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Regulation of Taste Bud Cell Differentiation by Notch Signaling Pathway

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Introduction

The Notch pathway is involved in determining cell fate within the nervous system and in various sensory organs (Lanford *et al.*, 1999; Cau *et al.*, 2000; Furukawa *et al.*, 2000; Ito *et al.*, 2000; Zine *et al.*, 2000). For example, *Mash1* is expressed in subsets of neuronal precursors in both the central nervous system (CNS) and the peripheral nervous system (PNS) (Guillemot *et al.*, 1993). Disruption of the Mash1 gene in mice results in the elimination of most olfactory and autonomic neurons, showing a role for Mash1 in the development of particular neural lineages (Guillemot *et al.*, 1993). In addition, Mash1 promotes differentiation during retinal development and is essential for proper ratios of neural cell types (Tomita *et al.*, 1996). Recently, Mash1 has been shown to be expressed in cells of the taste bud lineage, and that the expression of Mash1 in rat taste buds is dependent upon gustatory innervation (Seta *et al.*, 1999). However, involvement of the Notch signaling pathway, except for Mash1, in taste bud cell differentiation remained to be demonstrated.

In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation in fetal lingual epithelia, we have investigated the expression patterns of Notch and its ligands, Delta-like 1 (Dll1) and Jaggeds, hairy/enhancer of split (Hes1), and a mammalian homolog of the *achaete-scute* complex (Mash1) in fetal and adult mouse tongues using *in situ* hybridization. These genes are expressed in complex, dynamic patterns both in developing taste papillae and in taste cells within adult taste buds. The timing and pattern of early Notch signaling expression suggests a role for these genes in either sharpening the borders of developing papillae and/or specifying taste bud progenitors within papillae. Expression of Notch pathway genes in mature taste buds suggests that this signaling system may function in cell lineage decisions within taste buds.

Materials and methods

Tissue preparation

Timed pregnant CD-1 mice were obtained from Charles River (Wilmington, MA). The mice were overdosed with sodium pentobarbital, and E13–E18 embryos were surgically removed. The tongue tissues from embryos and adult mice were fixed overnight in 4% paraformaldehyde (PFA) in phosphate-buffer, pH 7.4, and embedded in OCT compound (Sakura, Torrance, CA). Cryostat sections (6–8 μm) were mounted on Superfrost slides (Fisher Scientific, Pittsburgh, PA) and stored in airtight boxes at –80°C.

In situ hybridization

Sections were washed in PBS and treated for 10 min with 0.2N HCl and for 5 min with proteinase K (1 μg/ml in TE). They were washed in PBS and refixed for 20 min in 4% PFA. They then were treated twice for 15 min with glycine (2 mg/ml in PBS). After washing with PBS, sections were prehybridized for 1 h at room temperature in hybridization buffer containing 50% formamide; 1.3× SSC; 5 mM EDTA; 0.5% CHAPS; 0.1% Tween 20; 1% blocking reagent (Roche Diagnostics GmbH, Germany); 100 μg/ml tRNA; 50 μg/ml heparin. Digoxigenin-labeled antisense and sense riboprobes were produced from plasmids containing Mash1, Dll1, Jagged1-2, Hes1 and Notch1-4. Hybridization was performed overnight at 60°C in hybridization buffer containing 0.5–1.0 μg/ml riboprobe. Excess probe was removed by sequential washes in 2× SSC, 0.1× SSC and MABT (0.1 M maleic acid, 0.15 M NaCl and 0.1% Tween 20) twice at room temperature. Sections were blocked for 1 h in 1% blocking reagent in MAB (0.1 M maleic acid and 0.15 M NaCl). Then sections were incubated for 2 h with anti-digoxigenin antibody conjugated to alkaline phosphatase diluted 1:250 in blocking solution. After rinsing with MABT, sections were equilibrated with color buffer containing 100 mM Tris, pH 9.5; 50 mM $MaCl_2$; 100 mM NaCl; and 0.1% Tween 20. Antibody was visualized by using the 4-Nitro blue tetrazolinium chloride/ 5-Bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) blue color reaction. Prior to photography or immunohistochemistry, sections were refixed in 4% PFA.

Immunohistochemistry

After *in situ* hybridization, some sections were analyzed for presence of the taste receptor cell markers PGP9.5 (ubiquitin carboxyl terminal hydrolase) or gustducin. Sections were rinsed in PBS and blocked for 2 h in 5% goat serum in PBS. Incubation with primary rabbit anti-PGP9.5 (1:300; Biogenesis, UK) or primary rabbit anti-gustducin (1:1000; Santa Cruz Biotechnology, USA) occurred overnight at 4°C in a humidified chamber. After rinsing with PBS, sections were incubated with Alexa Fluor 488 or 568 conjugated goat anti-rabbit IgG (Molecular Probes, USA) overnight at 4°C. Slides were rinsed with PBS and coverslipped with Fluoromount G (Southern Biotechnology Associates, USA).

Results and discussion

The results of our studies demonstrate that Notch-associated genes are expressed both in developing taste epithelia, and in the taste buds of adults. During the development of mouse circumvallate papillae, Notch signaling genes display temporal and spatial changes of expression. Notch-associated gene expression is initially broad in the lingual epithelium. But expression of many of these genes then resolves to the trench wall epithelium of developing circumvallate papillae, such that scattered labeled cells are located in the dorsal trench epithelium with more extensive labeling deep in the ventral trench. In adult taste buds, Notch-associated genes display spatial regulated expression pattern. Mash1 is expressed in basal cells of taste buds and in small number of fusiform (PGP9.5 positive) taste cells. But Dll1 is expressed in many fusiform taste cells, those expressing both PGP9.5 and gustducin. Notch1 is expressed basal cells adjacent to taste buds, and may identify precursor cells for basal cells in taste buds. On the other hand, Notch3 and Notch4 are expressed in fusiform taste cells and basal cells of taste buds, as are Hes1 and Jagged1/2.

In the present study, Notch-associated genes display spatial regulated expression pattern in adult taste buds. Mash1 is expressed in basal cells of taste buds and in small number of fusiform (PGP9.5 positive) taste cells. Immunoreactivity of PGP9.5, marker of paraneuron, is localized in the Type III or gustatory cells which make synapse with nerve terminal (Kanazawa and Yoshie, 1996). Our observation suggests that Mash1 may regulate the differentiation of PGP9.5 positive taste receptor cells. On the other hand, Dll1 is expressed in many fusiform taste cells, those expressing both PGP9.5 and gustducin. Gustducin, a G-protein implicated in both sweet and bitter transduction, occurs only in some Type II cells (Tabata *et al.*, 1995; Boughter *et al.*, 1997). Based on our observation of the colocalization Dll1 and PGP9.5/gustducin in adult taste buds, Dll1 expressing fusiform taste cells are taste receptor cells. Other Notch-associated genes, Notch3 and Notch4 are expressed in fusiform taste cells and basal cells of taste buds, as are Hes1 and Jagged1/2. Recent studies have revealed that Notch signaling pathway is involved in promoting the choice of cell fate in sensory organs. Notch1 and Hes1 are downregulated in neuron, but continue to be expressed in glial cells in their development in the retina (Furukawa *et al.*, 2000). In the inner ear developing hair cells express Jagged2, and Jagged2 activates Notch in neighboring cells, preventing those cells from developing as hair cells (Lanford *et al.*, 1999). Hes1 represses neuronal differentiation by suppression of proneural bHLH factors. The suppressive mechanisms of Hes1 on proneural bHLH factors are suggested to involve two way pathways: one suppressing formation of the Mash1/E2A complex though protein–protein interaction and the other repressing Mash1 transcription. Recent studies have disclosed that repressive bHLH factors such as Hes1 may be regulated by the Notch pathway (Jarriault *et al.*, 1995; Schroeter *et al.*, 1998). Mammalian taste buds contain several morphologically and biochemically distinguishable types of cells. On the basis of the observation of the ultrastructural studies, mammalian taste bud cells are classified into at least two cell types, supporting cells and sensory cells (Murray, 1969, 1973; Seta and Toyoshima, 1995). Our *in situ* hybridization analyses show Notch3, Notch4 and Hes1 express in subset of fusiform taste cells, and also Notch ligands, Jagged1, Jagged2 and Dll1 express in subset of taste cells. These expression data imply that subset of taste cells expressing Notch3, Notch4, and Hes1 may be supporting cells (non-receptor cells), and Notch-ligands expressing cells may be receptor cells expressing PGP9.5 and gustducin. These results suggest that the Notch signaling pathway may be involved in determination of taste receptor cell types within taste buds of adult.

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