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## Pou2f3/Skn-1a Is Necessary for the Generation or Differentiation of Solitary Chemosensory Cells in the Anterior Nasal Cavity

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### Abstract

Solitary chemosensory cells in the non-neuronal epithelium of the anterior nasal cavity have bitter taste cell-like molecular characteristics and are involved in the detection of noxious substances. Here, we demonstrate that Pou2f3/Skn-1a, which is necessary for generation of sweet, umami, and bitter taste cells, is also necessary for the generation or differentiation of solitary chemosensory cells.

### Keywords

transcription factor; Pou2f3; solitary chemosensory cell; differentiation; taste cell

Solitary chemosensory cells (SCCs) are epithelial sensory cells observed in vertebrates.<sup>1–3)</sup> They are distributed on the body surface in fish and in the respiratory epithelia in mammals. Although little is known about fish SCCs,<sup>4)</sup> knowledge of mammalian SCCs has been accumulating in the past several years. In mammals, SCCs express bitter receptors (Tas2rs), gustducin, phospholipase C-2 (PLC-2), and transient receptor potential channel M5 (TRPM5) and detect noxious substances such as bitter chemicals and acyl-homoserine lactone quorum-sensing molecules.<sup>2,5)</sup> Activated SCCs transmit chemical information to the trigeminal neurons *via* synapses and regulate respiratory responses to hazardous chemicals.<sup>2,5,6)</sup> Thus, SCCs function as a gatekeeper of biophylactic reaction(s), similar to the function of taste cells eliciting aversive behaviors. SCCs also express a sweet and umami receptor component (Tas1r3) enigmatically, but its function in SCCs is unknown.<sup>7)</sup> Like other epithelial chemosensory cells, such as taste cells, SCCs undergo continuous turnover.<sup>8)</sup> However, the mechanisms of development and differentiation of SCCs remain unclear.

Pou2f3, a POU homeodomain transcription factor also known as Skn-1a, is involved in the generation of sweet, umami, and bitter taste cells (so-called type II taste cells).<sup>9)</sup> Its loss-of-function mutation expanded the sour taste cell population at the expense of sweet, umami, and bitter taste cells. Thus, Pou2f3/Skn-1a functions as an early determinant of the

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differentiation to these taste cells, and there should be terminal selectors regulating the terminal differentiation to respective sweet, umami, and bitter taste cells.<sup>9)</sup>

Here we show that *Pou2f3/Skn-1a* is necessary for generation or functional differentiation of SCCs. The commonalities between SCCs and bitter cells, including intracellular signaling molecules and physiological functions to detect noxious substances, suggested that a common mechanism underlies the differentiation of these chemosensory cells. Intriguingly, SCCs co-express *Tas1r3* and *Tas2rs*, while *Tas1rs* and *Tas2rs* are expressed in different subsets of taste cells in taste buds. This further suggests a possibility that the mechanism of differentiation of SCCs would be the same as the early differentiation of the precursors prior to the terminal differentiation to bitter, sweet, and umami cells, which requires *Pou2f3/Skn-1a*. Therefore, we first investigated the expression of *Pou2f3/Skn-1a* mRNA in the anterior nasal cavity by *in situ* hybridization analysis. In this research, we used 1- to 2-month-old male C57BL/6J and *Pou2f3/Skn-1a*-knockout mice. Preparation of mouse tissues, materials, and methods were as described previously.<sup>7,10)</sup> All animal experiments were approved by the Institutional Animal Care and Use Committee of Monell Chemical Senses Center and Tokyo Institute of Technology.

*In situ* hybridization analysis showed that the scattered signals of *Pou2f3/Skn-1a* mRNA were detected in the epithelial cells of the mouse anterior nasal cavity (Fig. 1A). These signals were observed in a small population of cells in the non-neuronal epithelial cell layer of the vomeronasal organ and respiratory epithelium (Fig. 1B and C). The distribution and frequency of *Pou2f3/Skn-1a* mRNA signals were similar to those of the SCCs.<sup>2,7)</sup> Then, we carried out double-label *in situ* hybridization analysis to examine whether *Pou2f3/Skn-1a* is expressed in the SCCs. The signals of *Pou2f3/Skn-1a* mRNA overlapped with those of *Trpm5* and *Tas1r3* mRNAs, markers for the SCCs in the anterior nasal cavity<sup>7)</sup> (Fig. 1D–G). These results clearly show the expression of *Pou2f3/Skn-1a* in the SCCs.

To examine the impact of *Pou2f3/Skn-1a* on the SCCs, we examined the expression of genes in *Pou2f3/Skn-1a*-knockout mice. We detected the signals for *Tas1r3*, *Tas2r105*, *Tas2r108*, *Gnat3* (gustducin), *Plcb2*, and *Trpm5* mRNAs in the nasal epithelium of wild-type mice, all of which should be expressed in the SCCs<sup>2,7)</sup> (Fig. 2, top), but no signal of these mRNAs was observed in the *Pou2f3/Skn-1a*-knockout mice (Fig. 2, bottom). These results suggest that *Pou2f3/Skn-1a* is necessary for the generation or differentiation of the SCCs in the anterior nasal cavity. Sweet, umami, and bitter taste cells were absent in the taste buds of *Pou2f3/Skn-1a*-knockout mice.<sup>9)</sup> *Pou2f3/Skn-1a* is presumably involved in the generation of the SCCs in the anterior nasal cavity.

In this study, we found that *Pou2f3/Skn-1a* is essential for the generation or differentiation of SCCs as well as bitter taste cells.<sup>9)</sup> However, unlike bitter taste cells, SCCs co-express *Tas1r3* with *Tas2rs* and have synapses.<sup>2,7)</sup> It will be interesting to compare bitter cells and SCCs to identify mechanisms responsible for these differences. Taste cell-like chemosensory cells have been found in other tissues.<sup>11–14)</sup> Comparative analyses across these cells may reveal crucial mechanisms for generating a variety of closely related chemosensory cells.

## Acknowledgments

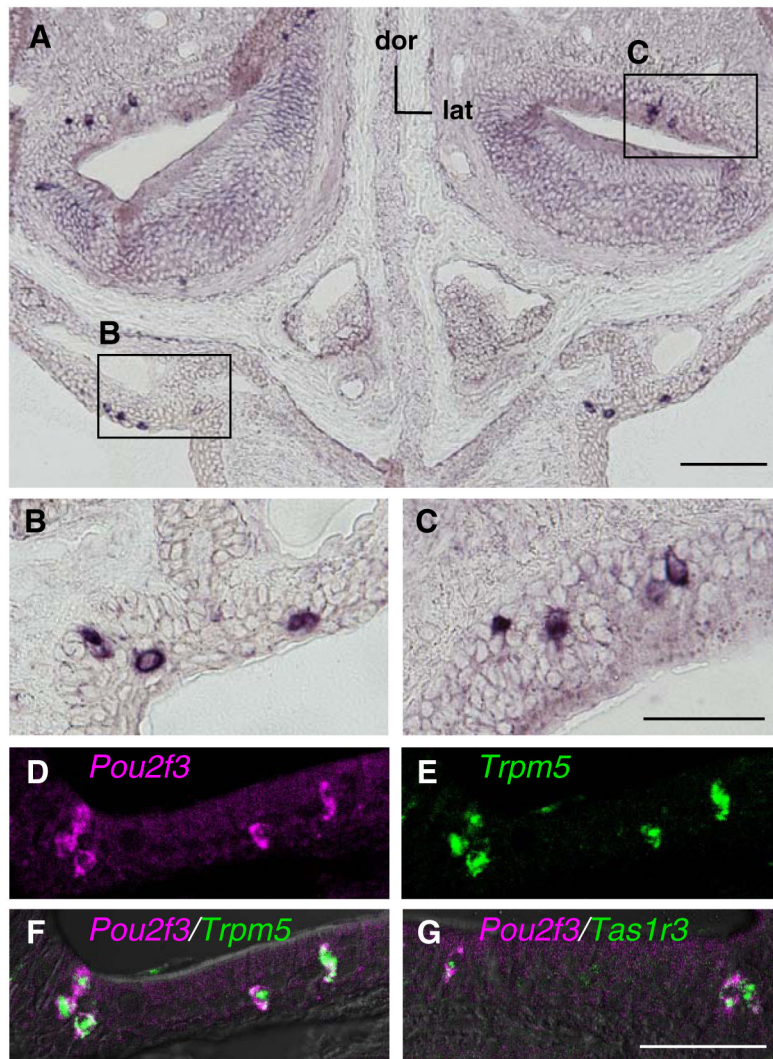
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## Abbreviations

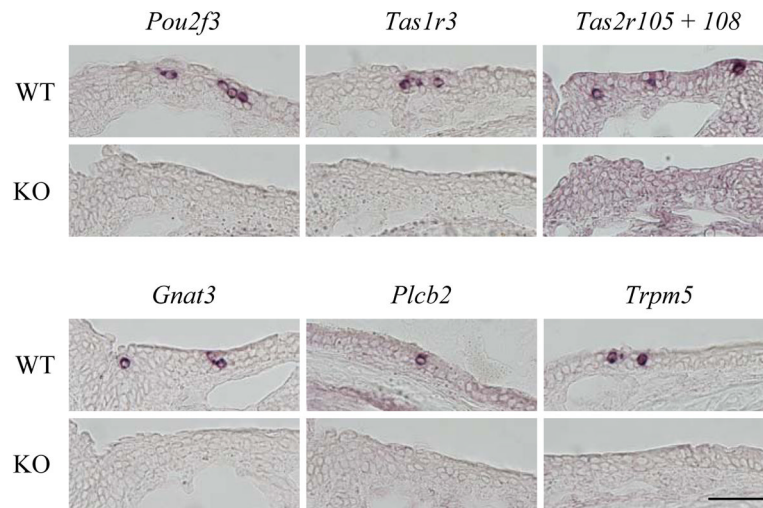
<b>SCC</b>	solitary chemosensory cell
<b>PLC- 2</b>	phospholipase C- 2
<b>TRPM5</b>	transient receptor potential channel M5

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**Fig. 1.** Expression of *Pou2f3/Skn-1a* in the SCCs in the Nasal Epithelia. Single-label (A–C) and double-label (D–G) *in situ* hybridization of *Pou2f3/Skn-1a* and genes expressed in SCCs in coronal sections of mouse anterior nose. dor, dorsal; lat, lateral. B and C, Magnified views of the *Pou2f3/Skn-1a* mRNA signals in the respiratory epithelia (B) and non-neuronal epithelial cell layer of the vomeronasal organ (C). D–G, Signals of *Pou2f3/Skn-1a* mRNA (magenta) were observed in cells expressing *Trpm5* (green, E and F) and *Tas1r3* (green, G) in the anterior nasal epithelia. Scale bars, 100  $\mu$ m in A and 50  $\mu$ m in C and G.



**Fig. 2.** Loss of the Expression of SCC Signaling Genes in *Pou2f3/Skn-1a*-Knockout Mice. *In situ* hybridization of signaling molecules in SCCs on sections of wild-type (WT, top) and *Pou2f3/Skn-1a*-knockout (KO, bottom) mice was carried out to examine the impact of *Pou2f3/Skn-1a* on SCCs. The mRNA signals of *Pou2f3/Skn-1a*, *Tas1r3*, mixed probes of *Tas2r105* and *Tas2r108*, *Gnat3*, *Plcb2*, and *Trpm5* observed in wild-type mice was completely absent in the *Pou2f3/Skn-1a*-knockout mice. Scale bar, 50  $\mu$ m.