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Sequential Arrival and Graded Secretion of Sema3F by Olfactory Neuron Axons Specify Map Topography at the Bulb

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SUMMARY

In the mouse olfactory system, the anatomical locations of olfactory sensory neurons (OSNs) roughly correlate with their axonal projection sites along the dorsal-ventral (D-V) axis of the olfactory bulb (OB). Here we report that an axon guidance receptor, Neuropilin-2 (Nrp2), and its repulsive ligand, Semaphorin-3F (Sema3F), are expressed by OSNs in a complementary manner that is important for establishing olfactory map topography. Sema3F is secreted by early-arriving axons of OSNs and is deposited at the anterodorsal OB to repel Nrp2-positive axons that arrive later. Sequential arrival of OSN axons as well as the graded and complementary expression of Nrp2 and Sema3F by OSNs help to form the topographic order along the D-V axis.

INTRODUCTION

In the mouse olfactory system, each olfactory sensory neuron (OSN) expresses one functional odorant receptor (OR) gene (Buck and Axel, 1991; Malnic et al., 1999; Serizawa et al., 2003). Furthermore, OSNs expressing a given type of OR converge their axons to a specific glomerulus in each mirror map in the right and left olfactory bulbs (OBs) (Mombaerts et al., 1996; Ressler et al., 1994; Vassar et al., 1994). During glomerular map formation, OR molecules play instructive roles in the projection of OSN axons (Feinstein and Mombaerts, 2004; Ishii et al., 2001; Wang et al., 1998). Recent studies demonstrated that ORs direct axon targeting along the anterior-posterior (A-P) axis by regulating the transcription of axon guidance molecules, such as Neuropilin-1 (Nrp1) and Semaphorin-3A (Sema3A), using OR-derived cAMP signals (Chesler et al., 2007; Col et al., 2007; Imai et al., 2006).

In contrast to A-P projection, there is a close correlation between the locations of OSNs in the olfactory epithelium (OE) and their axonal projection sites in the OB along the dorsal-ventral (D-V) axis (Astic et al., 1987). The preservation of the spatial relationship of cell bodies and their axonal target sites is widely seen in other brain regions (Luo and Flanagan, 2007; Petersen, 2007; Rubel and Fritzsch, 2002). R.W. Sperry's seminal experiment provided the basis for his "chemoaffinity model" in which target cells present chemical cues to guide projecting axons to their correct destinations (Sperry, 1963). This model has held true for the projection of axons in several different systems, such as the visual system (Lemke and Reber, 2005; McLaughlin and O'Leary, 2005). In the olfactory system, two sets of repulsive signaling systems, Nrp2/Sema3F and Robo2/Slits, have been proposed to participate in projection along the D-V axis in the OB (Cho et al., 2007; Cloutier et al., 2002, 2004; Nguyen-Ba-Charvet et al., 2008; Norlin et al., 2001; Prince et al., 2009; Walz et al., 2002). It has been reported that D-zone axons expressing a guidance receptor, Robo2, navigate to the D domain of the OB through the repulsive effects of the ligands Slit-1 and Slit-3, which are expressed in the V domain of the OB (Cho et al., 2007). These molecules are assumed to contribute to the separation of D and V domains (Cho et al., 2007).

In the OE, *OR* genes expressed by OSNs that project to the D domain of the OB are distributed throughout the D zone (Tsuboi et al., 2006). However, V-zone-specific *OR* genes exhibit spatially limited expression: each *OR* gene possesses its unique expression area, which is distributed in an overlapping manner along the dorsomedial-ventrolateral (DM-VL) axis of the OE (Miyamichi et al., 2005; Ressler et al., 1993; Vassar et al., 1993). The reliability of the relationship between D-V positioning of glomeruli and DM-VL locations of OSNs has been

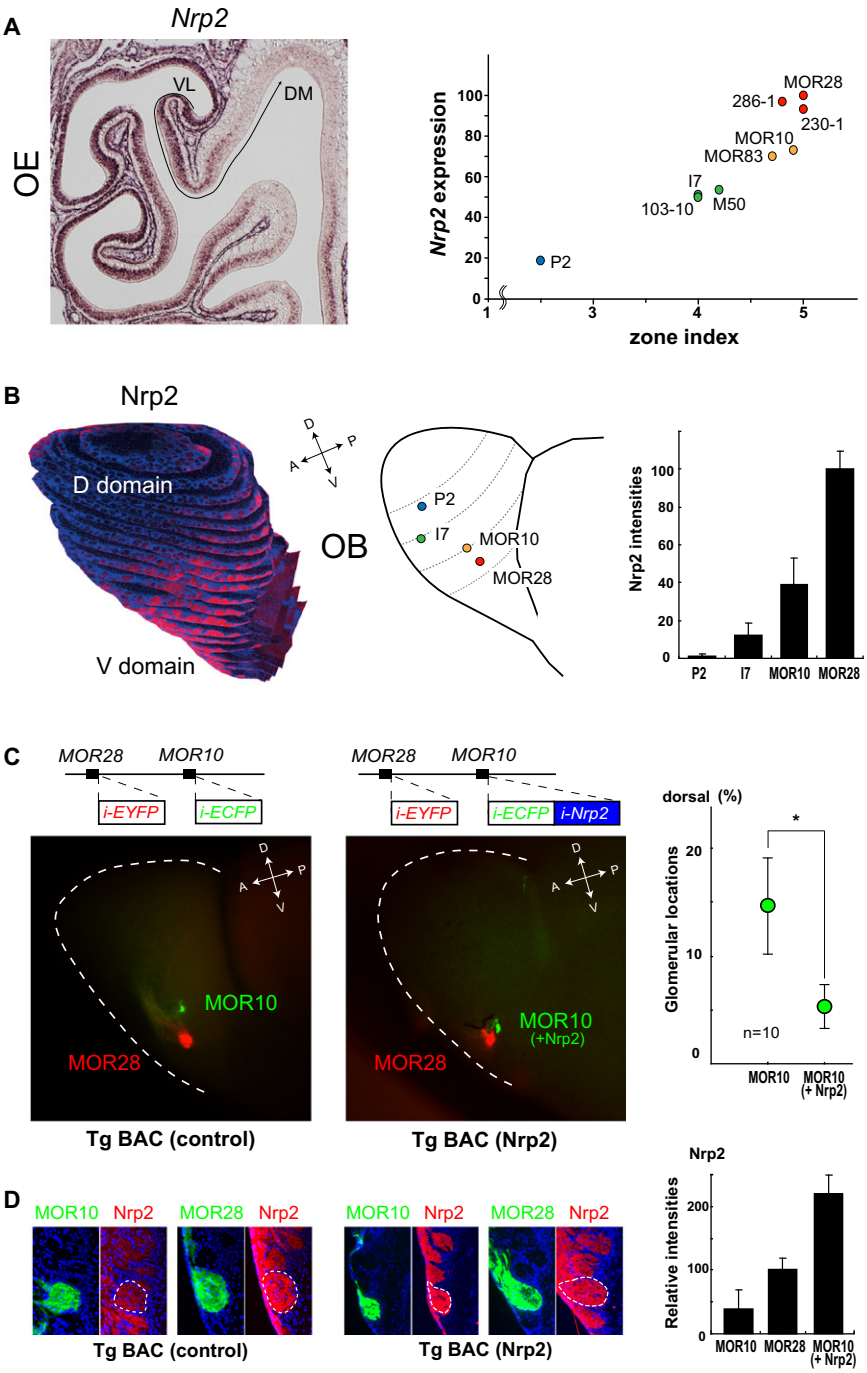

Figure 1. Nrp2 Expression Levels Correlate with Glomerular Positions along the D-V Axis in the OB

(A) Graded expression of the *Nrp2* in the OE. An OE section was hybridized with the *Nrp2* probe. *Nrp2* expression was high in the ventrolateral (VL) area and low in the dorsomedial (DM) area, as reported previously (Norlin et al., 2001) (left). *Nrp2* levels are correlated with zone indices (Miyamichi et al., 2005) for various *OR* genes in the OE (right). In situ hybridization data are shown in Figure S1A. *Nrp2* expression levels are normalized to those for MOR28-expressing cells (100%).

(B) Graded distribution of Nrp2 in the OB. Horizontal OB sections were immunostained with anti-Nrp2 antibodies and stacked at ~180 μm intervals (left). Glomerular locations for MOR28, MOR10, I7, and P2 were determined by immunostaining with OR-specific or anti-β-galactosidase antibodies and are shown in a schematic diagram of the OB (middle). Signal intensities of Nrp2 within glomeruli for each OR are compared (normalized to MOR28 glomeruli) (right). Error bars indicate standard deviation (SD) (n = 12).

(C) The gain-of-function experiment of Nrp2. Two transgenic (Tg) mice were made. One Tg BAC (control) contained *MOR28* and *MOR10* genes that were differently tagged with *IRES-gapEYFP* and *IRES-gapECFP*, respectively. The other, Tg BAC (Nrp2), drove expression of *IRES-Nrp2* downstream of the *ECFP*-tagged *MOR10*. In the whole-mount OB (lateral views), MOR10 glomeruli fluoresced green, whereas MOR28 glomeruli fluoresced red. Increased Nrp2 expression caused a ventral shift of MOR10 glomeruli. The relative glomerular locations for MOR10 (green) and MOR28 (red) were compared between control animals (left) and transgenic animals expressing excess Nrp2 (right). *p < 0.01 (student's t test). Error bars indicate SD (n = 10 for each line).

(D) Nrp2 levels in MOR10 and MOR28 glomeruli. Glomeruli were identified with anti-GFP antibodies. To measure Nrp2 levels, adjacent OB sections were immunostained with anti-Nrp2 antibodies. Signal intensities of Nrp2 in MOR10 and MOR28 glomeruli are compared in the right (normalized to those of MOR28). Error bars indicate SD (n = 8).

D, dorsal; V, ventral; A, anterior; P, posterior. See also Figure S1.

demonstrated (Astic et al., 1987; Miyamichi et al., 2005). How is this positional information of neurons in the OE translated to their target sites in the OB during olfactory map formation? Here we report that a repulsive ligand, Sema3F (Chen et al., 1997; Giger et al., 1998), is secreted by early-arriving OSN axons and is deposited in the anterodorsal OB to serve as a guidance cue to repel late-arriving OSN axons that express Nrp2 receptor. Sequential arrival of projecting axons and complementary expression of Nrp2 and Sema3F by OSNs appear to contribute to D-V patterning in the OB.

RESULTS

Graded Expression of Nrp2

As shown in Figure 1A, *Nrp2* is expressed at high levels in the VL area of the OE but at low levels in the DM area. Double in situ hybridization revealed that the level of *Nrp2* transcription within each cell is correlated with the expressed OR species (Figures 1A, right and Figure S1A available online). In addition, there appears to be a direct relationship between the amount of *Nrp2* transcript or Nrp2 protein in OSNs and their projection sites