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Identification of Midbrain Floor Plate Radial Glia-Like Cells as Dopaminergic Progenitors

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KEY WORDS
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ABSTRACT
The floor plate (FP), a signaling center and a structure rich in radial glia-like cells, has been traditionally thought to be devoid of neurons and neuronal progenitors. However, in the midbrain, the FP contains neurons of the dopaminergic (DA) lineage that require contact with radial glia-like cells for their induction. We, therefore, decided to explore the interaction relationship between radial glia and neurons during DA neurogenesis. Taking advantage of a novel FP radial glia-like cell culture system and retroviruses, DA neurons were lineage traced *in vitro*. *In utero* BrdU pulse-chases extensively labeled the midbrain FP and traced DA neurons both *in vivo* and in FP cultures. Moreover, from E9.5 to E13.5 the midbrain FP contained dividing cells only in the most apical part of the neuroepithelium, in cells identified as radial glia-like cells. We, therefore, hypothesized that midbrain FP radial glia-like cells could be DA progenitors and tested our hypothesis *in vivo.* Lineage tracing of DA progenitors with EGFP in Tis21-EGFP knock-in mice, and genetic fate mapping in GLAST::CreERT2/ZEG mice identified the neuroepithelium of the midbrain FP, and specifically, GLAST+ radial glia-like cells as DA progenitors. Combined, our experiments support the concept that the midbrain FP differs from other FP regions and demonstrate that FP radial glia-like cells in the midbrain are neurogenic and give rise to midbrain DA neurons.

INTRODUCTION

The floor plate (FP), the most ventral part of the neural tube has been classically considered as a structure devoid of neurons and neuronal progenitors, but rich in radial glia-like cells (Kingsbury, 1920). This structure is often referred to as a signaling or organizing center because it secretes factors such as Sonic Hedgehog (Shh), which is involved in specifying neuronal and glial identity (Jessell, 2000), and Netrin, an axon guidance molecule (Colamarino and Tessier-Lavigne, 1995). Shh generates a ventral to dorsal signaling gradient that regulates the expression of transcription factors and specifies neuronal identity (Briscoe et al., 1999). Shh, netrin, and forkhead box A2 (FoxA2; Placzek, 1995) are expressed in the FP from the diencephalon to the spinal cord, but the FP is not a homogeneous structure and there are important cellular and molecular differences along the anteroposterior axis (Placzek and Briscoe, 2005). Some of the cellular differences between the midbrain and other FP regions were reported by His as long ago as 1888 (reviewed by Kingsbury, 1920), who observed that neuroblasts and neurons were intermingled with radial glia-like cells in the midbrain FP. During early stages of development, the FP is the source of ventral patterning signals, such as Shh (Ericson et al., 1995), and at latter stages, radial glia-like cells in the midbrain FP serve as a scaffolds for dopaminergic (DA) neuron migration (Kawano et al., 1995; Shults et al., 1990), a function that they share with radial glia cells outside the FP (Rakic, 2003). Non-FP radial glia has been instead described as a source of neuronal differentiation factors such as retinoic acid for striatal neurons (for review see Campbell and Götz, 2002). More recently, non-FP radial glia in diverse brain regions were demonstrated to undergo neurogenic divisions (for review see, Ever and Gaiano, 2005; Gotz and Huttner, 2005; Kriegstein and Götz, 2003; Fishell and Kriegstein, 2003). However, it remains to be determined whether FP radial glia-like cells, a cell until now considered as non-neurogenic, can also undergo neurogenesis.

The position occupied by DA progenitors in the neural tube has been debated for many years. Different areas of the brain such as the diencephalon (Marin et al., 2005), the isthmus (Marchand and Poirier, 1983), or different parts of the midbrain such as the basal plate/

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lateral FP, (Hynes et al., 1995a,b), the FP (Kawano et al., 1995), or both these areas (Hanaway et al., 1971) have been proposed as possible positions occupied by DA progenitors. In recent years, emerging evidence has indicated that the midbrain FP expresses proneural genes. One of them, the basic-helix-loop-helix transcription factor, neurogenin2 (Ngn2), is expressed in the midbrain FP during the neurogenenic period (from E10.75 to E13.5), and is required for DA neurogenesis (Andersson et al., 2006a; Kele et al., 2006). In addition, the identification, functional characterization, and expression analysis of several other transcription factors such as Lmx1b, Lmx1a, Msx1/2, and FoxA2 (Andersson et al., 2006b; Ferri et al., 2007; Kittappa et al., 2007; Smidt et al., 2000) or secreted factors such as Wnt1 (Prakash et al., 2006) have suggested that DA progenitors lie within the midbrain FP. Moreover, region-specific genetic elements of Wnt1 or Gli1, expressed in the VM neuroepithelium, fate map DA neurons (Zervas et al., 2004). In a recent study, Ono et al., (2007) reported that sorting of cells expressing *Corin*, a transmembrane protein expressed in the FP, give rise to DA neurons after differentiation *in vitro*. However, the cell type(s) that undergo DA neurogenesis in the midbrain FP *in vivo* remain to be identified. In the past, three different cell types have been identified as neuronal progenitors during neural development *in vivo*: neuroepithelial cells, radial glia cells, and basal/subventricular-zone progenitors (for reviews see Götz and Huttner, 2005; Huttner and Kosodo, 2005). In this study, we exclusively focused on examining whether a fourth cell type, radial glia-like cells in the midbrain FP, are neurogenic *in vivo* and whether they contribute to the DA lineage. We found that radial glia-like cells with nuclei in the apical part of the FP neuroepithelium actively divide. Moreover, lineage tracing and genetic fate mapping experiments indicate that FP radial glia-like cells are neurogenic and have the capacity to give rise to midbrain DA neurons *in vivo*. Thus, we conclude that midbrain FP radial glia-like cells can undergo neurogenic divisions and give rise to postmitotic migratory DA precursors that rapidly differentiate into DA neurons.

MATERIALS AND METHODS

Animals

All the experiments described were performed according to the guidelines of the European Community (Council Directive 86/609/EEC), the Society for Neuroscience (January 1985). Animal manipulation and experimental methods were approved by the local ethical committee. Wild-type CD-1 pregnant mice (25–35 g) were obtained from Charles River (Uppsala). Tis21-EGFP (C57BL/6 background) and GLAST::Cre ERT2/ZEG mice have been previously described (Haubensak et al., 2004; Mori et al., 2006).

In Utero BrdU Labeling

Timed mated female mice (CD1 or Tis21-EGFP) with E10.5-12.5 embryos (where E0.5 is noon after the female is put with the male) were injected intraperitoneally with 50 μg BrdU per gram of body weight, injected at 10 mg/mL in phosphate-buffered saline (PBS) and killed 2 h (for characterization of VM RG and Tis21-EGFP fate mapping) or 1.5 h later (for *in vitro* pulse-chasing). For cumulative labeling of proliferating cells with BrdU, pregnant females with E10.5 and/or E9.5 embryos were injected with BrdU (every 4 h, three times/day). BrdU labeled embryos were analyzed at E12.5 as described below.

Immunohistochemistry

Mice embryos were fixed for 4 h in cold 4% paraformaldehyde/PBS, cryoprotected in 20–30% sucrose/PBS at +4°C overnight, embedded in OCT and 14–18 μM cryosections were obtained, blocked in PBS +5% serum from the secondary antibody species and incubated with primary antibodies diluted in PBST (PBS/3%BSA/0.3% Triton) overnight at +4°. After blocking, sections were incubated in the secondary antibody Cy2-, Cy3-, or Cy5-conjugated, 1:400; Jackson Lab diluted in PBST for 4 h at room temperature (RT). For BrdU detection, the first antibody was fixed with 70% EtOH/20% acetic acid/10% water for 7 min at RT, PBS washed and incubated 15 min at RT in 2 N HCl/PBS. Sections were counterstained with Hoechst 33258 (Sigma) in PBS 0.3% Triton for 5 min, PBS washed and mounted in PBS/glycerol (1:9). Antibodies used were mouse monoclonal RC2 (1:200; DSHB), anti-nestin (1:150; DSHB), anti-FoxA2 (1:10; DSHB), anti-human Sox2 (1:200; R&D), and anti-GFP (1:500, Chemicon), rabbit polyclonal Nurr1 (1:200; Santa Cruz), anti-tyrosine hydroxylase (TH) (1:100; Pel-Freeze), antiphosphohistone H3 (1:100, Upside) anti-phosphorylated vimentin (Ser55) (4A4) (1:100; BioSide), and anti-Pitx3 (1:1,000; gift from Smidt M.P.) and the guinea pig polyclonal antiglutamate transporter GLAST antibody (1:2,500; Chemicon). Sections were viewed using a Zeiss Axiovert 100M microscope, or Zeiss Axioplan 2 confocal (Tis21-EGFP work), photographed using a Kodak MDS 290 camera and Openlab or LSM510 Software and assembled into figures using Adobe Photoshop 7.0.

Retroviral Labeling of Radial Glia-Like Cells Progeny

Ecotropic Phoenix packaging cells were transfected with pLPC-CMV-GFP, selected and maintained in DMEM medium + serum. The cells (100% GFP+) were washed and allowed to condition a small volume of N2 medium for 48 h. The medium was then filtered through a 45-μm sterile filter, FGF2 was added to 10 ng/mL and this complete medium was added to primary E10.5 VM RG plated 24 h previously in N2+FGF2 (10 ng/mL). Polybrene was not used, as it killed the radial glia-like cells. After 24 h, the cells were washed with warm N2 and the original warmed N2+FGF2 was returned to the

cells. Three days later, the culture was fixed, examined for GFP expression and stained for TH and for GFP.

Immunocytochemistry

Medium was gently removed from the culture well and the cells were fixed for 15 min at RT with 4% PFA/PBS, extensive PBS washes followed and a short blocking incubation and the staining continued as described earlier. For staining of intermediate filaments in acutely cultured cells, the fixed cells were incubated at −20°C in chilled 80% ethanol/20% acetic acid for 15 min and extensively washed in PBS before staining.

Tamoxifen Administration in GLAST::CreERT2/ZEG Mice

Tmx (Sigma, T-5648) was dissolved in corn oil (Sigma, C-8267) at 37°C for several hours to prepare the 20 mg/mL solution (Zervas et al., 2004). For Cre activity induction, 3 mg Tmx was administrated per mouse by oral gavage to E10.5 GLASTCre Ert2/Zeg pregnant mothers (Mori et al., 2006).

RESULTS

Most Midbrain DA Neurons are Born as Postmitotic Precursors Between E9.5 and E10.5

DA neurons appear from embryonic day 10 (E10) to E14 in mouse, peaking in numbers at E11.5 (Vitalis et al., 2005). These cells appear for the first time in the FP region of the VM. We therefore characterized the VM FP at E10.5, the onset of DA neurogenesis. We found that cells in the VM midline expressed radial glia markers (nestin, RC2 and the glutamate astrocyte transporter (GLAST), Fig. 1A–C) and high levels of FoxA2 (Fig. 1F), a regulator of FP development (Sasaki and Hogan, 1994). In our study, we considered the GLAST+ domain as the FP (yellow dotted line in 1C), in which only the medial domain is strongly labeled. The FoxA2 domain (yellow dotted line in 1F) extended into the basal plate. Mitotic figures were only detected at the ventricular surface of the VM by Hoechst staining (asterisks, Fig. 1D). These and many more ventricular surface cells in the FP were labeled with the 4A4 antibody (asterisks, Fig. 1E), which recognizes an epitope of vimentin that is phosphorylated by cdc2 kinase at Ser55 during radial glia mitosis (Kamei et al., 1998). The 4A4 antibody sometimes labeled single cells from their soma at the ventricular surface to their end-foot at the pial surface, confirming that these mitotic cells are radial glia-like cells. *In utero* BrdU pulsing was used to identify the cells that proliferate in the E10.5 VM. A single pulse of BrdU followed by a 2 h chase was sufficient to label many more cells within the VM FP compared to the basal plate, showing that they are actively dividing and going through S phase of the cell cycle at this age (Fig. 1G,I). This short BrdU pulse also labeled a small number of cells before they underwent mitosis at the ventricular surface, giving BrdU+ mitotic figures (asterisk, Fig. 1G). The BrdU labeling identified a clear 'proliferative zone' within the VM during DA neurogenesis. The HMG box transcription factor Sox2 was expressed by all cells in this proliferative zone (Fig. 1H). Antibodies against the neuron-specific ELAV-like proteins Hu C/D or β-III tubulin recognized ventral cells in the postmitotic zone (Fig. 1G and not shown), which included cells positive for the orphan nuclear receptor Nurr1 and tyrosine hydroxylase+ (TH+) cells of the DA lineage (Fig. 1H,I). There was a very sharp boundary between the proliferative and postmitotic zones. Later, at E11.5, a two hour in utero BrdU pulse labeled a smaller percentage of VM cells (Fig. 1J). At this stage, Nurr1+ cells aligned over the distal part of RC2+ radial glia-like cells processes and GLAST+ cells (Fig. 1K,L). We noted that at E11.5 Nurr1+ cells emerge from the entire FP, including the GLAST- lateral FP domain and the GLAST+ medial FP domain (Fig. 1L). At E12.5, a 2 h *in utero* BrdU pulse labeled cells occupying a thinner proliferative zone close to the ventricle (data not shown). In animals pulsed with BrdU (every 4 h three times per day, at E9.5 and at E10.5) 90% of the labeled cells expressed TH/Pitx3 (paired-like homeodomain transcription factor 3), two specific markers of midbrain DA neurons. Most of the DA neurons born between E9.5 and E10.5 were found in the FP domain at E12.5. While few of them were found in the medial FP, the majority of the labeled DA neurons were found in the lateral FP domain (Fig. 1M,N, pink nuclei surrounded by green cytoplasm).

A Novel Method to Culture FP and VM Radial Glia-Like Cells

To characterize the neurogenic potential of VM radial glia-like cells *in vitro,* we first developed a novel method to culture radial glia-like cells (see supplementary information). In brief, E10.5-E12.5 VM tissue cultivated on uncoated dishes at 200,000 cells/cm^2 in N2-supplemented medium resulted in the enrichment of RC2+ cells to 98–100% purity at 2 h after plating. However, many of these cells died or differentiated by 5 days *in vitro* and only 8% RC2+ cells were still detectable after 5 days *in vitro* (Supplementary Fig. 1A–D). Addition of FGF2 (10 ng/mL), but not EGF to the culture, reduced the number of pyknotic nuclei and increased the number of RC2+ cells (Supplementary Fig. 1E,F). These cells were also characterized by the expression of notch (Gaiano et al., 2000; Gaiano and Fishell, 2002), nestin, and diazepam binding inhibitor (DBI, acyl-CoA binding protein) (Yanase et al., 2002), *wnt1* and *wnt5a* (Supplementary Fig. 2) as well as *glast* and *blbp* (data not shown). Despite FGF2 treatment, these cells rapidly differentiated and gave rise first to neurons (TuJ1+ and MAP2+ cells that elaborated extensive processes) (Supplementary Fig. 1G,H), and then to GFAP+ astrocytes. After 5 days *in vitro*, 3% of the cells were DA neurons (TH+ and TuJ1+). Moreover, cultures prepared from more stringent midline dissection of the VM FP gave

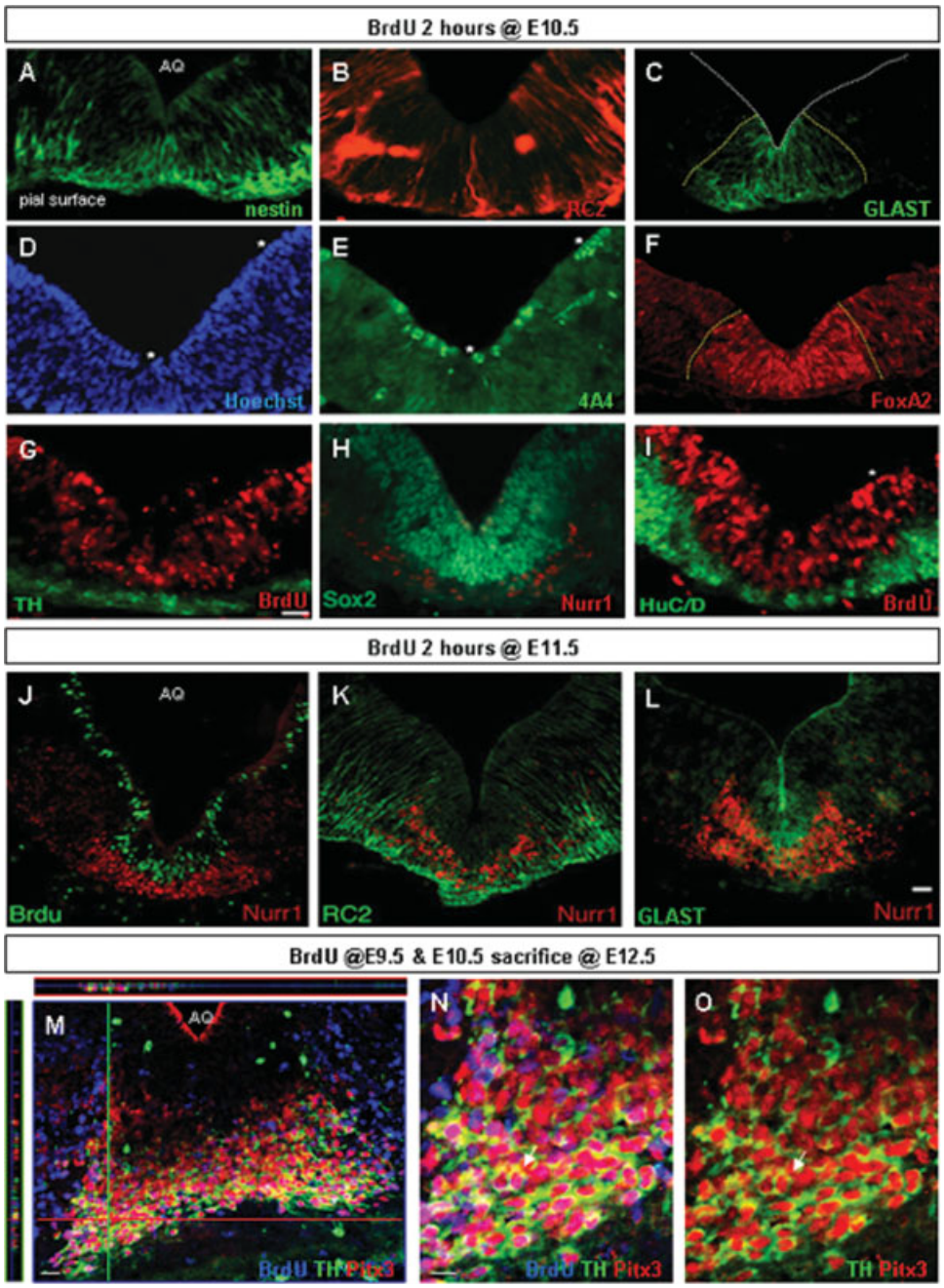

Fig. 1. Characterization of the ventral midbrain during DA neurogenesis. **A–I**: Radial glia-like cells and neurons at E10.5. Radial glia-like cells were immunostained for Nestin (**A**) and RC2 (**B**) in coronal cryosections through the VM. FP radial glia-like cells were GLAST+ (**C**) and FoxA2+ (**F**). The white dotted line (in C) labels the apical edge of the neuroepitelium. The yellow dotted line labels the boundary between FP, basal plate (in C), note that FoxA2 extends to the basal plate (line in F more lateral than in C). Hoechst DNA counterstaining revealed mitotic figures only at the ventricular surface (asterisks, **D**). These mitotic figures were 4A4+, a marker of radial glia (asterisks, **E**). A single BrdU pulse labels a clear proliferative zone of cells after 2 h chase (**G**,I), occasionally, mitotic figures are BrdU+ (asterisk I). Sox2+ cells lie within the proliferative zone (**H**) and ventrally lie TH+ DA neurons (G), postmitotic Nurr1+ DA precursors and neurons (H), and postmitotic neurons stained for Hu C/D (I). **J–L:** Radial glia-like cells and neurons at E11.5. Less VM cells are labeled with a 2 h BrdU pulse, compared to E10.5 (J). RC2 clearly labels RG spanning the VM (**K**) and Nurr1+ DA cell nuclei are aligned in close apposition to RC2+ (K) and GLAST+ RG processes (L). **M–O:** DA neurons at E12.5 labeled with BrdU at E9.5 or E10.5. Cells labeled with 3 BrdU pulses at E9.5 and E10.5 were immunostained with DA neuron markers. 90% of the DA neurons (TH+/Pitx3+) were labeled with BrdU (note the pink colour of Pitx3+/BrdU+ nuclei surrounded by a green-yellow TH+ cytoplasm). Arrow in (**N**, O,) shows the BrdU/TH/Pitx3 triple positive cell shown in z plane of M. AQ: aqueduct. Scale bars: 20μm (A–M), 10 μm (N–O).

rise to higher numbers of DA neurons (data not shown). We next decided to examine whether there is a lineage relation between VM radial glia-like cells and DA neurons. We first traced the progeny of VM radial glia-like cells *in vitro* in two ways: *in utero* BrdU pulse-chasing and retroviral labeling.

Mitotic FP Radial Glia-Like Cells Generate VM DA Neurons *In Vitro*

E10.5 VM FP radial glia-like cells pulsed *in vivo* with BrdU (1.5 h) and cultured for 2 h in FGF2 (10 ng/mL) revealed 99–100% of the BrdU+ cells as either RC2+ (Fig. 2A–C) or nestin+ (Fig. 2D–F). When replicate cells were cultured for a further 3 days and then fixed and stained for BrdU and TH, and double BrdU+/TH+ cells were detected (Fig. 2G,H), suggesting that FP radial glia-like cells which had been in S phase during the 1.5 h *in utero* pulse, may give rise to DA neurons *in vitro*. Interestingly, many of the BrdU+/TH+ cells lay in pairs close to one another suggesting that FGF2 may have induced symmetrical division *in vitro* (Fig. 2G,H). Next, we took advantage of the fact that FGF2-treated VM FP radial glia-like cells undergo mitosis in these cultures (Fig. 2I–K), and infected them with a GFP retrovirus in order to trace their progeny. When E10.5 VM

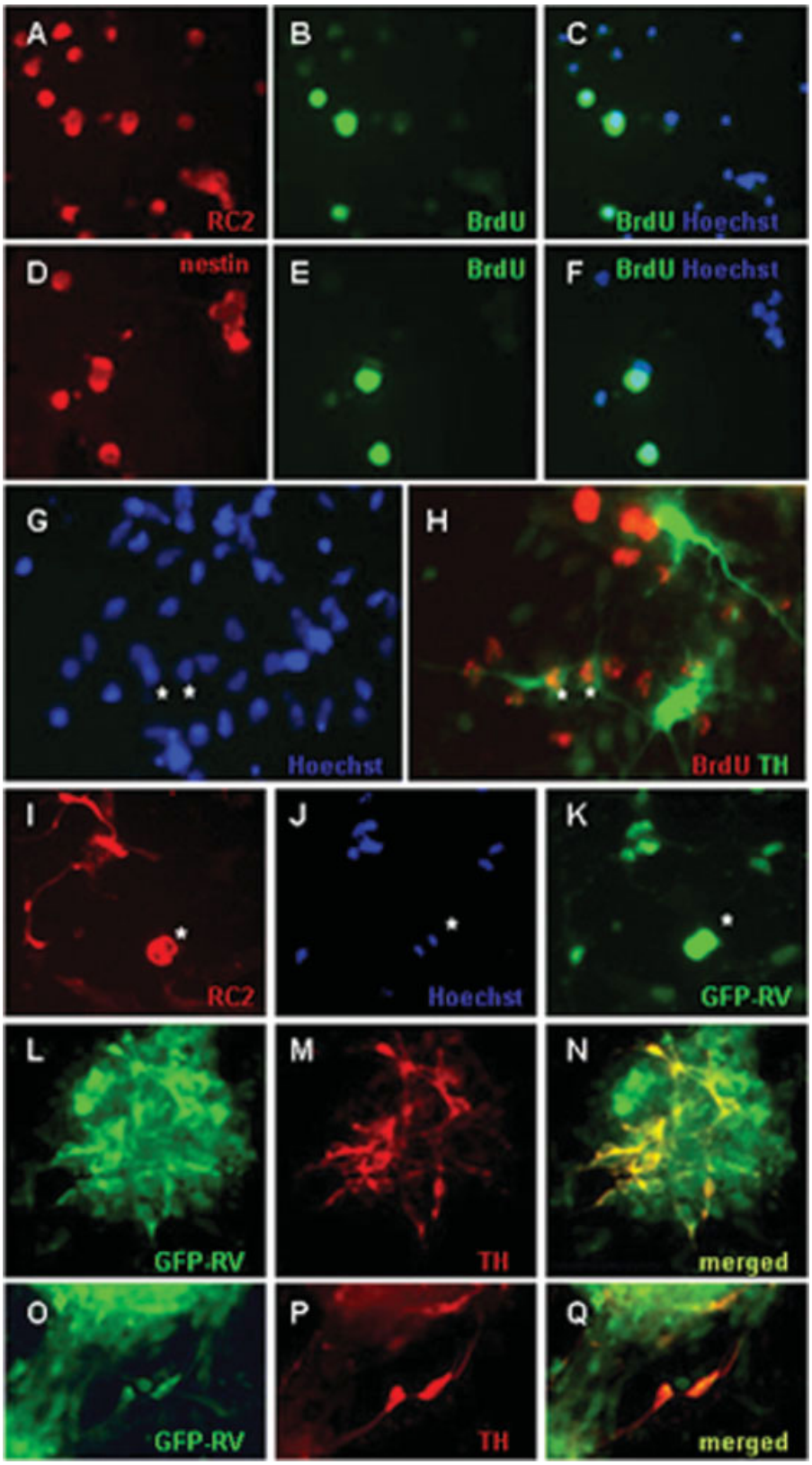

Fig. 2. Lineage tracing of VM radial glia-like cells *in vitro*. **A–H**: VM radial glia-like cells labeled with BrdU at E10.5 *in vivo*, differentiate into TH+ DA neurons *in vitro*. Embryonic VMs were cultured in N2+FGF2 (10 ng/mL) for 1.5 h after BrdU injection. Only cultures with 99–100% of BrdU+ cells were examined for co-localization of the radial glia marker RC2 (A–**C**), or nestin (**D–F**). Cells labeled with BrdU in utero differentiated into TH+ DA neurons (asterisks G, H). Not all TH+ cells were BrdU+ (H) presumably these were post-mitotic during the in utero pulse. **I–J:** Mitotic VM radial glia-like cells infected in vitro with a pLPC-CMV-GFP retrovirus differentiate into TH+ DA neurons. Four hours after the infection, E10.5 mitotic RC2+ VM radial glia-like cells were GFP+ (I–**K**). Three days after, GFP+/ TH+ DA neurons were seen in both in clusters or isolated (**L–Q**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

radial glia-like cells cultures were examined 72 h after infection with a GFP retrovirus *in vitro*, GFP+/TH+ neurons were detected (Fig. 2L–Q). Thus, our results suggested that mitotic radial glia-like cells from the embryonic VM generated DA neurons *in vitro*. However, in order to unequivocally demonstrate whether the FP cells

generate DA neurons, we performed lineage tracing and genetic fate mapping experiments *in vivo*.

Apical Neurogenic Progenitors in the Midbrain FP Give Rise to DA Neurons *In Vivo*

To address whether midbrain FP cells give rise to DA neurons *in vivo*, we first performed lineage tracing analysis in Tis21-EGFP knock-in mice (Haubensak et al., 2004) and then fate mapping in GLAST::CreERT2/ZEG mice (Mori et al., 2006). *Tis21* is an antiproliferative gene expressed in the neural tube as cells switch from proliferative to neurogenic divisions (Iacopetti et al., 1999). Tis21 mRNA is expressed in the neuroepithelium, but not in the intermediate or marginal zones of the VM (Iacopetti et al., 1999). However, the EGFP protein expressed via the *Tis21* promoter is very stable and is inherited following a neurogenic division (Haubensak et al., 2004); therefore, it can be used to identify the progeny of such divisions in the neuroepithelium and beyond. We first analyzed whether at E11.5 FP Tis21-EGFP+ cells expressed Sox2, a transcription factor expressed in the VM neuroepithelium (Kele et al., 2006, supplemental Fig. 1B). Interestingly, we found that 44.5% of the Tis21-EGFP+ cells in the FP were Sox2+ and that 26% of the Sox2+ cells were Tis21-EGFP+ (Fig. 3A,B,D). This finding suggested that it is only one every four Sox2+ cells in the FP that undergo neurogenic divisions at E 11.5.

It has been previously described that neurogenic divisions may occur in both the apical and basal portions of the neuroepithelium (Haubensak et al., 2004). Importantly, different neurogenic cell types such as neuroepithelial cells and radial glia divide in apical position, and basal progenitors divide in basal or subventricular position (Götz and Huttner, 2005). Interestingly, we found that, phosphohistone H3, a marker of mitotic cells, was expressed almost exclusively in the apical portion of the FP neuroepithelium in the VM (Fig. 3C). Moreover, a BrdU pulse labeled most of the Sox2 cells in the VM FP of EGFP-Tis21 at E11.5 mice, but mitotic triple positive cells (BrdU+/Sox2+/EGFP-Tis21+) were exclusively found at the apical ventricular surface of the FP (Fig. 3D), suggesting that VM neurogenesis occurs via neuroepithelial or radial glia-type apical progenitors. However, in the basal plate of the midbrain neuroepithelium, phosphohistone H3 labeled many apical but also some subapical cells (Fig. 4G).

When the lineage of Tis21-EGFP+ cells in the marginal zone was traced, the progeny of Tis21-EGFP+ cells was found migrating between RC2+ and GLAST+ FP radial glia-like cells processes (Fig. 3E,F) and co-expressed markers of the DA lineage such as Nurr1 (Fig. 3G,H). We found that 19% of the EGFP+ cells were Nurr1+ and that 17.5% of the Nurr1+ population was also EGFP+ at E11.5 (Fig. 3I). As Nurr1+ cells differentiate into TH+ DA neurons, we tested whether TH and Tis21-EGFP were co-expressed. Despite the decay of EGFP as cells differentiate, we found that 15% of the EGFP+ cells in the VM expressed TH (27% of the TH+ population in the E11.5 VM, Fig. 3J–L). Combined, these results suggested that cells in the DA lineage derive from apical Tis21+ progenitors in the midbrain FP. We then examined whether such population of cells that divide apically in the VM FP are radial glia-like cells.

Midbrain FP Radial Glia-Like Cells Divide in Apical Position

We first characterized the temporal and spatial distribution of the radial marker, GLAST, in the VM during early and late DA neurogenesis (Fig. 4A–E). At E9.5 the first GLAST+ cells labels appear in the FP. At E10.5, at the onset of the DA neurogenesis, anti-GLAST antibodies labeled radial glia-like cells extending from an invagination of the ventricle in the ventral midline, radially towards the pial surface, labeling the medial FP. At E11.5 a weaker GLAST domain labeling appeared more laterally, in the lateral FP. During the final stages of neurogenesis (12.5 and 13.5), the medial FP remains clearly and a strongly labeled, but a dorsal GLAST+ domain appears in the basal plate. A more detailed analysis of the midbrain FP clearly showed two compartments: containing cells with radial glia-like morphology, a medial FP, that co-expressed GLAST together with high FoxA2 at E10.5 (Fig. 4F); and a lateral FP, that contains $GLAST^{low}$ at E11.5 and E12.5 (Fig. 4C,D). We then examine whether radial glia-like cells in the VM FP divide at early and late stages of neurogenesis (E10.5 and E12.5). For this study, we performed double immunohistochemistry with antibodies against GLAST, in combination with PH3 or 4A4 (that labels dividing radial glia). Interestingly, double+ cells (GLAST+/PH3+ and GLAST+/4A4+) were found in an apical position in the FP at the onset (E10.5) and at later stages (E12.5) of DA neurogenesis (Fig. 4G–J). Outside of the FP, PH3 and 4A4 positive cells were also found but they were GLAST negative until E10.5 (Fig. 4G,H). Subapical divisions were only observed in the basal plate (GLAST-/PH3+ Fig. 4G). Combined, these experiments suggested that radial glia-like cells in the FP divide apically and that apical cells in the FP give rise to DA neurons. This raised the question, are then FP radial glia-like DA progenitors?

FP Radial Glia-Like Cells Give Rise to DA Neurons *In Vivo*

To provide direct evidence that midbrain FP radial glia-like cells generate DA neurons *in vivo*, we performed a genetic fate mapping analysis of GLAST::CreERT2/ZEG mice (Mori et al., 2006). Here the inducible form of the Cre recombinase (CreERT2) was targeted to the GLAST locus (Mori et al., 2006). GLAST is one of the best markers of radial glia cells (Hartfuss et al., 2001) and a strong medial FP marker in the midbrain at

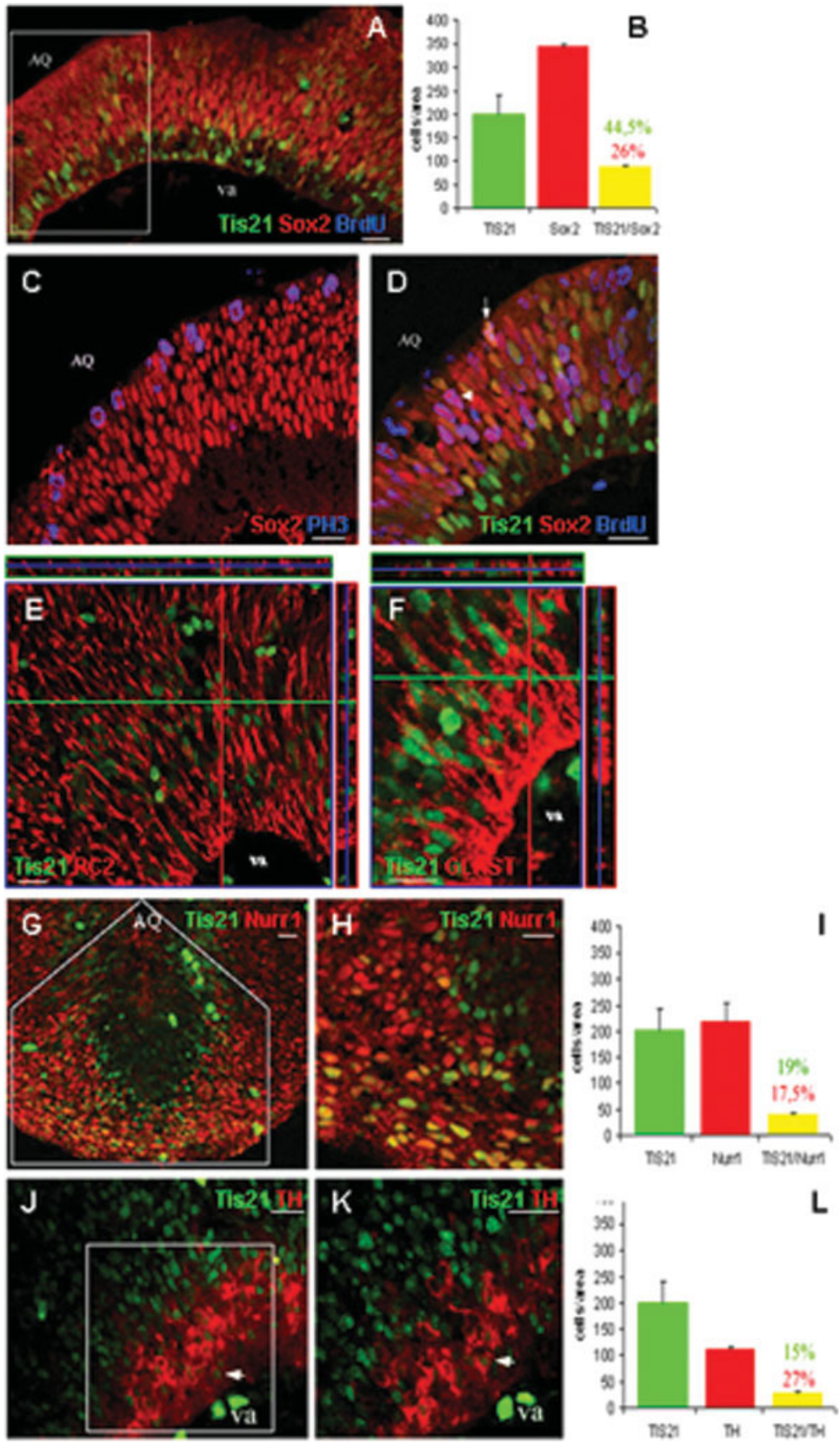

Fig. 3. Lineage tracing of midbrain DA neurons *in vivo* using the Tis21-EGFP knock-in. **A–F**: Expression of Tis21 in neuronal progenitors, including radial glia-like cells, at E11.5. (A) Sagittal (A–F, **J–L**) and coronal (**G**,**H**) sections through the FP were stained with antibodies against: GFP (green), the neural progenitor and RG marker Sox2 (red), and BrdU (blue, after a 2 h in utero pulse). Cells Tis21+ and Sox2+ (yellow) were quantified (**B**, average ± SEM; $n = 18$) in a standard area of 47,000 μm^2 (highlighted in G). (**D**) Higher magnification of the area in A. Arrow in D shows a cell triple positive for Tis21/Sox2/BrdU and arrowhead shows a Sox2+/Brdu+ cell. 44.5% of Tis21+ cells co-expressed Sox2 and 26% of Sox2+ cells co-expressed Tis21. (**C**) All progenitors (Sox2+, red) undergoing mitosis (phosphohistone H3, blue) were found in the apical/subapical ventricular zone of the midbrain FP. (**E**,**F**) Tis21+ cells were found on RC2+ fibers (red in E) and GLAST+ cell bodies (red in F). Confocal Z-stacks showed that Tis21+ cells (green) were also GLAST+ (F). (G–L) *Tis21-EGFP+ progenitors gave rise to DA neurons in vivo.* **(G–I)** Coronal sections through the E11.5 mouse FP (area in G) were immunostained for the postmitotic DA marker Nurr1 (red). Tis21+/EGFP (green). Double positives (yellow, 19% of the Tis21+) are shown at a higher magnification in (**H**) and quantified (average ± SEM) in I. (J–L) TH+ DA neurons (red) expressed weak Tis21+/EGFP (15% of the Tis21+, arrow). **K:** is an enlargement of the region in J. (**L**) Quantification (average ± SEM) of Tis21-EGFP+/TH+ cells (yellow). Scale bar: 20 μm. AQ: aqueduct. va: ventral area.

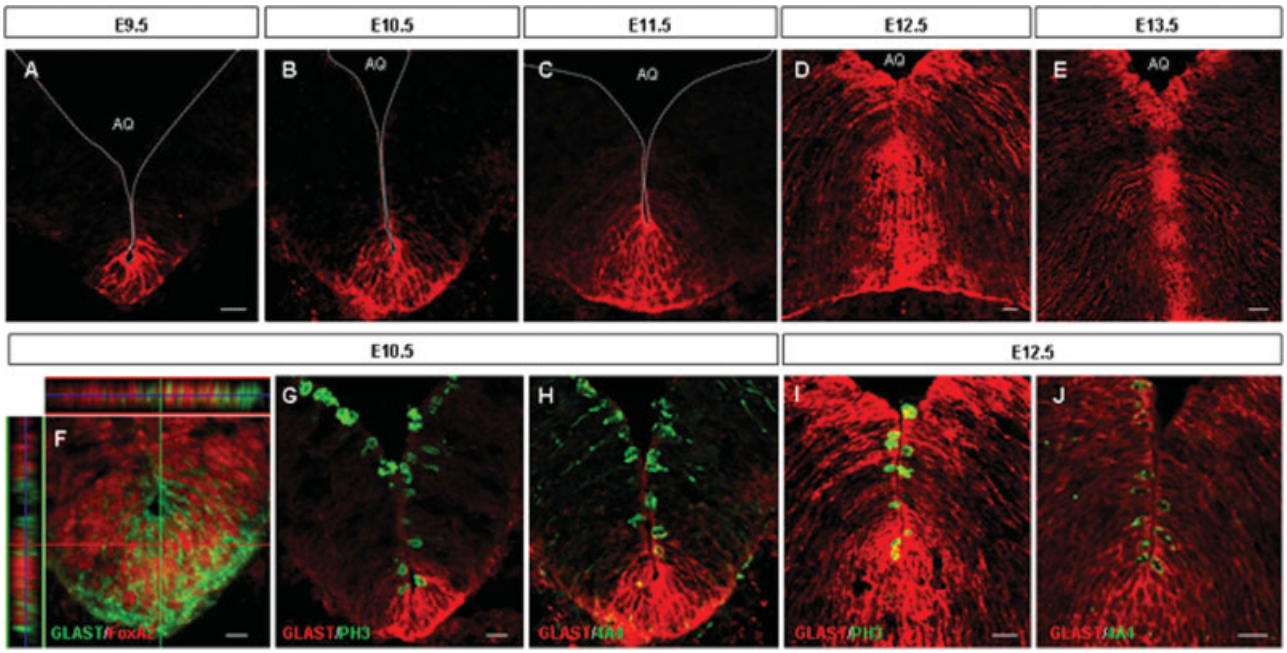

Fig. 4. Characterization of GLAST+ radial glia-like cells in the VM during DA neurogenesis. Coronal cryosections from E9.5 to E13.5 were immunostained with the radial glia marker GLAST. **A–E:** The Glast+ domain occupied by radial glia-like cells expands from the medial FP (E9.5-10.5) to the lateral FP (E11.5) and the basal plate at E12.5 and E13.5 (strong dorsal band below the aqueduct (AQ)). The white dotted line (in A–**C**) labels the apical edge of the neuroepitelium. **F:** Confocal Z-stacks from E10.5 coronal sections showed that GLAST+ cells (green) coexpressed the FP marker FoxA2 (red). Phosphohistone H3 (green in **G**, **I**) and the mitotic radial glia marker 4A4 (green in **H**, **J**) labeled proliferating radial glia inside and outside the FP at E10.5 (G–H) and at E12.5 (I–J). Scale bars: 20 μm (A–**D**, G–J); 40 μm (E); 10 μm (F). AQ: aqueduct.

E9.5-E10.5 (Fig. 4A,B). In these mice, tamoxifen (Tmx) stimulation translocates a Cre protein fused to the ligand binding domain of the modified estrogen receptor (ERT2) from the cytoplasm to the nucleus, where it mediates recombination (Metzger et al., 1995). We first performed three BrdU pulses separated by 4-h intervals at E10.5 to estimate the total number Nurr1+ cells born at E12.5. Around 42% of Nurr1+ cells in the FP domain were labeled (Fig. 5A,B). Moreover, we found that the generation of DA neurons was very rapid and several Nurr1+/BrdU+ DA neurons labeled at E10.5 were already migrating laterally in the marginal zone (Fig. 5A, cell shown in *z* axis) at E12.5.

We next examined at E12.5 the number and fate of cells recombined in the midbrain FP of embryonic GLAST::CreERT2/ZEG mice after Tmx treatment at E10.5. This experiment allowed us to identify a large number of EGFP+ (recombined) cells first localized in the neuroepithelium and then in the marginal zone of the VM including the FP at E12.5. GLAST+ radial glia-like cells also gave rise to non-DA cells (red), positioned lateral to the FP domain (Fig. 5G). More than 35% of the GLAST-EGFP+ cells co-expressed Nurr1, a marker of DA precursors and neurons (Fig. 5C–F), and also Pitx3, a marker specific for VM DA neurons (Fig. 5G,H). Comparison of the proportion of postmitotic Nurr1+ DA cells born at E10.5 (42% BrdU+/Nurr1+ cells after 3 pulses) with the percentage of Nurr1+ DA cells derived from GLAST-EGFP+ cells (35%, GLAST+/Nurr1+ cells after Tmx injection at E10.5), revealed that about (83%) of the Nurr1+ cells at E12.5 were born from GLAST-EGFP+ cells at E10.5. Figure 5G clearly shows the sequence of events during DA neurogenesis. Starting from the neuroepithelium (right, unstained) towards the pial surface (down and left), we first find Nurr1+/Pitx3−/EGFP− DA precursors and Nurr1+/Pitx3+/EGFP− DA neurons born after E10.5 (EGFP-, light blue). And third, we find Nurr1+/Pitx3+/EGFP+ DA neurons born at E10.5 (EGFP+, pink), some of which are again migrating laterally (to the left). Thus, our *in vivo* results show that GLAST+ radial glia-like cells labeled at E9.5-E10.5 in the midbrain FP are DA progenitors that give rise first to Nurr1+/TH-/Pitx3− DA precursors and then to Nurr1+/TH+/Pitx3+ DA neurons.

DISCUSSION

FP Radial Glia-Like Cells are Midbrain DA Progenitors

The main goal of our study was to determine whether radial glia-like cells in the VM FP are DA progenitors and can give rise to DA neurons. Our analysis of the midbrain FP shows that cells expressing radial glia markers (GLAST::CreERT2/ZEG and RC2/GLAST) and exhibiting typical radial-like morphology pass through the S phase of the cell cycle, undergo mitosis at E9.5-10.5 in the ventricular surface (4A4, PH3) and undergo apical neurogenic divisions to give rise first to DA pre-

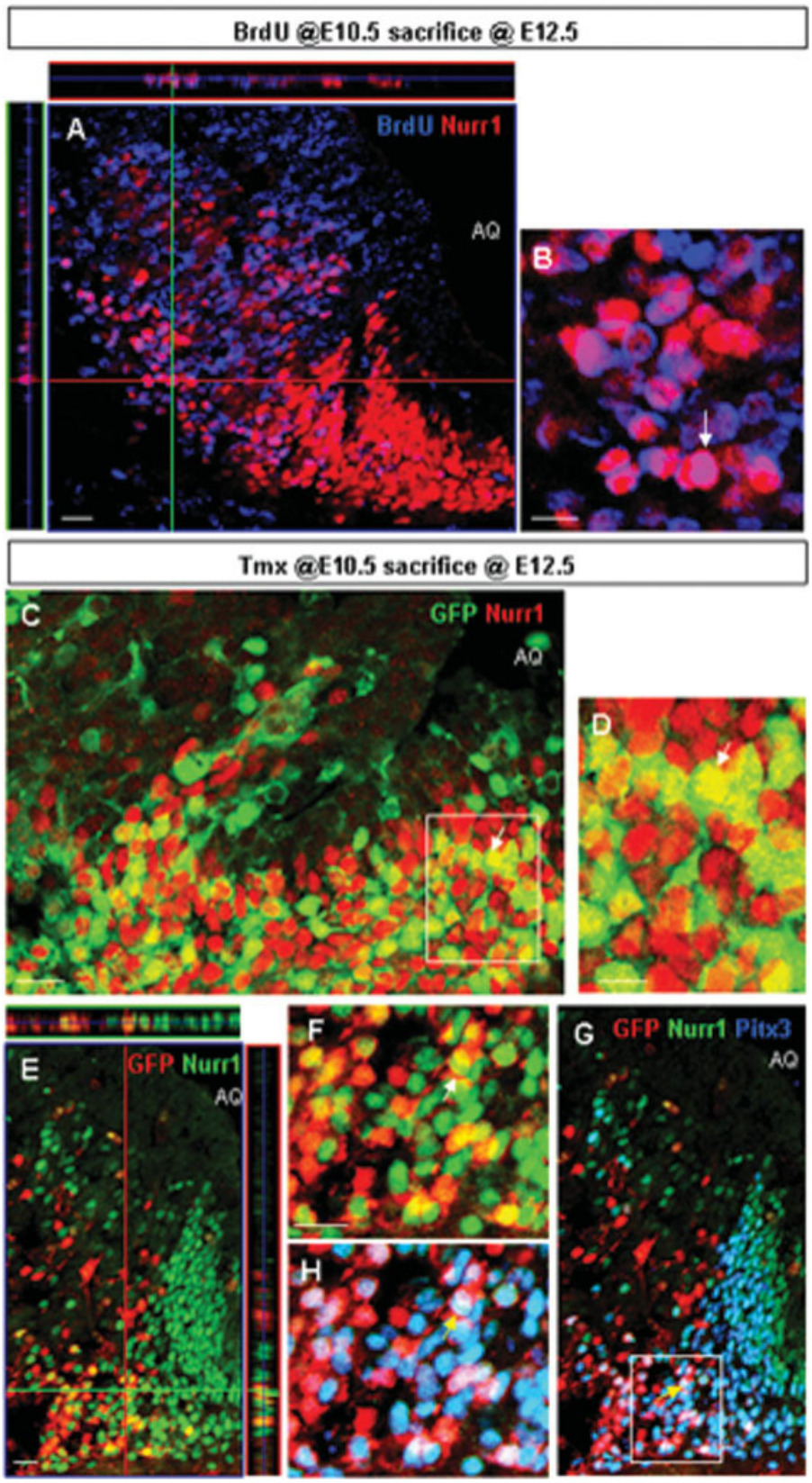

Fig. 5. Lineage tracing of DA neurons in the VM *in vivo*. **A**,**B**: Birth dating of DA precursors and neurons in the VM. The VM of animals receiving 3 BrdU pulses at E10.5 were immunostained at E12.5 for Nurr1, a DA precursor and neuron marker. (A) shows one side of the VM with the midline to the right. 42% of the Nurr1+ cells are labeled with BrdU. An example of BrdU+/Nurr1 + cell migrating laterally is shown in the z plane in A, at higher magnification in (**B**) (arrow). **C–H:** Fate mapping of midbrain DA neurons with GLAST::-CreERT2/ZEG mice. Embryonic Cre activity was induced by E10.5 with Tmx. Pups were analyzed at E12.5. Large numbers of EGFP+ cells (GLAST+ FP radial glia-like cells derived cells) localized in the midbrain FP. More than 35% of them co-expressed Nurr1 (red in C, **D**; green in **E–H**) and the marker for VM DA neurons, Pitx3 (blue in **G**–H). D, **F** and H are enlargements of cells in C, E and G. Arrows in F–G point to the GFP+/Nurr1+ cell in the *z* plane of E. Scale bars: 20 μm (A, C, E–H) 10 μm (B, D). AQ: aqueduct.

cursors (Nurr1+/Pitx3−) and then to DA neurons (Nurr1+/Pitx3+). We also report that midbrain radial glia-like cells in the FP express the antiproliferative and proneurogenic gene, *Tis21,* and that DA neurons are generated from recently divided radial glia-like cells. *In vivo,* the protein stability of EGFP in Tis21-EGFP mice allowed us to trace the DA lineage, from the proliferating progenitors to the Nurr1+ postmitotic DA precursors and TH+ DA neurons. Thus, our results confirm that *Tis21* is expressed by nearly all neuronal progenitors in the midbrain (Iacopetti et al., 1999). Moreover, we identify Tis21-EGFP+ cells and GLAST-EGFP+ radial glia-like cells in the midbrain FP as DA progenitors that undergo apical neurogenic divisions *in vivo*. While subapical divisions were detected with PH3 antibodies in the basal plate of the midbrain, they were absent in the neighbor VM FP during early and late neurogenesis (E10.5 and E12.5). Other structures such as the cerebral cortex generate most neurons by basal symmetrical divisions, a mechanism that allows the rapid amplification of neurons (Haubensak et al., 2004). The finding that the VM FP generates DA neurons only by apical division is surprising because it indicates that there is no rapid amplification of neurons in the VM FP.

We also found that the radial glia marker GLAST was highly expressed and restricted to the medial FP at E10.5 (Fig. 4). Since both GLAST and FoxA2 expression is expanded more laterally, outside the FP after E10.5, we choose the GLAST promoter at E10.5 for direct fate mapping of radial glia-like cells from medial FP *in vivo.* Treatment of GLAST::CreERT2/ZEG transgenic mice with Tmx at E10.5 to labeled the progeny generated from GLAST+ FP radial glia-like fate mapped the entire DA lineage as showed by the co-localization of EGFP with Nurr1 and Pitx3. This result clearly demonstrated that GLAST+ radial glia-like cells in the VM FP give rise to DA neurons *in vivo*. The stable expression of EGFP in recombined cells in GLAST::CreERT2/ZEG mice, allowed to trace Nurr1+/Pitx3− postmitotic DA precursors and Nurr1+/Pitx3+ DA neurons. At E12.5, comparison of the EGFP+ radial glia-like cells (recombined at E10.5) with the number of BrdU+ cells (injected also at E10.5), suggested that a large proportion of the VM DA cells generated at E10.5 (83%), originate from FP radial glia-like cells *in vivo*. The DA progenitors that we identify using cell type specific genetic elements (GLAST promoter-enhancer) occupy a similar position and/or developmental time as DA progenitors identified by FACS sorting of Corin+ cells in the VM midline at E9.75 (Ono et al., 2007), or by fate mapping of DA neurons (Zervas et al., 2004). Very few midbrain DA neurons were fate mapped in Gli1-CreER^{T2} mice at E7.5 and E8.5 compared to Wnt1-CreER^{T} mice at E8.5-E11.5. Interestingly, we found that midbrain FP radial glia-like cells express high levels of Wnt-1 (Supplementary Fig. 2). Thus, our fate mapping of midbrain DA neurons with GLAST::CreERT2/ZEG suggests that this population of progenitors that may partially overlap with Wnt1-CreER^{T}+ progenitors. Interestingly, DA progenitors give rise to Nurr1+ cells that emerge to form a peak at either side of the midline (Fig. 1L), immediately below the two stripes of Wnt1 in the FP neuroepithelium at E10.5-E11.5 (Zervas et al., 2004 and unpublished observation). It is therefore possible that radial glia-like cells in the medial part of the FP (the midline) either contribute less to neurogenesis, or that DA precursors are repelled away from the midline, as described for netrin and oligodendrocyte progenitors in the spinal cord (Jarjour et al., 2003).

Combined, our data and that in the literature suggest: (1) That midline neuroepithelial progenitors that transiently express Gli1 (E7.5-8.5), and Wnt1 (at E8.5) generate a few early DA neurons, and (2) That subsequently DA neurons are generated by GLAST+ radial glia-like progenitors in the FP, in a domain that we speculate could overlap with Corin+ cells in the midline and with Wnt1+ cells laterally. Thus, it is possible that the functional and anatomical diversity of midbrain DA neurons is in part contributed by the molecular diversity of DA progenitors observed in these studies.

Neurogenesis and Differentiation in the Midbrain FP

Our results identify midbrain FP radial glia-like cells as DA progenitors and reinforce previous findings showing that such cells express several proneural genes, such as Ngn2, which is required for DA neurogenesis (Andersson et al., 2006a; Kele et al., 2006). At least three other genes that regulate neurogenesis including Mash1, a *bHLH* gene; Hes5, a notch effector; and δ-like-1, a notch ligand, are also expressed in the midbrain FP. All of these genes are expressed from E11.5 to E13.5 and their expression is specifically down regulated upon deletion of ngn2 (Kele et al., 2006). The loss of Ngn2 in the FP results in an initial loss of DA neurogenesis, that is partially rescued by re-expression of Mash1 at a later stage (Kele et al., 2006). Importantly, the expression of these proneural genes in the midbrain FP occurs while the FP still expresses both Shh and FoxA2 (Echelard et al., 1993; Ferri et al., 2007), suggesting that FP and neurogenic functions are not incompatible. On the contrary, the induction of DA neurons requires both the highest levels of Shh (Hynes et al., 1995b; Ye et al., 1998), and direct contact with FP cells (Hynes et al., 1995a). Moreover, ectopic expression of FoxA2 in *En1-FoxA2* transgenic mice, results in the induction of a dorsal midbrain FP that contains DA neurons (Hynes et al., 1995a). Thus, FoxA2, is an essential component for FP radial glia, both for ventral patterning and for DA neurogenesis (Ferri et al., 2007; Hynes et al., 1995a).

We previously reported that glial-derived factors (Wagner et al., 1999), including Wnt1 and Wnt-5a (Castelo-Branco et al., 2006), regulate the proliferation, neurogenesis of DA progenitors and the differentiation of Nurr1+/TH− postmitotic DA precursors into Nurr1+/TH+ DA neurons to a different extent (Castelo-Branco et al., 2003). Here, we show that both Wnt1 and Wnt-5a are expressed in VM FP radial glia-like cell cultures (Supplementary Fig. 2). Thus, our results indicate that VM FP radial glia-like cells may work: (1) in an autocrine or paracrine manner to regulate their own func-

tion (via Wnt1 and Wnt5a), or (2) in a paracrine manner (via Wnt5a) to instruct the differentiation of Nurr1+/TH− DA precursors into Nurr1+/TH+ DA neurons, while migrating over the radial process.

The idea that midbrain FP radial glia-like progenitors express Wnt1 and the known function of Wnt1 suggests that radial glia-derived Wnt1, directly or indirectly, may play an important role in DA neurogenesis. In agreement with this, we previously found that deletion of Wnt1 induces a severe loss of FP radial glia-like cells and a subsequent loss of DA neurons (Prakash et al., 2006). Moreover, we also found that the overexpression of Wnt1 (En1-Wnt1 transgenic mice) induces ectopic DA neurons in the hindbrain FP (Prakash et al., 2006), suggesting the intriguing possibility that Wnt1 may contribute to the neurogenic conversion of non-neurogenic FP radial glia in the hindbrain. Since the induction of DA neurons is limited to the FP, it is likely that Shh also plays an important role in this process. Thus, combined, the data suggest that the function of the FP is not disturbed by neurogenesis, arguing against the dogma that the FP has to be a non-neurogenic region. Indeed, Shh regulates the expression of Msx1 and FoxA1/2, two upstream positive regulators of Ngn2 (Andersson et al., 2006b; Ferri et al., 2007).

Radial Glia-Like Cells in the Midbrain FP: A Complete Developmental Unit

To summarize, our data and that in the literature suggest that FP radial glia-like cells perform more functions than previously anticipated. These cells carry out classical FP organizer functions (Ericson et al., 1995; Hynes et al., 1995a,b; Placzek and Briscoe, 2005), and classical radial glia roles such as guiding neuronal migration (Kawano et al., 1995; Rakic, 1972; Shults et al., 1990). Moreover, our results demonstrate for the first time, that midbrain FP radial glia-like cells can undergo neurogenesis *in vivo* and that midbrain DA neurons can be generated by FP radial glia-like cells. These activities define midbrain FP radial glia-like cells, and distinguish them from other FP and non-FP radial glia. Moreover, all the data available suggest that midbrain FP radial glia-like cells regulate most aspects of embryonic DA neuron development, from patterning and neurogenesis, to migration and differentiation, constituting a complete and unique developmental unit.

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REFERENCES

Andersson E, Jensen JB, Parmar M, Guillemot F, Björklund A. 2006a. Development of the mesencephalic dopaminergic neuron system is compromised in the absence of neurogenin 2. Development 133:507–516.

Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, Perlmann T, Ericson J. 2006b. Identification of intrinsic determinants of midbrain dopamine neurons. Cell 124:393–405.

Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell TM, Rubenstein JLR, Ericson J. 1999. Homeobox gene *Nkx2.2* and specification of neuronal identity by graded Sonic hedgehog signalling. Nature 398:622–627.

Campbell K, Götz M. 2002. Radial glia: Multi-purpose cells for vertebrate brain development. Trends Neurosci 25:235–238.

Castelo-Branco G, Sousa KM, Bryja V, Pinto L, Wagner J, Arenas E. 2006. Ventral midbrain glia express region-specific transcription factors and regulate dopaminergic neurogenesis through Wnt-5a secretion. Mol Cell Neurosci 31:251–262.

Castelo-Branco G, Wagner J, Rodriguez FJ, Kele J, Sousa K, Rawal N, Pasolli HA, Fuchs E, Kitajewski J, Arenas E. 2003. Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. Proc Natl Acad Sci USA 100:12747–12752.

Colamarino SA, Tessier-Lavigne M. 1995. The role of floor plate in axon guidance. Annu Rev Neurosci 18:497–529.

Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP. 1993. Sonic Hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75:1417–1430.

Ericson J, Muhr J, Jessell TM, Edlund T. 1995. Sonic hedgehog: a common signal for ventral patterning along the rostrocaudal axis of the neural tube. Int J Dev Biol 39:809–816.

Ever L, Gaiano N. 2005. Radial 'glial' progenitors: neurogenesis and signaling. Curr Opin Neurobiol 15:29–33.

Ferri ALM, Lin W, Mavromatakis YE, Wang JC, Sasaki H, Whitsett JA, Ang SL. 2007. Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. Development 134:2761–2769.

Fishell G, Kriegstein AR. 2003. Neurons from radial glia: The consequences of asymmetric inheritance. Curr Opin Neurobiol 13:34–41.

Gaiano N, Fishell G. 2002. The role of Notch in promoting glial and neural stem cell fates. Annu Rev Neurosci 25:471–90.

Gaiano N, Nye JS, Fishell G. 2000. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. Neuron 26:395–404.

Götz M, Huttner WB. 2005. The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6:777–788.

Hanaway J, McConnell JA, Netsky MG. 1971. Histogenesis of the substantia nigra, ventral tegmental area of Tsai and interpeduncular nucleus: An autoradiographic study of the mesencephalon in the rat. J Comp Neurol 142:59–73.

Hartfuss E, Galli R, Heins N, Götz M. 2001. Characterization of CNS precursor subtypes and radial glia. Dev Biol 229:15–30.

Haubensak W, Attardo A, Denk W, Huttner WB. 2004. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. Proc Natl Acad Sci USA 101:3196–3201.

Huttner WB, Kosodo Y. 2005. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. Curr Opin Cell Biol 17:648–657.

Hynes M, Porter JA, Chiang C, Chang D, Tessier-Lavigne M, Beachy PA, Rosenthal A. 1995b. Induction of midbrain dopaminergic neurons by Sonic hedgehog. Neuron 15:35–44.

Hynes M, Poulsen K, Tessier-Lavigne M, Rosenthal A. 1995a. Control of neuronal diversity by the floor plate: Contact-mediated induction of midbrain dopaminergic neurons. Cell 80:95–101.

Iacopetti P, Michelini M, Stuckmann I, Oback B, Aaku-Saraste E, Huttner WB. 1999. Expression of the antiproliferative gene TIS21 at the onset of neurogenesis identifies single neuroepithelial cells that switch from proliferative to neuron-generating division. Proc Natl Acad Sci USA 96:4639–4644.

Jarjour AA, Manitt C, Moore SW, Thompson KM, Yuh SJ, Kennedy TE. 2003. Netrin-1 Is a chemorepellent for oligodendrocyte precursor cells in the embryonic spinal cord. J Neurosci 23:3735–3744.

Jessell TM. 2000. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nat Rev Genet 1:20–29.

Kamei Y, Inagaki N, Nishizawa M, Tsutsumi O, Taketani Y, Inagaki M. 1998. Visualization of mitotic radial glial lineage cells in the developing rat brain by Cdc2 kinase-phosphorylated vimentin. Glia 23:191–199.

Kawano H, Ohyama K, Kawamura K, Nagatsu I. 1995. Migration of dopaminergic neurons in the embryonic mesencephalon of mice. Brain Res Dev Brain Res 86:101–113.

Kele J, Simplicio N, Ferri AL, Mira H, Guillemot F, Arenas E, Ang SL. 2006. Neurogenin 2 is required for the development of ventral midbrain DA neurons. Development 133:495–505.

Kittappa R, Chang WW, Awatramani RB, McKay RD. 2007. The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol 2007 5:e325.

Kingsbury BF. 1920. The extent of the floor-plate of His and its significance. J Comp Neurol 32:113–135.
Kriegstein AR, Götz M. 2003. Radial glia diversity: a matter of cell fate. Glia 43:37–43.
Marchand R, Poirier LJ. 1983. Isthmic origin of neurons of the rat substantia nigra. Neuroscience 9:373–381.
Marin F, Herrero MT, Vyas S, Puelles L. 2005. Ontogeny of tyrosine hydroxylase mRNA expression in mid- and forebrain: Neuromeric pattern and novel positive regions. Dev Dyn 234:709–17.
Metzger D, Clifford J, Chiba H, Chambon P. 1995. Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. Proc Natl Acad Sci USA 92:6991–6995.
Mori T, Tanaka K, Buffo A, Wurst W, Kuhn R, Götz M. 2006. Inducible gene deletion in astroglia and radial glia—a valuable tool for functional and lineage analysis. Glia 54:21–34.
Ono Y, Nakatani T, Sakamoto Y, Mizuhara E, Minaki Y, Kumai M, Hamaguchi A, Nishimura M, Inoue Y, Hayashi H, Takahashi J, Imai T. 2007. Differences in neurogenic potential in floor plate cells along an anteroposterior location: Midbrain dopaminergic neurons originate from mesencephalic floor plate cells. Development 134:3213–3225.
Placzek M. 1995. The role of the notochord and floor plate in inductive interactions. Curr. Opin Genet Dev 5:499–506.
Placzek M, Briscoe J. 2005. The floor plate: multiple cells, multiple signals. Nat Rev Neurosci 6:230–240.
Prakash N, Brodski C, Naserke T, Puelles E, Gogoi R, Hall A, Panhuysen M, Echevarria D, Sussel L, Weisenhorn DM, Martinez S, Arenas E, Simeone A, Wurst W. 2006. A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors in vivo. Development 133:89–98.
Rakic P. 1972. Mode of cell migration to the superficial layers of fetal monkey neocortex. J Comp Neurol 145:61–83.
Rakic P. 2003. Elusive radial glial cells: Historical and evolutionary perspective. Glia 43:19–32.
Sasaki H, Hogan BLM. 1994. HNF-3β as a regulator of floor plate development. Cell 76:103–115.
Shults CW, Hashimoto R, Brady RM, Gage FH. 1990. Dopaminergic cells align along radial glia in the developing mesencephalon of the rat. Neuroscience 38:427–436.
Smidt MP, Asbreuk CH, Cox JJ, Chen H, Johnson RL, Burbach JP. 2000. A second independent pathway for development of mesencephalic DA neurons requires Lmx1b. Nat Neurosci 3:337–341.
Vitalis T, Cases O, Parnavelas JG. 2005. Development of the dopaminergic neurons in the rodent brainstem. Exp Neurol 191(Suppl 1):104–112.
Wagner J, Akerud P, Castro DS, Holm PC, Canals JM, Snyder EY, Perlmann T, Arenas E. 1999. Induction of a midbrain dopaminergic phenotype in Nurr1-overexpressing neural stem cells by type 1 astrocytes. Nat Biotechnol 17:653–659.
Yanase H, Shimizu H, Yamada K, Iwanaga T. 2002. Cellular localization of the diazepam binding inhibitor in glial cells with special reference to its coexistence with brain-type fatty acid binding protein. Arch Histol Cytol 65:27–36.
Ye W, Shimamura K, Rubenstein J, Hynes M, Rosenthal A. 1998. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93:755–766.
Zervas M, Millet S, Ahn S, Joyner AL. 2004. Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. Neuron 43:345–357.