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Pax6 regulates Tbr1 and Tbr2 expressions in olfactory bulb mitral cells

Fumiaki Imamura [a], Charles A. Greer [a,b,*]

[a] Department of Neurosurgery, Yale University School of Medicine, New Haven, CT 06520, USA
[b] Department of Neurobiology, Yale University School of Medicine, New Haven, CT 06520, USA

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ABSTRACT

Tracking olfactory bulb mitral cell development with BrdU labeling, we find that mitral cells are generated from Pax6+ radial glial cells in the ventricular zone of the embryonic olfactory bulb. Unlike cortical projection neurons, postmitotic mitral cell precursors express both Tbr1 and Tbr2. Our tracking experiments revealed that down-regulation of Pax6 preceded up-regulation of Tbrs, and that Tbr1 emerged earlier than Tbr2. Using *in utero* electroporation, we also show that Pax6 negatively regulates the expression of Tbr1 and Tbr2 in postmitotic mitral cell precursors. Exogenous expression of Pax6 in embryonic olfactory bulb postmitotic precursors decreased the number of cells that progressed to a mitral cell fate. In contrast, exogenous expression of Pax6 resulted in an increase of GABAergic and/or dopaminergic interneurons. These results indicate that Pax6 is a regulator of fate determination of precursor cells.

Introduction

Mitral and tufted cells, the glutamatergic olfactory bulb (OB) projection neurons, receive synaptic input from olfactory sensory neuron axons and transmit information to the olfactory cortex (Mori et al., 1999). Mitral cells are generated from ventricular zone (VZ) progenitors in the anterior telencephalic vesicle (Blanchart et al., 2006; Imamura et al., 2011). Postmitotic mitral cell precursors migrate radially toward the intermediate zone (IZ) where they differentiate into mitral cells. However, the molecular mechanisms regulating mitral cell differentiation remain enigmatic. Here, we studied the mechanisms of differentiation by focusing on transcription factors, Tbr1, Tbr2, and Pax6.

In developing neocortex, Pax6 is expressed by radial glial cells and is an intrinsic fate determinant of their neurogenic potential (Hack et al., 2004; Haubst et al., 2004; Heins et al., 2002). During cortical pyramidal neuron development Pax6 is down-regulated in radial glial-derived intermediate progenitor cells (IPCs). Down-regulation of Pax6 is associated with an up-regulation of Tbr2, while a down-regulation of Tbr2 results in an up-regulation of Tbr1 in postmitotic pyramidal cells. Therefore, there is a transcription factor expression sequence Pax6→Tbr2→Tbr1 in the differentiation of radial glia→IPC→postmitotic pyramidal neuron (Englund et al., 2005). Postmitotic Tbr1+ pyramidal cells do not express Pax6 or Tbr2.

Pax6 is also expressed in the anterior tip of telencephalic vesicle (Hebert et al., 2003; Walther and Gruss, 1991). Like developing neocortex, it was recently suggested that OB mitral and tufted cells are generated from Neurog2+ cells derived from Pax6+ cells in the VZ (Winpenny et al., 2011). These data also suggested the existence of cells expressing both Pax6 and Tbr2, but not Tbr1, in the VZ. However, unlike cortical pyramidal neurons, postmitotic mitral cell precursors in the IZ express not only Tbr1, but also Tbr2 (Bulfone et al., 1995, 1999; Faedo et al., 2002; Mizuguchi et al., 2012). Absence of either Tbr1 or Tbr2 in postmitotic mitral cell precursors causes comparable defects in mitral cell development, indicating that both molecules are necessary for the cells to progress toward a mitral/tufted cell phenotype (Arnold et al., 2008; Bulfone et al., 1998; Sessa et al., 2008). Thus, there should be a unique mechanism that regulates the expression of Tbr1 and Tbr2 in postmitotic mitral cell precursors in the developing OB.

Here, we used the mouse to determine the temporal and spatial expression patterns of Pax6, Tbr1, and Tbr2 in developing mitral cells. We first establish that Pax6 and Tbrs show diametrical expression patterns during mitral cell development. Using *in utero* electroporation to control Pax6 expression, we also show that exogenous expression of Pax6 in postmitotic mitral cell precursors impairs both Tbr1 and Tbr2 expressions and therefore, mitral cell fate. Interestingly, as a consequence of ectopic Pax6 expression, mitral cell precursors changed their fate and expressed molecular phenotypes characteristic of OB interneurons including dopaminergic and GABAergic periglomerular cells. These data demonstrate the importance of transcription factor expression pathways and that mitral cell fate is critically dependent upon down-regulation of Pax6 and the ensuing up-regulation of Tbr2 and Tbr1.

* Corresponding author at: Department of Neurosurgery, Yale University School of Medicine, P.O. Box 208082, New Haven, CT 06520-8082, USA.
E-mail address: charles.greer@yale.edu (C.A. Greer).

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Results

Expressions of Pax6, Tbr1, and Tbr2 in mitral cell precursors

To begin probing the molecular signaling pathways that regulate mitral cell fate, we first examined the developmental expression of the candidate transcription factors Pax6, Tbr1, and Tbr2 in mitral cell precursors. Because we previously showed that mouse mitral cells were generated predominately between embryonic day (E) 10 and E13, with a peak of genesis at E11, (Blanchart et al., 2006; Imamura et al., 2011), we made a single injection of BrdU into pregnant mothers at E11, and then double labeled for Pax6, Tbr1, and Tbr2 at 2 h (E11), 1 day (E12), 2 days (E13), 3 days (E14), or 4 days (E15) post-BrdU (Fig. 1). Progenitor cells divide asymmetrically to produce a postmitotic cell as well as a second progenitor cell. Both daughter cells would be labeled with BrdU. Nevertheless, because the general cell cycle duration in the VZ of E11 cerebral cortex is about 8 h (Takahashi et al., 1995), we can reasonably conclude that almost all BrdU-labeled cells examined at each of the sacrifice times were generated around E11.

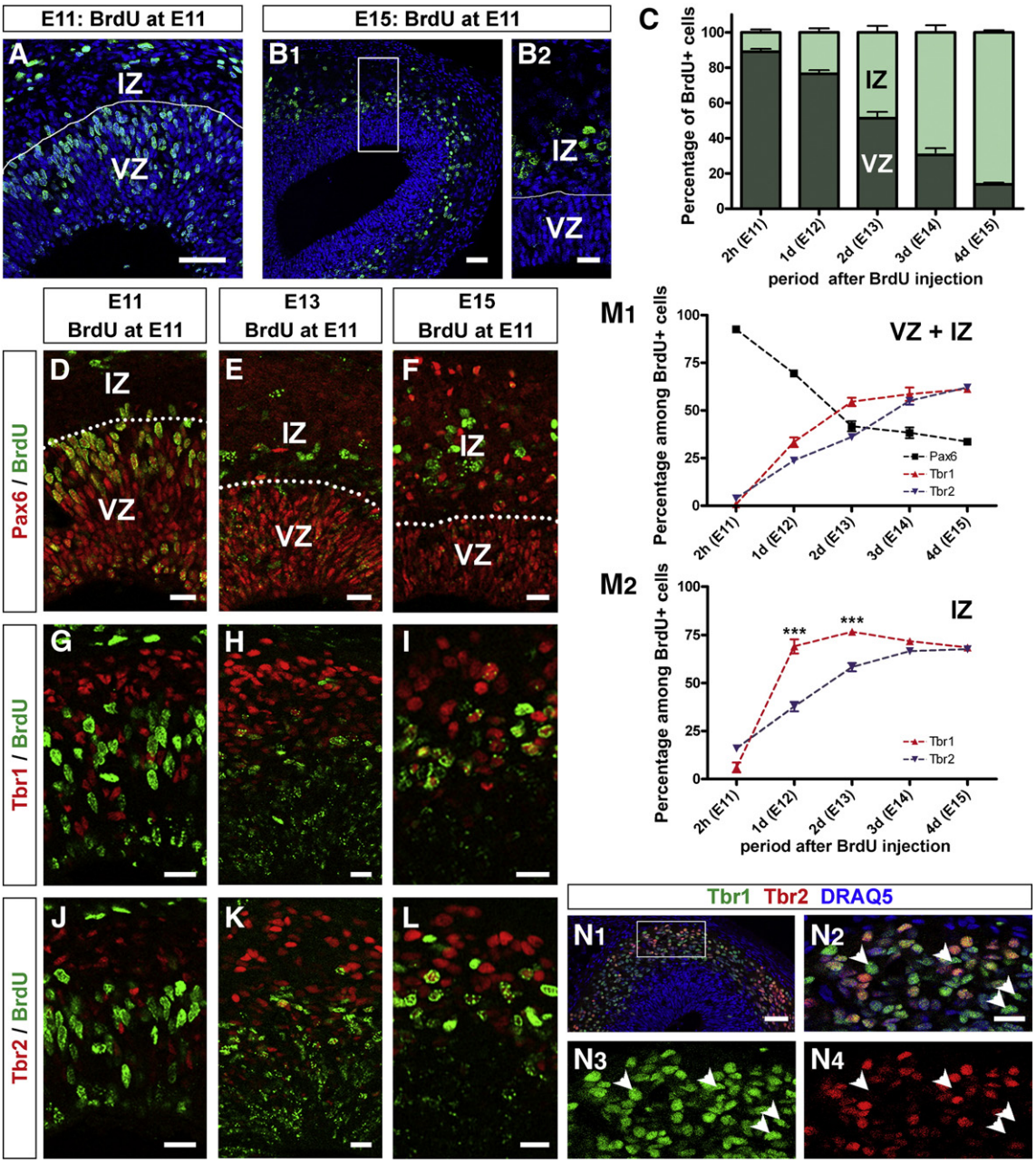

Fig. 1. Expression of Pax6, Tbr1, and Tbr2 in mitral cell precursors in embryonic olfactory bulbs. (A, B) Horizontal sections of E11 (A) and E15 OBs (B). BrdU (Cy2, green) was intraperitoneally injected into pregnant mothers at E11. All nuclei were stained with DRAQ5 (blue). The OBs were divided into VZ and IZ based on the morphology of cell nuclei. (C) Graph showing the distribution of BrdU-labeled cells between VZ and IZ at 2 h (E11), 1 day (E12), 2 days (E13), 3 days (E14), and 4 days (E15) after BrdU injection. (D–L) Expression of Pax6 (D–F; Alexa 555, red), Tbr1 (G–I; Alexa 555, red), and Tbr2 (J–L; Alexa 555, red) were immunohistochemically examined at 2 h (E11; D, G, J), 2 days (E13; E, H, K) and 4 days (E15; F, I, L) after injection. Distributions within OBs and co-labeling with BrdU (green) were examined. VZ: ventricular zone; IZ: intermediate zone. (M) Graphs showing percentages of BrdU-labeled cells expressing Pax6 (black), Tbr1 (red), or Tbr2 (blue) at each time point after BrdU injection. The percentages were calculated among BrdU-labeled cells in both VZ and IZ (M1) and only IZ (M2). In total, decrease of percentage of Pax6+ cells occurs in parallel with increases of Tbr1+ and Tbr2+ cells. Percentages of Tbr1+ cells in IZ are significantly higher than those of Tbr2+ cells at E12 and E13 (***$p<0.0001$; $n=6$) (unpaired *t* test). (N) Expression patterns of Tbr1 (Alexa 488, green) and Tbr2 (Alexa 555, red) in E13 OB. There are many cells expressing only Tbr1 (N2–N4; arrowheads) while all Tbr2+ cells are expressing Tbr1. Error bars, s.e.m. Scale bars, 50 μm in (A) and (B1); 20 μm in (B2), (D–L), and (N2); and 100 μm in (N1).