh* expression. To compare the distributions of *Shh* expression and BrdU signals, a single cell and a cell cluster composed of multiple cells expressing *Shh* were equally treated as one signal.

Immunohistochemistry after *in situ* hybridization

After the detection of *in situ* hybridization signals with BCIP/NBT, the slides were placed in PBS containing 1% blocking reagent (Roche Diagnostics) for 1 h. Sections were incubated with anti-neural cell adhesion molecule (NCAM) rabbit polyclonal antibody (Chemicon, Temecula, CA) in TBS (100 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 1% blocking reagent at 4°C overnight. After they were washed in TBS, the sections were incubated with fluorescence-labeled anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 1 h and then washed in TBS. *In situ* hybridization images were overlaid with immunohistochemical fluorescent images using Photoshop (Adobe Systems).

Results

The change in gene expression after the cranial nerve IXth transection: rapid loss in basal cells, continuous expression in fusiform cells

To clarify the nerve-dependence of gene expression in taste buds, we performed bilateral transection of the IXth cranial nerve and examined the change in gene expression (Figures 1 and 2). At 6 h after denervation, the expression of *Shh* was decreased markedly in the basal cells of the taste buds, and a

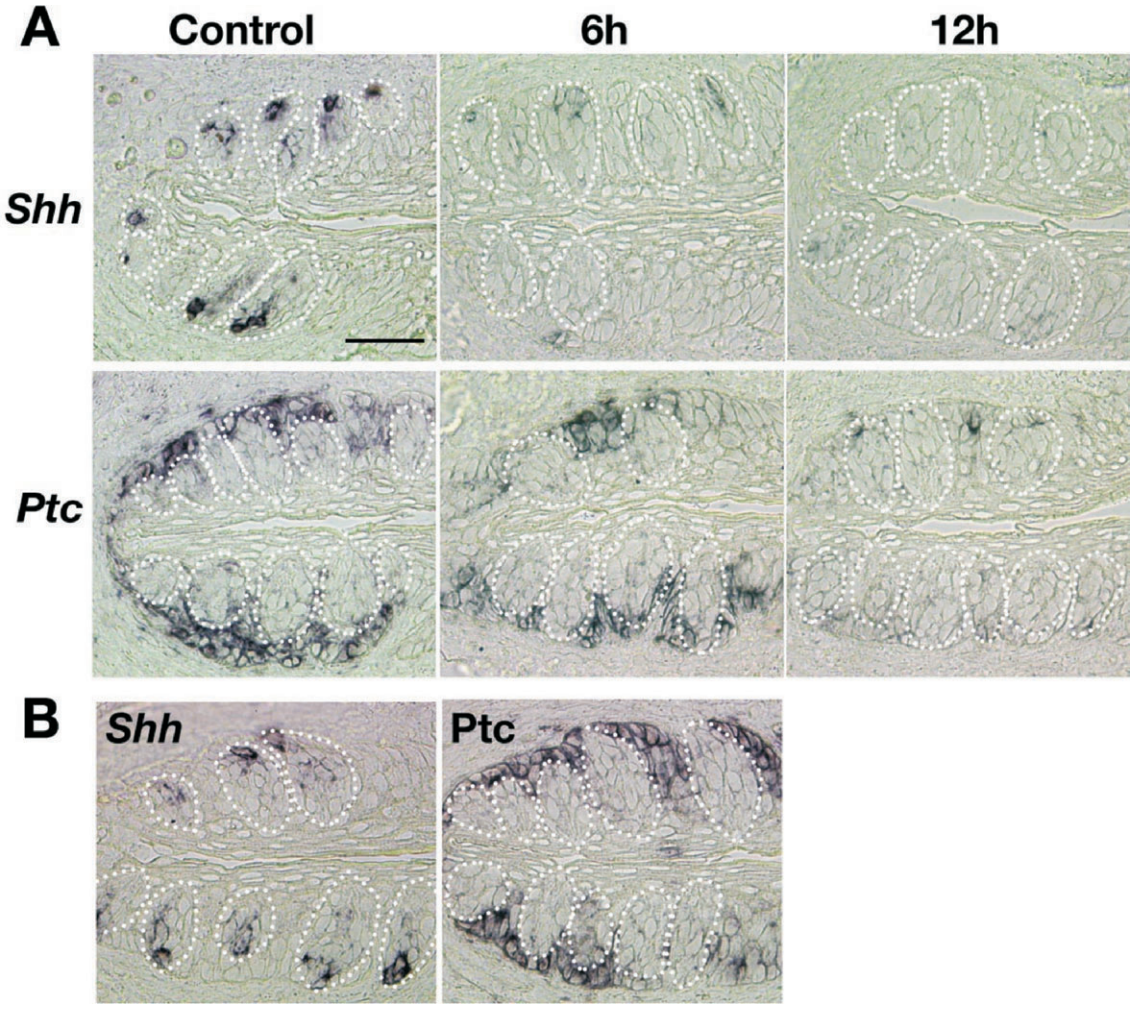

Figure 1 The change in the expression of *Shh* and *Ptc* in circumvallate papillae after bilateral transection of the IXth cranial nerve. **(A)** The expression of Shh and Ptc at 6 and 12 h after bilateral transection of the IXth cranial nerve. **(B)** The expression of *Shh* and *Ptc* at 24 h after bilateral transection of the Xth cranial nerve. The broken line indicates the outline of each taste bud. Scale bar (in A) is 50 μm for A, B.

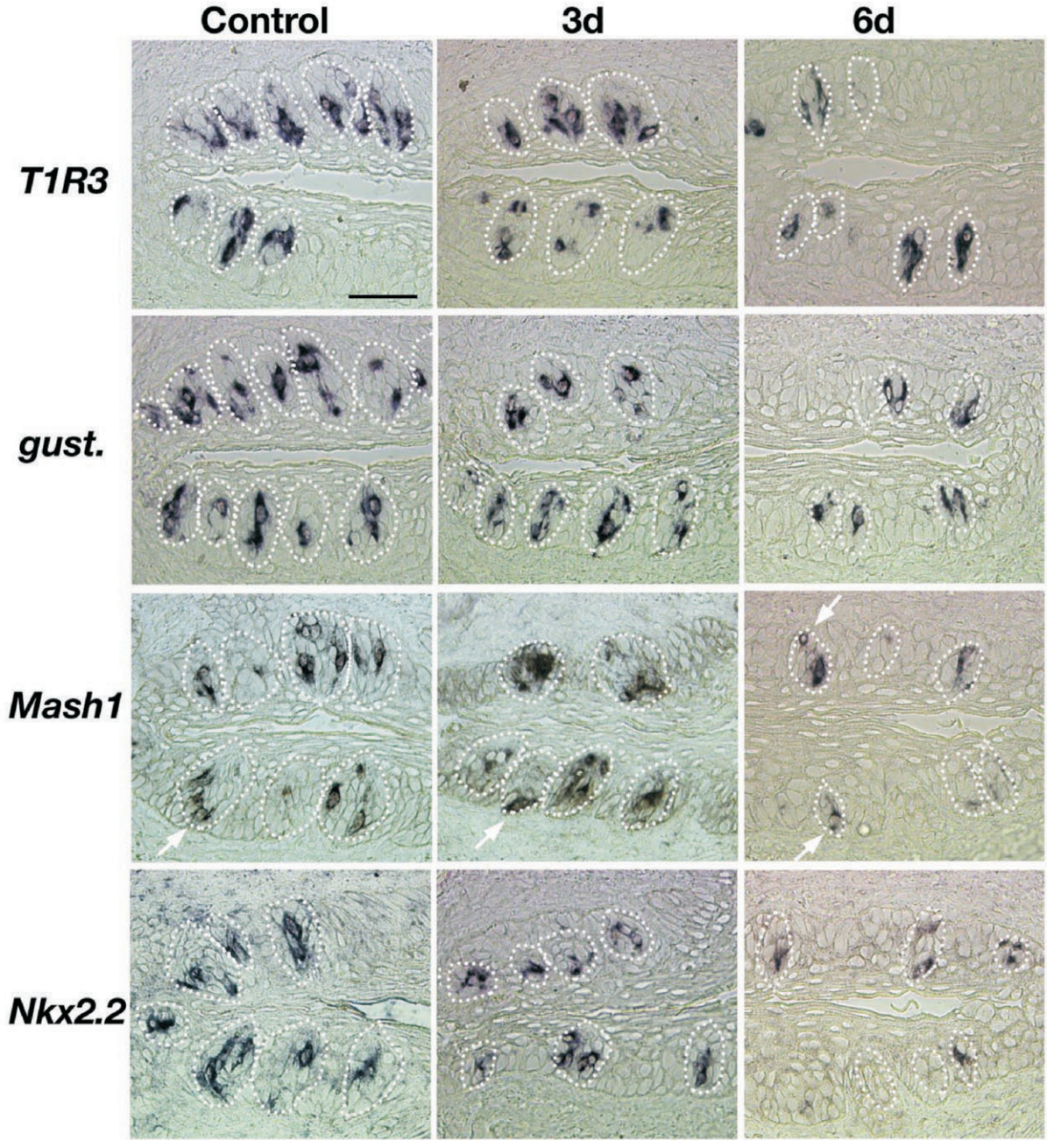

Figure 2 The change in *T1r3*, *gustducin*, *Mash1* and *Nkx2.2* expression in taste buds of circumvallate papillae at 3 and 6 days after bilateral transection of the IXth cranial nerve. The signal for *Mash1* in basal cells (arrows) was detected even at 6 days after denervation. The broken line indicates the outline of each taste bud. Scale bar is 50 μm.

decline in *Ptc* expression was observed (Figure 1A). At 12 h after denervation, *Ptc* expression almost disappeared following the loss of *Shh* expression. This rapid loss of *Shh* and *Ptc* expression was specifically caused by the IXth nerve transection, and no change was observed at 24 h after the Xth nerve transection (Figure 1B). In contrast, expression of the taste reception-related genes *T1r3* and *gustducin*, and that of the transcription factors *Mash1* and *Nkx2.2*, was detected at almost the same intensity as that in the control, even after denervation (Figure 2). At 6 days, although taste buds were observed to be thin, strong signals for these genes were still detected. Post-surgical expression of *Mash1* was detected not only in fusiform cells but also in round cells in the basal region of the taste buds. These *Mash1*-expressing round cells are likely to become fusiform cells in the course of the normal maturation process (Miura *et al.*, 2003). The number of positive cells for these genes was decreased after denervation, reflecting the decrease in the size of the taste buds. There was no obvious difference in the rate at which the number of these gene-expressing cells decreased, while

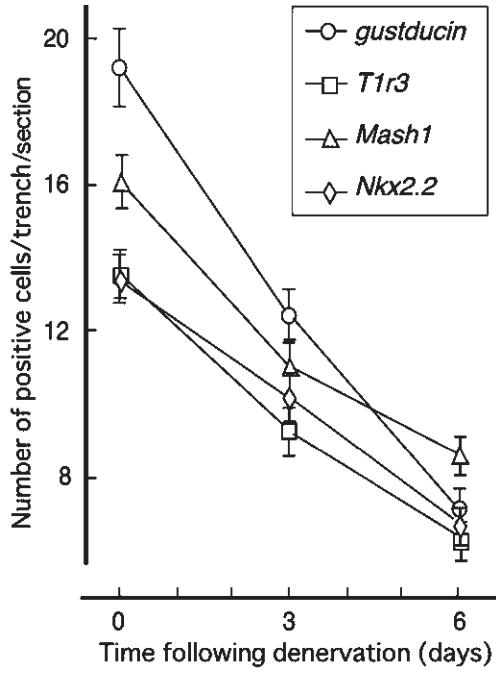

Figure 3 The change in the number of gustducin, *T1r3*, *Mash1* and *Nkx2.2*-expressing cells in a trench of circumvallate papillae after bilateral transection of the IXth nerve. Each spot represents the means ± SEMs from 33–40 sections of trenches.

the rate for *gustducin*-expressing cells seemed to be slightly more rapidly than that for other genes at 6 days after denervation (Figure 3). At 8 days after denervation, most of the taste bud structure had disappeared, though the signals of these genes were detected in dispersed cells in the circumvallate trench (Figure 4). These signals seem to remain from the signals in taste buds, which had disappeared at that time. The intensity of the signals appeared to not have decreased.

Basal cell-specific gene expression during regeneration

To examine the basal cell-specific gene expression during taste bud regeneration, we crushed the bilateral IXth nerve, and then examined the expression of *Shh* and *Ptc* at 11 days after nerve crush, together with NCAM immunoreactivity, which shows the presence of NCAM-positive taste cells and nerve fibers. In a previous study in rats, analysis of NCAM immunoreactivity after bilateral IXth nerve crush showed that taste bud regeneration begins at 10–12 days after the crushing (Smith *et al.*, 1994). We examined the epithelium at 11 days after nerve crush, and the expression of *Shh* was again detected in the basal cells of circumvallate papillae, despite the absence of a discernible taste bud structure (Figure 5A,C,E). The *Shh*-expressing population varied from one cell to small clusters of a few cells. The *Ptc* signal was observed in the serial sections, although the intensity of *Ptc*-expression around *Shh*-expressing cells varied from very weak and almost undetectable (Figure 5B) to relatively strong and clustered (Figure 5F). Nerve fiber was observed to be in contact with *Shh*-expressing cells under the basement membrane, indicating a correlation between *Shh* expression and reinnervation (Figure 5C,F). In addition, a small number of cells immunoreactive to NCAM antibody

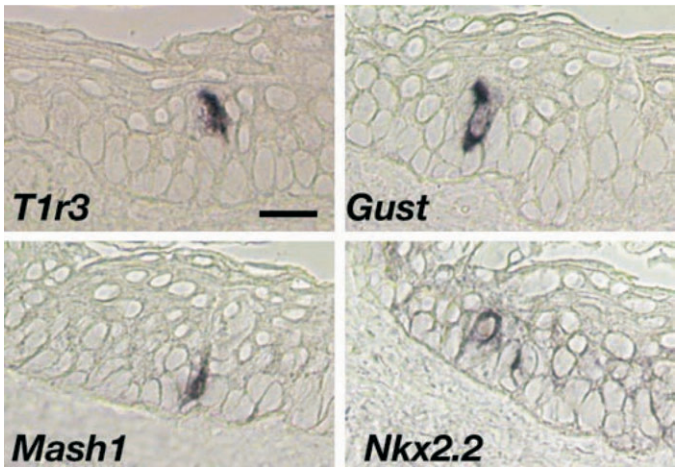

Figure 4 Gene expression at 8 days after bilateral IXth nerve transection. The signals for *T1r3*, *gustducin*, *Mash1* and *Nkx2.2*, respectively, were detected, though the taste bud structure had almost disappeared. Scale bar is 20 μm.

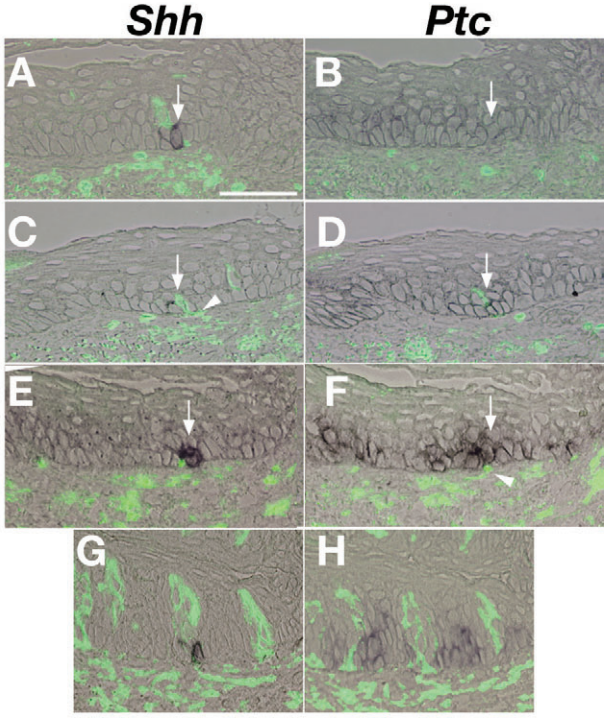

Figure 5 *Shh* and *Ptc* expression in circumvallate papillae epithelium at 11 days after bilateral IXth nerve crush. The expression of *Shh* **(A,C,E,G)** and *Ptc* **(B,D,F,H)** was examined by *in situ* hybridization. The adjacent panels correspond to adjacent sections. Immunohistochemistry to NCAM (green) was performed subsequent to *in situ* hybridization. (A–F) At 11 days after the operation. Arrows indicate the position of the signal for *Shh*. Arrowheads show where the nerve fiber touches a *Shh*-expressing cell. (G, H) Control image for a normal adult mouse that did not undergo the nerve crush operation. Scale bar is 50 μm (in A) for (A)–(H).

were also detected near *Shh*-expressing cells (Figure 5A,D). The advance in taste bud regeneration as represented by gene expression varied at this stage, which may reflect differences in the arrival timing of regenerating nerves.

BrdU uptake in *Shh*-expressing basal cells in taste buds

Our observations suggest that *Shh* expression in the basal cells of taste buds is strongly dependent on the taste nerve, and that this expression is turned on in a very early phase of taste bud regeneration. The question therefore arises as to whether the *Shh*-expressing cell itself is mitotically active in order to supply the taste buds with cells. We injected BrdU and examined the location of BrdU uptake in comparison with sites of *Shh* and *Ptc* expression in circumvallate papillae, using the double-color detection method. At 1 h after injection, the BrdU signals were detected primarily on the basal side of the epithelium where *Ptc* was expressed (Figure 6A). The *Ptc*-expressing region was located adjacent to *Shh*-expressing cells, and an overlap of *Shh* and *Ptc* was also observed to some extent (Figure 6B). Double-color detection revealed that only 2.3% (11/476) of *Shh*-expressing basal cells had the BrdU signal, while 58% (67/115) of *Shh*-expressing cell clusters had the BrdU signal in adjacent cells (Figure 6C). In addition, we examined the BrdU signal in *Shh*-expressing cells from 1 h to 4 days after BrdU injection (Figure 7). At 3 h after injection, the ratio of BrdU-positive cell among the *Shh*-expressing cells was almost same as that at 1 h: 2.1% (5/239) were BrdU positive. At 12 h, BrdU signals were rapidly increased in *Shh*-expressing cells: 13.4% (28/209) of *Shh*-expressing cells were BrdU positive. At 2 days after injection, the ratio of BrdU-positive cells in *Shh*-expressing cells reached a peak level, and 24.4% (38/156) of Shh-expressing cells were BrdU-positive (Figure 6D). The ratio of BrdU-positive cells in Shh-expressing cells then gradually decreased (Figure 7).

Discussion

The nerve dependency of gene expression in taste buds remains unclear, although the induction of gene expression by taste nerves seems to be critical for the maintenance of taste buds in mammals.

In the present study, the *Shh* expression in basal cells was shown to decrease markedly immediately within 6 h of denervation, indicating a strong dependency of its expression on the nerve. This rapid loss of *Shh* expression appears to reflect direct control by the taste nerve rather than control mediated by other tissues. *Ptc* expression disappeared just after the loss of *Shh* following denervation. The time lag of loss of gene expression between *Shh* and *Ptc* seems to reflect the time period of depletion of the *Shh* signal, which is needed for *Ptc* expression (Goodrich *et al.*, 1996; Marigo *et al.*, 1996), after denervation. The contribution of the proliferating basal cell population within taste buds has previously been reported (Delay *et al.*, 1986). Therefore, the quick response of *Shh* expression in basal cells to denervation and reinnervation implies the participation of *Shh*-expressing cells in the supply of cells for taste bud maintenance. However, our BrdU tracing results revealed that most *Shh*-expressing cells are mitotically silent. The basal cells adjacent to *Shh*-expressing cells were found to have high mitotic activity, suggesting that these proliferative cells in the *Ptc*-

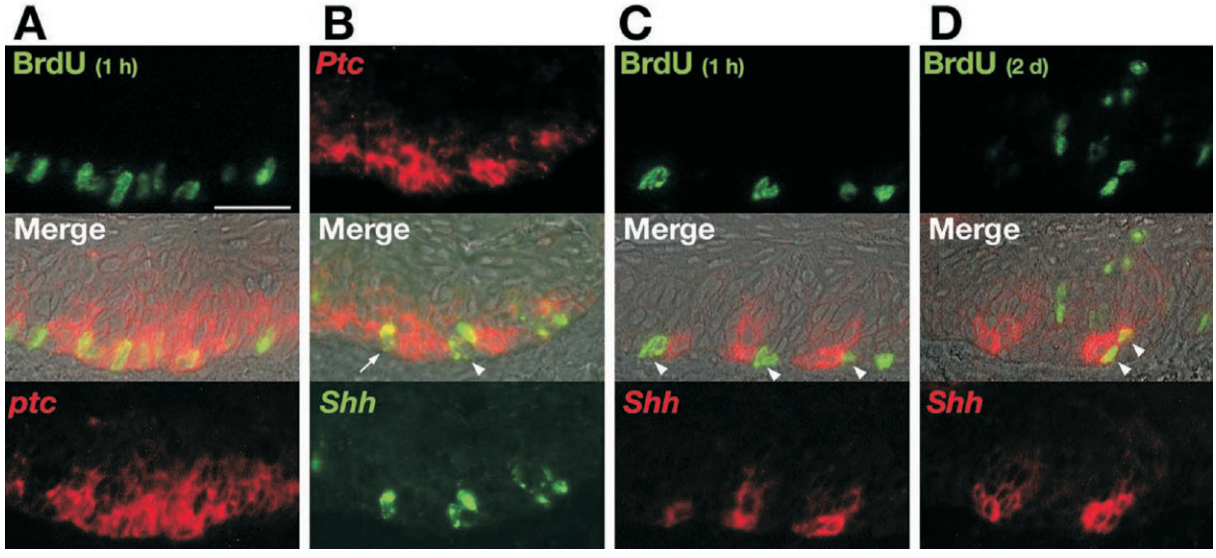

Figure 6 The distribution of BrdU signal in comparison with the expression of *Shh* and *Ptc* in circumvallate papillae. **(A)** BrdU signal (green) at 1 h after BrdU injection in comparison with Ptc expression (red). **(B)** Double-color *in situ* hybridization of *Shh* (green) and *Ptc* (red). The arrowhead indicates *Shh* expression adjacent to *Ptc* expression, and the arrow indicates the overlap expression of *Shh* and *Ptc*. **(C)** BrdU signal (green) at 1 h after BrdU injection in comparison with *Shh* expression (red). Arrowheads indicate the BrdU signals adjacent to *Shh*-expressing cells. **(D)** At 2 days after BrdU injection, arrowheads indicate BrdU-positive Shh-expressing cells. Scale bar is 50 μm (in A) for (A)–(D).

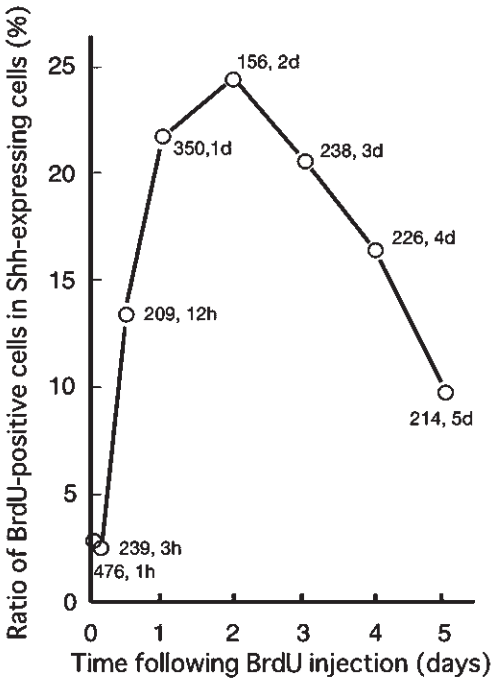

Figure 7 The change in the ratio of BrdU-positive cells in *Shh*-expressing cells. The numbers near each point denote the number of *Shh*-expressing cells counted and the time after BrdU injection for that point.

expressing region may correspond to the basal cell population, as reported by Delay *et al.* (1986). On the other hand, the rapid increase and subsequent decrease of BrdU signal in *Shh*-expressing cells after BrdU injection was observed. As for *gustducin*-expressing cells, the BrdU signal has been reported to become detectable at 2.5 days after injection (Cho *et al.*, 1998), while, in our analysis, the ratio of BrdU-signal in *Shh*-expressing cells had reached a peak level and began to decrease at the time of the first detection of BrdU-signal in *gustducin*-expressing cells. These findings suggest that the *Shh*-expressing cells may be in the early transient developmental stage of taste cell differentiation in taste buds. The mitotic cell population in *Shh*-expressing cells seems to be too small to explain the rapid increase in BrdU signal in *Shh*-expressing cells, as the number of BrdU-positive cells increased 6.4-fold in 9 h (from 2.1% at 3 h to 13.4% at 12 h). It therefore seems that the *Shh*-expressing cell population can be categorized into two groups. One is the mitotic cell population. Although this is a very small population of *Shh*-expressing cells, it is possible that a particular cell lineage in taste buds may be derived from these mitotic cells. The other is the cell population group derived from other proliferating cells distinct from *Shh*-expressing cells, a potential candidate of which seems to be *Ptc*-expressing cells. The overlap expression of *Shh* and *Ptc* might reflect the early stage of *Shh*-expressing cells derived from *Ptc*-expressing cells. *Shh* signaling has been shown to play an important role in a variety of different processes during vertebrate development (Ingham and McMahon, 2001), and regulation of neuronal progenitor cell proliferation by *Shh* has been reported in both the neonatal and adult brain (Wechsler-Reya and Scott, 1999; Lai *et al.*, 2003; Oliver *et al.*, 2003; Lewis *et al.*, 2004). In the cerebellum, *Shh* has been reported to be expressed in Purkineje neurons and to induce the proliferation of granule neuron progenitors expressing *Ptc* (Wechsler-Reya and Scott, 1999; Dahmane and Altaba, 1999; Lewis *et al.*, 2004). Each gene expression was segregated and seems to occur in separate cell lineages. In the case of taste buds, *Shh* signaling might be related to the proliferation of taste bud progenitor cells expressing *Ptc*, as in the case in the cerebellum. However, the cell lineages of *Shh*- and *Ptc*-expressing cells seem to be closely related to each other, and the majority of *Shh*-expressing cells may be derived from the cells expressing *Ptc* unlike the case in the cerebellum. And *Shh*-expressing cells themselves may be not only a signal center of the proliferation of progenitor cells, but also a transient precursor in the taste bud.

In contrast to the basal cell-specific *Shh* expression, denervation has almost no effect on the intensity of the expression of taste reception-related genes, *T1r3* and *gustducin*, and even at 8 days after denervation, when most of the taste bud structure had disappeared, strong signals for these genes were observed. If the expression of each gene depended on the taste nerve, a more rapid and coordinated decline in expression levels would be observed, as in *Shh* and *Ptc*. It is likely that expression of each gene is continued nerve-independently until the removal of each cell, since mRNA cannot remain without transcription for a period as long as 8 days. Our observations suggest the autonomous, nerve-independent, control of gene expression in differentiated taste bud cells, which mechanism seems to be consistent with the results of *in vitro* culture of rat taste buds without nerves, in which taste bud-specific markers were immunohistochemically detected after a few days in culture (Kishi *et al.*, 2001; Ruiz *et al.*, 2001). Notably, autonomous control is present in taste cells expressing *Mash1* and *Nkx2.2*, as in *T1r3* and *gustducin*-expressing cells. The expression of these transcription factors overlapped each other and has been assumed to precede those of taste reception-related genes, and to be downregulated in mature taste cells (Kusakabe *et al.*, 2002; Miura *et al.*, 2003). In this case, if the maturation process of taste cells continues after denervation, it is expected that the expression of marker genes for premature taste buds would disappear before that of taste reception-related genes. However, no such sequential loss of gene expression was observed, and the loss of the expression of each gene was mostly parallel. It is possible that denervation might lead to arrest of the maturation process in taste buds. This idea seems to be supported by the *Mash1* expression in round cells in the basal region of taste buds at 6 days after denervation, as these cells have been speculated to become fusiform cells expressing *Mash1* in the maturation process (Miura *et al.*, 2003) and are expected to disappear in an early phase after denervation if the maturation process continues after

denervation. In normal taste buds, aged cells are thought to be removed by programmed cell death (Zeng and Oakley, 1999; Zeng *et al.*, 2000). Cell death in taste buds has been reported to increase after denervation, though it has not been clarified whether this increase is based on enhancement of normal cell death in the ageing process or the occurrence of cell death over a wider range of maturation stages apart from the normal cell death process (Takeda *et al.*, 1996). Our data seem to support the latter possibility, because the sequential loss of gene expression in taste buds was not observed after denervation. In addition, *Shh* has been reported to contribute to the survival of certain cell types (Borycki *et al.*, 1999; Litingtung and Chiang, 2000). Thus, it may be possible for *Shh* in taste buds to function as a factor contributing to the survival of taste bud cells, and quick loss of *Shh* signaling might be involved in the occurrence of cell death. On the other hand, further studies of cell lineage in taste buds are required to eliminate the alternative possibility that the parallel decrease in gene expression after denervation might reflect the independent cell lineage of each gene-expressing cell.

In summary, our data provide evidence of the autonomous regulation of gene expression in mature and presumptive premature taste bud cells, and of the strong nerve dependence of basal cells in taste buds, raising the possibility of basal cell-specific gene expression as a primary target of the taste nerve in taste bud maintenance. The possibility of *Shh*-expressing basal cells as a transient precursor was also shown by BrdU-labeling experiments. Further analyses of basal cells of taste buds are expected to yield a better understanding of the molecular mechanism of taste bud maintenance.

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