Chem. Senses 30 (suppl 1): i50–i51, 2005 doi:10.1093/chemse/bjh108

# Shh Signaling and Regulatory Gene Expression in Mouse Taste Buds

**Hirohito Miura[1], Hiromi Kato[1], Yuko Kusakabe[1], Mizuho Tagami[1], Jun Miura-Ohnuma[1], Tetsuya Ookura[1], Yoichiro Shindo[2], Yuzo Ninomiya[3] and Akihiro Hino[1]**

[1]National Food Research Institute, [2]Asahi Breweries Ltd and [3]Kyushu University

*Correspondence to be sent to: Hirohito Miura, e-mail; hmiura@affrc.go.jp*

**Key words:** Mash1, Nkx2.2, Patched1 (Ptc), Sonic hedgehog (Shh), taste bud

## Introduction

In mammals, taste buds arise from local epithelium and are maintained by continuous cell renewal (Beidler and Smallman, 1965; Farbman, 1980; Delay *et al.*, 1986; Stone *et al.*, 1995). The life span of taste cells is estimated to be ~10 days. Denervation causes the degeneration of taste buds, indicating that the taste nerves trophically maintain the taste buds. It is assumed that cell proliferation, differentiation and death for taste buds are regulated in a coordinated fashion. However, the mechanism of each process in taste buds remains to be clarified.

## Shh signaling in taste buds

*Sonic hedgehog* (*Shh*) is an evolutionarily conserved intercellular signaling molecule and *Patched1* (*Ptc*) is its receptor (Ingham and McMahon, 2001). *Shh* signaling is known to mediate various inductive events, including cell proliferation and differentiation, in animal development. We have examined the expression of *Shh* signaling-related genes in taste buds of adult mice. The expression of *Shh* was found in the basal cells in taste buds, and *Ptc* expression was observed in the surrounding region of the *Shh* expression (Figure 1; Miura *et al.*, 2001). This *Shh* and *Ptc* expression pattern was observed in every taste papilla (Figure 2). While *Ptc* expression itself can indicate the tissues responding to *Shh* during development (Goodrich *et al.*, 1996; Marigo *et al.*, 1996), the expression of *Shh* target gene *Gli1* was also observed in almost same region where *Ptc* was expressed, indicating that the cells responded to *Shh* signal (Figure 1). Analysis of BrdU incorporation showed mitotic cells were observed mainly in *Ptc*-expressing region, while mitotic cells in and around taste buds have been thought to contain the taste bud progenitors (Beidler and Smallman, 1965; Delay *et al.*, 1986). In addition, the expression of a downstream gene of *Shh*, *Nkx2.2,* was observed in a subset of taste cells (Miura *et al.*, 2003). *Nkx2.2* is a homeobox, which is induced by *Shh* in the ventral region of neural tube and is required for the specification of ventral neuron (Briscoe *et al.*, 2000). These data raise the possibility that *Shh* signal may be involved in taste cell differentiation and that *Nkx2.2*-expressing cells in taste buds might be derived from the *Ptc*-expressing cells, which received *Shh* signal. The transection of cranial nerve IXth caused the

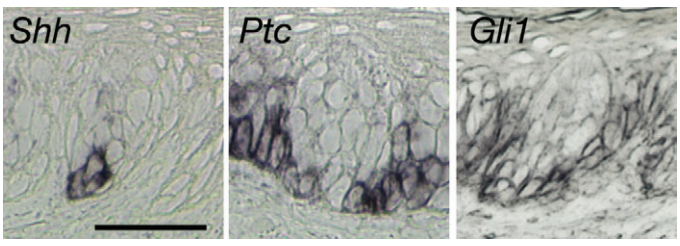


**Figure 1** The expression of Shh, Ptc and Gli1. The expression of Shh was restricted to the basal cells in taste buds. Ptc was observed in the basal side of the epithelial cell around Shh expression. The expression of Gli1 was observed in almost same region where Ptc was expressed. Scale bar indicate 50 μm.

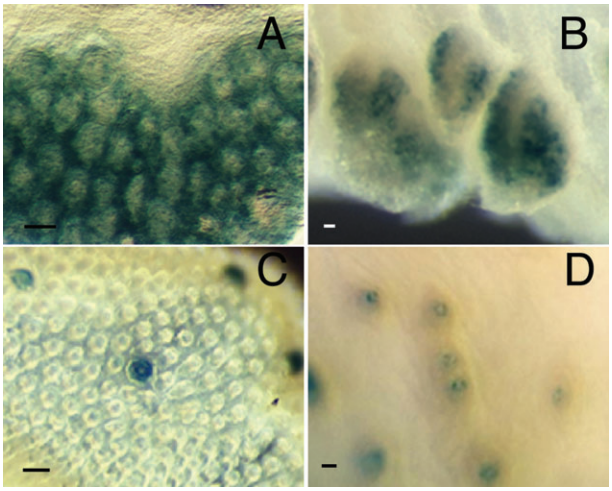


**Figure 2** The expression of Ptc in taste papillae and soft palate. **(A)** Circumvallate papilla; **(B)** foliate papilla; **(C)** fungiform papilla; **(D)** soft palate. In order to detect the *Ptc* expression with high sensitivity in whole mount, *Ptc*+/– mice, in which part of *Ptc* exon I and all of exon II were replaced with *lacZ* gene, were used, and the *Ptc* expression was detected by X-gal staining (Goodrich *et al.*, 1996). (A–C) The lingual epithelia were peeled off by collagenase and elastase treatment and used for staining. *Ptc* expression was observed in every taste bud. Scale bars indicate 50 μm.

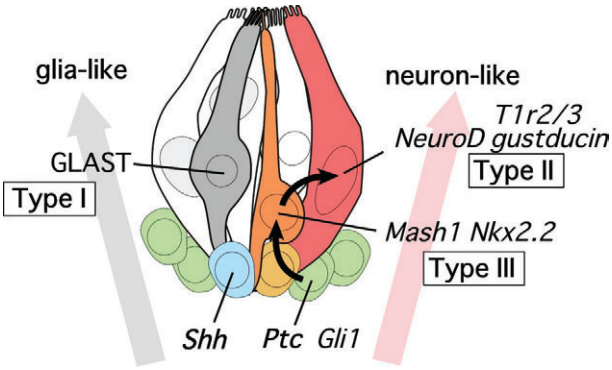


**Figure 3** Hypothetical cell lineage in taste buds. Shh may be involved in expression of Ptc and Nkx2.2. Type II cell may be derived form type III cell, while some of type III cell may be in type III cell state throughout cell life. (See text for further details.)

rapid loss of *Shh* expression in the basal cells in taste buds of circumvallate papillae, suggesting the possible involvement of *Shh* signaling in the taste bud maintenance (Miura *et al.*, 2001).

## Regulatory genes in taste buds and possible cell lineage

On the other hand, most *Nkx2.2*-expressing taste cells co-expressed *Mash1*, a bHLH transcription factor required for neuronal differentiation in CNS and olfactory epithelium (OE) (Miura *et al.*, 2003). We have previously reported that, in taste buds, the expression of *Mash1* segregated with those of taste reception-related genes, *gustducin* and *T1r2*, and that *Mash1* expression preceded taste reception-related gene expressions during development (Kusakabe *et al.*, 2002). Based on these data, we speculated that *gustducin*- and *T1r2*-expressing cells might be derived from *Mash1*-expressing cells. The expression of *NeuroD* in *gustducin*-expressing cells (Suzuki *et al.*, 2002) appears to be consistent with this idea by consideration of the sequential expression of bHLH transcription factors in neuronal differentiation in olfactory epithelium: *Mash1* is expressed in the early phase, and induces the expression of the other bHLH genes including *NeuroD* (Cau *et al.*, 2002). Based on ultrastructural characteristics, the taste bud cells have been categorized into four cell types—basal cell, type I (dark) cell, type II cell and type III (light) cell—and some immunohistochemical markers have been shown to correlate with each cell types: *gustducin* and NCAM are specific for type II and type III, respectively (Yee *et al.*, 2001). However, it is not clear whether these cell types represent separate cell lineages or transient cell states of one cell lineage (Pumplin *et al.*, 1997; Delay *et al.*, 1986). We found almost of all *Mash1*-expressing taste cells were NCAM-positive (unpublished data). Therefore, the hypothesis that *gustducin*-expressing cells derived from *Mash1*-expressing cells proposes the possible cell lineage of type II cell from type III cell. *Mash1* and *NeuroD* are bHLH factors critical for neuronal differentiation, while Type I cell has been considered as a glia-like cell (Lindemann, 1996; Lawton *et al.*, 2000). Recently, the expression of the genes related to Delta-Notch signaling in taste buds has been reported (Seta *et al.*, 2003). This signaling is known to be involved in neuron–glia cell fate determination, and therefore it may be possible for the Delta-Notch signaling to be involved in a cell-fate choice between neuron-like cell and glia-like cell in taste buds. Our hypothesis of taste cell lineage is summarized schematically in Figure 3.

## Acknowledgements

The authors thank Dr. Matthew P. Scott for providing with *Ptc* mutant mice. This study was supported by Bio-oriented Technology Research Advancement Institution.

## References

**Beidler, L.M.** and **Smallman, R.L.** (1965) *Renewal of cells within taste buds.* J. Cell Biol., 27, 263–272.

**Briscoe, J., Pierani, A., Jessell, T.M. and Ericson, J.** (2000) *A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube*. Cell, 101, 435–445.

**Cau, E., Casarosa, S. and Guillemot, F.** (2002) *Mash1 and Ngn1 control distinct steps of determination and differentiation in the olfactory sensory neuron lineage*. Development, 129, 1871–1880.

**Delay, R.J., Kinnamon, J.C. and Roper, S.D.** (1986) *Ultrastructure of mouse vallate taste buds: II. Cell types and cell lineage.* J. Comp. Neurol., 253, 242–252.

**Farbman, A.I.** (1980) *Renewal of taste bud cells in rat circumvallate papillae*. Cell Tissue Kinet., 13, 349–357.

**Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A. and Scott, M.P.** (1996) *Conservation of the hedgehog/patched signaling pathway from flies to mice—induction of a mouse patched gene by hedgehog*. Genes Dev., 10, 301–312.

**Ingham, P.W.** and **McMahon, A.P.** (2001) *Hedgehog signaling in animal development: paradigms and principles*. Genes Dev., 15, 3059–3087.

**Kusakabe, Y., Miura, H., Hashimoto, R., Sugiyama, C., Ninomiya, Y. and Hino A.** (2002) *The neural differentiation gene Mash-1 has a distinct pattern of expression from the taste reception-related genes gustducin and T1R2 in the taste buds*. Chem. Senses, 27, 445–451.

**Lawton, D.M., Furness, D.N., Lindemann, B. and Hackney, C.M.** (2000) *Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds.* Eur. J. Neurosci., 12, 3163–3171.

**Lindemann, B.** (1996) *Taste reception.* Physiol. Rev., 76, 719–766.

**Marigo, V., Scott, M.P., Johnson, R.L. Goodrich, L.V. and Tabin, C.J.** (1996) *Conservation in hedgehog signaling: induction of a chicken patched homolog by Sonic hedgehog in the developing limb*. Development, 122, 1225–1233.

**Miura, H., Kusakabe, Y., Sugiyama, C., Kawamatsu, M., Ninomiya, Y., Motoyama, J. and Hino, A.** (2001) *Shh and Ptc are associated with taste bud maintenance in the adult mouse.* Mech. Dev., 106, 143–145.

**Miura, H., Kusakabe, Y., Kato, H., Miura-Ohnuma, J., Tagami, M., Ninomiya, Y. and Hino, A.** (2003) *Co-expression pattern of Shh with Prox1 and that of Nkx2.2 with Mash1 in mouse taste bud.* Gene Expr. Patterns, 3, 427–430.

**Pumplin, D.W., Yu, C. and Smith, D.V.** (1997) *Light and dark cells of rat vallate taste buds are morphologically distinct cell types*. J. Comp. Neurol., 378, 389–410.

**Seta, Y., Seta, C. and Barlow, L.A.** (2003) *Notch-associated gene expression in embryonic and adult taste papillae and taste buds suggests a role in taste cell lineage decisions*. J. Comp. Neurol., 464, 49–61.

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

**Suzuki, Y., Takeda, M. and Obara, N.** (2002) *Expression of NeuroD in the mouse taste buds.* Cell Tissue Res., 307, 423–428.

**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.