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mDll1 and *mDll3* Expression in the Developing Mouse Brain: Role in the Establishment of the Early Cortex

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The Delta/Notch signalling system is involved in several developmental processes. During fly neurogenesis, *Delta* expression defines the fate of neuronal precursors and inhibits neighboring *Notch*-expressing cells from acquiring a neural fate, a process known as *lateral inhibition*. In vertebrates, recent evidence demonstrates that Notch activation can positively determine cell fate and affect neuronal process extension. Nevertheless, Delta-like expression patterns during brain development are relatively unknown. Using a transgenic mouse, which expresses LacZ under the *mDll1* promoter, we show by immunofluorescence that in the developing telencephalon *mDll1* is expressed in undifferentiated cells in close contact with radial glial cells. Based on in situ hybridization data on *mDll1* and *mDll3* mRNA expression and on the immunohistochemical detection of β-galactosidase in the Dll1-lacZ transgenic mouse, we suggest that *mDll1* and *mDll3* are involved in the establishment of the early cortical plate and that *mDll1*-expressing cells are in close contact with radial glial cells, thereby modulating the latter population, which is known to express Notch1. Furthermore, we suggest that the decrease in *mDll1* mRNA found toward the end of gestation could be related, first, to the slowing of neurogenesis and, second, to the differentiation of the radial glial cell population into astrocytes. J. Neurosci. Res. 64:590–598, 2001.

Key words: neurogenesis; cortex; development; *mDll1*; *mDll3*; telencephalon

INTRODUCTION

Neural development is a complex and highly regulated process. During embryogenesis, inductive interactions generate a specialized neuroepithelium. Throughout neurulation, this epithelium folds, giving rise to a hollow structure, the neural tube, where cells divide close to the lumen in a region called the *ventricular zone* (VZ). As daughter cells migrate from their position in the VZ towards the pial surface, along a special type of elongated radial glial cell (Hatten, 1999), the initial pseudostratified epithelium thickens in an orderly way. In the increasingly complex developing central nervous system (CNS), most neurons are borne close to the inner surface of the ventricles and have to migrate (radially or tangentially) to their final position (for review see Hatten, 1999).

Delta/Serrate/LAG-2 (DSL) transmembrane proteins regulate cell differentiation through the Notch receptors. In *Drosophila*, Delta/Notch signalling is required for the production of neural precursors (Artavanis-Tsakonas et al., 1995) through lateral inhibition: Cells fated to become neuroblasts express a surface protein (Delta), which activates Notch on neighboring cells, preventing the later from adopting the same fate (for review see Beatus and Lendahl, 1998). Vertebrate homologues for the fly *Delta* gene have been identified in *Xenopus* (Chitnis et al., 1995; Jen et al., 1997), chick (Henrique et al., 1995), and mouse (Bettenhausen et al., 1995; Dunwoodie et al., 1997; Shutter et al., 2000). Two mouse homologues (*mDll1* and *mDll3*) are expressed in the neurectoderm during early embryogenesis and were suggested to cooperate during mouse development (Dunwoodie et al., 1997). Mouse *Dll1* is expressed in the spinal cord postmitotic cells, prior to their differentiation into neurons (Henrique et al., 1995; Myat et al., 1996). Moreover, ectopic expression of *mDll1* inhibits the production of neurons in the neural plate and retina (Chitnis et al., 1995; Dorsky et al., 1997; Henrique et al., 1997). This evidence supports the hypothesis that *mDll1* expression is a marker for neuronal precursors, and data from Dunwoodie et al. (1997) suggest that *Dll3* expression could be involved in an increasing commitment towards neuronal fate, in the spinal cord. Nevertheless, recent evidence also shows a role for Delta/Notch vertebrate homologues in positively determining a glial cell fate in the nervous system (Morrison et al., 2000; Gaiano et al., 2000). Given these results, we present the hypothesis that Dll1 and Dll3 could be involved in cortical development through an effect in both neurogenesis and gliogenesis. In spite of the accumulating evidence that Delta/Notch signalling plays important roles during CNS development, the mRNA expression patterns during cortical development for two major Notch1 li-

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gands, *mDll1* and *mDll3*, are relatively unknown. Using digoxigenin-labeled riboprobes, we show that *mDll1* and *mDll3* mRNAs are present in specific mouse cortical layers. Moreover, the decline in *mDll1* mRNA at the end of gestation coincides with a decline in neurogenesis and with an increase in gliogenesis. Using a transgenic mouse that expresses LacZ under the control of the *mDll1* promoter, we show that β-galactosidase-positive cells are undifferentiated but in close contact with radial glial cells. This observation leads to the suggestion that *mDll1* expressed by undifferentiated cells and/or neuronally committed cells could be the ligand involved in the interactions with the Notch1 receptor expressed by radial glia cells (as described by Gaiano et al., 2000). Taking into account the expression patterns described in this paper, we suggest that *mDll1* and *mDll3* are involved in the initial layering of the cortex and that *mDll1* expression could maintain the radial glial scaffold while needed, pointing to different functions for this molecule.

MATERIALS AND METHODS

Tissue Preparation and In Situ Hybridization for *mDll1* and *mDll3*

Embryos aged from E9.5 to E18.5 postcoitum (pc) were collected. Tissue was fixed in 4% paraformaldehyde (PFA), cryoprotected in 30% sucrose, and cut on a cryostat. In situ hybridization was carried out following the procedures described by Myat et al. (1996). The fluorescein- or digoxigenin-labeled antisense RNA probes were synthesized using standard procedures from plasmids containing coding cloned sequences from *mDll1* and *mDll3*. For all experiments, the mating day was considered to be embryonic day 0 (E0), and the birth day was considered to be postnatal day 0 (P0).

Bromodeoxyuridine Injections

Pregnant dams were injected once with bromodeoxyuridine (BrdU; 60 mg/kg) at gestational ages 12.5, 14.5, 16.5, and 17.5 days pc. The mice were sacrificed 30 min after the injection and the embryos fixed by immersion in 4% PFA for 2–12 hr.

Immnuhistochemistry

Sections were washed in PBS and blocked with 10% sheep serum and 3% BSA in PBS, and the antibodies were applied overnight in the same solution at 4°C. The following antibodies were used: glial fibrillary acidic protein (GAFP; 1:200; Dako, Carpenteria, CA), antitubulin III (1:100; Mab; Sigma, St. Louis, MO), RC2 (1:10; Mab; Developmental Biology Hybridoma Bank, Ames, IA), nestin (Mab rat 401 from Developmental Biology Hybridoma Bank), β-galactosidase (1:500; ICN, Costa Mesa, CA), and anti-BrdU (1:100; Sigma). Sections were washed in PBS, incubated in the appropriate fluorescent secondary antibody [anti-mouse Cy3 and anti-rabbit Cy3 (1:200; Jackson, West Grove, PA), anti-rabbit fluorescein isothiocyanate (FITC; 1:100; Chemicon, Temecula, CA), anti-mouse IgM TRITC (Harlan SeraLab, Indianapolis, IN)] for 1 hr at room temperature, washed, and mounted in Hydromount (National Diagnostics, Manville, NJ) or Vectashield. Some sections were stained using the ABC kit (Vector, Burlingame, CA). For BrdU staining, sections were incubated in 2 M HCl for 30 min at 37°C, washed in PBS, and stained following the same protocol as for other primary antibodies.

Generation of *mDll1-lacZ* Transgenic Mice

The transgene was constructed by inserting a 4.8 kb *Sal*1-*Xho*1 *mDll1* promoter sequence upstream of the *lacZ* coding sequence in pBS II KS- (Stratagene, La Jolla, CA). To prepare the DNA for microinjection, the transgene was excised from the vector by double restriction digest with *Sal*1 and *Not*1 and gel purified using Quiaex II (Quiagen, Chatsworth, CA). Transgenic mice were produced by pronuclear injection of CD1 one-cell-stage embryos (Hogan et al., 1994) and screened by PCR of tail genomic DNA with a set of primers specific to amplify a 300 bp fragment present in the transgene, spanning from the *mDll1* promoter region to the lacZ coding sequence. Two stable transgenic lines were derived by crossing founder transgenic males with CD1 females.

RESULTS

Dll1 and *Dll3* Are Expressed at Early Stages of Cortical Development

Previous data showed *mDll1* and *mDll3* expression in the early developing murine CNS (Lindsell et al., 1996; Dunwoodie et al., 1997). To analyze further the expression in specific areas at later stages of CNS development, we prepared 14 μm cryostat sections from E10.5–E18.5 pc animals. At E12.5 pc and at E13.5/E14.5 pc, both genes are expressed in the developing cortex (Figs. 1, 2) from the VZ up to the preplate (PP)/cortical plate (CP) for *mDll1* and in the PP only for *mDll3*. At E13.5 pc *mDll1* mRNA can also be detected in a second layer outside the VZ (Fig. 2A,B), whose function is unclear, whereas *mDll3* mRNA is found only in the intermediate zone (IZ)/CP (Fig. 2C,D). At later developmental stages, the expression of both genes declines, although both *mDll1* and *mDll3* mRNAs can be detected along the ventricles at E16.5 pc (Fig. 3). Table I displays and summarizes the mRNA expression pattern for *mDll1* and *mDll3* (detected by in situ hybridization) and the β-galactosidase distribution (detected by immunohistochemistry) in the Dll1-lacZ transgenic mouse.

mDll3 and *mDll1* Are Expressed in Concentric Layers

In the developing E12.5 cortex, *mDll1* and *mDll3* are expressed in concentric layers (Fig. 4A,B). Dunwoodie et al. (1997) suggested that cells undergoing neuronal differentiation would go through a first wave of *mDll1* expression, followed by a second wave of *mDll3* expression. To check this possibility, we attempted to detect both mRNAs in the same cells (Fig. 5). Overlap between cells that express *mDll1* and *mDll3* was evaluated by double in situ hybridization using *mDll1* mRNA marked with digoxigenin and *mDll3* mRNA marked with fluorescein. Cells labelled for one probe or the other are present in adjacent areas of the lateral ventricle VZ and SVZ at E12.5 and E14.5 pc, and the two concentric layers do not seem to overlap (Fig. 5A,B). However, it is not possible to

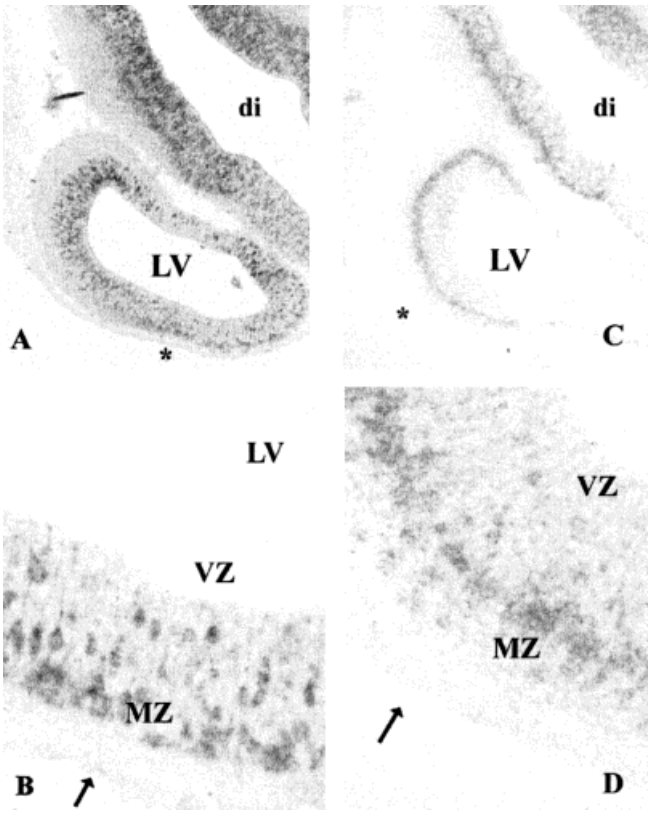

Fig. 1. *mDll1* (**A,B**) and *mDll3* (**C,D**) expression across the forebrain (A,C) at E11.5 pc, showing the same relative distribution as in E10.5 pc [*mDll1* mRNA across the neuroepithelium (B) and *mDll3* mRNA basally located in the neuroepithelium (D)]. Stars in A and C indicate cortical areas shown at higher magnification in B and D. LV, lateral ventricle; di, diencephalon; VZ, ventricular zone; MZ, marginal zone.

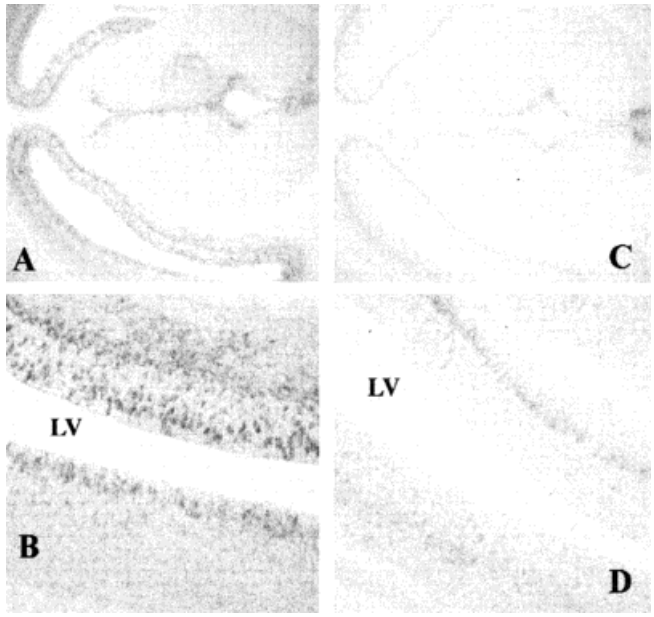

Fig. 2. *mDll1* mRNA and *mDll3* mRNA expression in horizontal sections of wild-type E13.5 pc mouse forebrain. *mDll1* and *mDll3* expression is detected along the ventricular system (**A,C**) and in the developing cortex (**B,D**). *mDll1* mRNA is found in two concentric layers in the frontal cortex (A) and in the cortical septal area (B; top half), it can be seen in the VZ and outer IZ/SP. *mDll3* mRNA is expressed in the CP/SP (C,D).

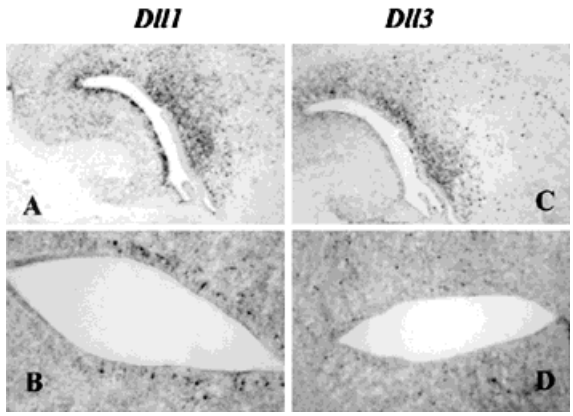

Fig. 3. *mDll1* and *mDll3* expression in the E16.5 pc mouse forebrain (horizontal sections). *mDll1* is expressed in the VZ of the lateral (**A**) and third (**B**) ventricles. High expression is detected in the extension of the lateral ventricles into the olfactory bulb (not shown). *mDll3* mRNA is present in the corresponding regions (**C,D**) and in dispersed cells around the ventricular system.

exclude that 1) these genes are expressed in completely different populations or 2) *mDll3* is expressed in the same cells but only after *mDll1* stops being transcribed. Cortical expression of β-galactosidase in the E12.5 pc Dll1-lacZ transgenic mouse occurs in the same layer where *mDll3* mRNA is detected (cf. Fig. 4B and C), a finding that supports the later hypothesis.

mDll1 and *mDll3* mRNAs Are Present in Nondividing Cells in the Embryonic CNS

Previous studies have shown that *mDll1*- and *mDll3*-expressing cells are postmitotic in the spinal cord of E12.5 pc embryos (Henrique et al., 1995; Dunwoodie et al., 1997). To check whether *mDll1* and *mDll3* are expressed only in postmitotic cells in the developing brain, we injected BrdU into pregnant females at E12.5, E14.5, and E16.5 pc. The animals were then killed, and the embryonic tissue was processed for in situ hybridization, followed by immunohistochemistry to detect BrdU incorporation. In the embryonic brains, no BrdU was detected in *mDll1* or *mDll3* mRNA-positive cells (Fig. 6A–D). The same result was obtained in the ventricular wall of the telencephalon for both *mDll1* and *mDll3* at two different embryonic ages, E12.5 and E14.5 pc. When an antibody against histone H3 was used to identify dividing cells, no overlap was detected with *mDll1* mRNA-expressing cells (data not shown). Therefore, this evidence also indicates that *mDll1* is expressed only in postmitotic cells in the developing brain.

TABLE I. *Dll1* and *D113* mRNA Expression Detected by In Situ Hybridization in Normal Mice and β-Galactosidase (βgal) Expression Detected by Immunohistochemistry in Dll1-lacZ Transgenic Mice During Mouse Forebrain Development⋆

	E12			E14			E16		
	Dll1 RNA	Dll3 RNA	βgal	Dll1 RNA	Dll3 RNA	βgal	Dll1 RNA	Dll3 RNA	βgal
Telencephalon									
Cortex									
VZ	+++	−	−	++	−	−	+	+	−
IZ	+	−	−	+	−	−	+/−		+/−
PP	++	++	++						
SP				+/−	++				
CP				+/−	++	+	+/−	−	−
MZ	++	−	++	−	−	−	−	−	−
Ganglionic eminence	+++	+++	+	++	++	+	+/−	+/−	+
Hippocampus									
Hip. plate							+/−		
IMZ							−		
OMZ							+/−		
Dentate gyrus							−		
Diencephalon									
Thalamus	+++	+++	++	++	++	+	+/−	+/−	+
Hypothalamus	+++	+++	++	++	++	+	+/−	+/−	+

⋆+++, Very high expression; ++, high expression; +, low expression; +/−, very low expression; −, no expression.

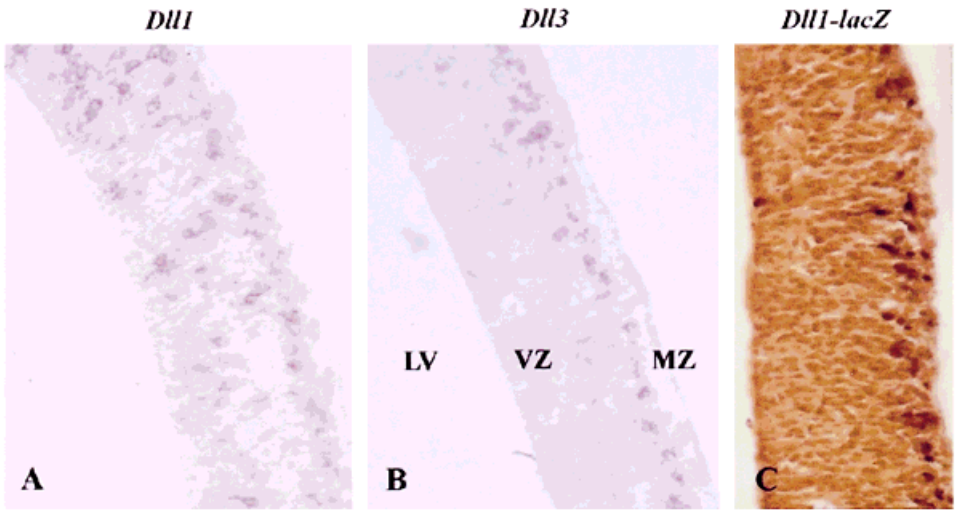

Fig. 4. *mDll1* and *mDll3* cortical expression in the E12.5pc mouse as detected by in situ hybridization. *mDll1* mRNA is present in the VZ, IZ, and MZ (**A**); *mDll3* mRNA is expressed only in the MZ (**B**). Immunohistochemical detection of β-galactosidase expression in the Dll1-lacZ transgenic brain shows reporter gene expression in the MZ (**C**). For all sections, the lateral ventricle is on the left, and the pial surface on the right.

However, in the more complex and multilayered cortical environment, the possibility exists that some of these cells reenter the mitotic cycle, once *mDll1* mRNA is no longer detectable. To check for the possibility that *mDll1* could be expressed during secondary neurogenesis, we injected BrdU in Dll1-lacZ transgenic animals at three embryonic ages (as described in Materials and Methods) and then stained for BrdU incorporation and β-galactosidase expression. None of the cells expressing the reporter gene was doubly stained for BrdU (Fig. 6E,F), suggesting that cells did not reenter the mitotic cycle after *mDll1* transcription stops. Had β-galactosidase-positive cells divided while migrating towards the pial surface, at least some BrdU would be present in the reporter-expressing cells, even if diluted. Moreover, in Figure 6E (arrows), some β-galactosidase-positive cells can be seen migrating out towards the pial surface, and these cells do not incorporate BrdU. This result supports the hypothesis that *mDll1* is expressed in postmitotic cells only. Moreover, it shows, for the first time, that *mDll1* is expressed in cortical postmitotic cells during brain development. In fact, previous evidence was obtained only from spinal cord. Nevertheless, it remains to be explained why a second layer of *mDll1* expression occurs away from the VZ in some areas of the developing cortex (Fig. 2A,B). This expression pattern raises the possibility that Dll1 and Notch signalling have different functions in neighboring anatomic sites.

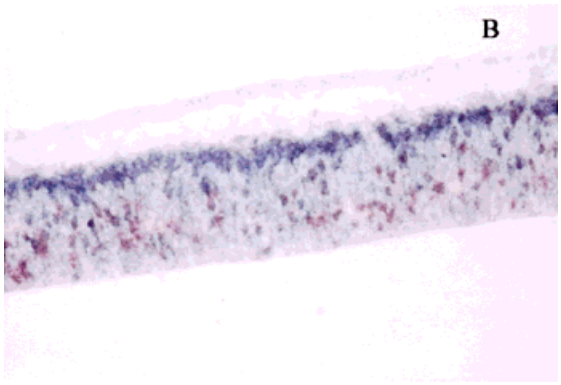

Fig. 5. Double in situ hybridization in E12.5 mouse cortical sections (sagital) using Dll1 and Dll3 riboprobes marked with digoxigenin and fluorescein, respectively. Dll1 expression is shown in brown, and Dll3 expression is shown in blue. The pial surface faces upward.

The possibility that *mDll1* is involved in glial fate determination by being expressed on glial progenitors is reduced, in that most of these cells are dividing during late gestation, at times when very little *mDll1* is expressed (probably in very specific neuronal populations). Nevertheless, an effect of the *mDll1*-expressing "neurons" in determining neighboring cells fates cannot be excluded. Recent evidence (Gaiano et al., 2000) shows a role for Notch1 activation in promoting radial glial cell identity in the forebrain, challenging the classical views on Notch/Delta signalling, which considered a role for Notch activation only in blocking differentiation. Moreover, Notch has been shown to promote differentiation in adipocytes (Garces et al., 1997), keratinocytes (Lowell et al., 2000), and granulocytes (Schroeder and Just, 2000).

Most Embryonic CNS Cells Expressing *LacZ* Under the *mDll1* Promoter Are Not Positive for RC2, Nestin, and Antitubulin III but Are Found in Close Contact With Nestin-Positive Radial Glial Cells

Although previous evidence indicates that *mDll1* is expressed in postmitotic neuronal cells only in the spinal cord (Henrique et al., 1995; Myat et al., 1996), a role for *mDll1* in the generation of other neural cell types has not been excluded. In fact, a role in oligodendrocyte differentiation has been described for the Notch/Delta signalling system (Wang et al., 1998; for review see Lee et al., 2000). Transient Notch activation causes a switch from neurogenesis to gliogenesis in neural crest stem cells (Morrison et al., 2000), and *Notch1* signalling is believed to promote radial glial identity in the murine forebrain (Gaiano et al., 2000) and Müller glia differentiation in the retina (Furakawa et al., 2000). Two possibilities arise. First, *Dll1*-expressing cells could be a heterogeneous population and give rise to both neurons and glial cells, in contrast to the classical view, which holds that *Dll1*-positive cells are neuronal cells only. Second, *Dll1*-expressing cells could in fact be neuronally fated cells only.

To clarify these issues, we attempted to characterize the phenotype of *Dll1*-expressing cells during CNS development, using a transgenic mouse that expresses LacZ under the control of the *Dll1* promoter. In the *Dll1-LacZ* transgenic brains, β-galactosidase can be detected by immunohistochemistry in cells in which *mDll1* mRNA is no longer detectable. β-Galactosidase-positive cells are found in the same regions where *mDll1* mRNA is detected but away from the VZ. This latter finding is expected, insofar as these cells migrate away from their birthplace and *mDll1* mRNA is probably not as long-lived as the β-galactosidase reporter protein (Fig. 3C, Table I). Bipolar β-galactosidase-positive cells can be seen outside the VZ, extending processes toward the cortical pial surface (Fig. 6E).

To check for the presence of both glial and neuronal precursors in the β-galactosidase-expressing population, we used antibodies against specific cell types (RC2 for radial glial cells, nestin for early glia including radial glial cells, and anti-β-tubulin isotype III for neuronal cells). β-Galactosidase-positive cells do not express β-tubulin isotype III close to the ventricle (Fig. 7A) or even further away from the VZ (Fig. 7B). Nestin has been generally used as a marker for early glial and radial glial cells (Anton et al., 1999), although some authors consider it to be a nonspecific marker (Messam et al., 2000). The shape of the nestin-positive cells strongly suggests that these are radial glial cells (Fig. 7C,D). The β-galactosidase-positive cells do not express nestin in the floor of the fourth ventricle (Fig. 7C, D) or in the cortex (Fig. 7E) and are also negative for RC2 (Fig. 8). Therefore, immunofluorescence staining with the available markers revealed no double staining of β-galactosidase-positive cells with radial glial and neuronal markers. From the transgenic mouse studies, we conclude that the undifferentiated cells that express the reporter gene (which gives rise mainly to neuronal cell types, as suggested by the β-galactosidase cortical expression in E12.5 pc embryos) are not radial glial cells. In fact, the *lacZ*-expressing cells are nestin-negative, whereas the radial glial cells are nestin-positive. Nevertheless, the two cell types are in close contact, as can be seen in Figure 7C,D. This evidence strongly supports the recent suggestion by Gaiano et al. (2000) that radial glial cell fate is influenced by Notch1 signalling. We propose that Dll1 is available in the neighboring migrating cells, as suggested by the expression of the reporter gene under the control of the *mDll1* promoter (Fig. 7C,D). This undifferentiated migrating population would then affect the behavior of the underlying radial glial cell "scaffold."

DISCUSSION

mDll1 and *mDll3* Play a Role in Cortical Neurogenesis

During mouse embryonic development, neurogenesis occurs between E8 and P0, whereas gliogenesis is believed to occur mainly at the end of gestation and during the first 2 postnatal weeks. Although Dll1 and Dll3 were previously known to play a role in early neurogenesis (Dunwoodie et al., 1997), we show that Dll1 and Dll3 are

also involved in cortical neurogenesis, which occurs from E10 to E16. We show expression in different prospective cortical layers for both genes. Furthermore, the large cortical β-galactosidase-positive cells present in the marginal zone (MZ) in the E12.5 pc Dll1-lacZ transgenic animal resemble Cajal-Retzius cells, the first postmitotic neurons known to populate the cortex. This suggests that, as for the spinal cord, Dll1 is involved in the generation of early neurons. Indirect evidence comes from the decrease in *mDll1* mRNA expression during the last days of gestation, an event that coincides with the slowing in neurogenesis. The few dispersed β-galactosidase-positive cells detected in the transgenic brain after birth (data not shown) at a

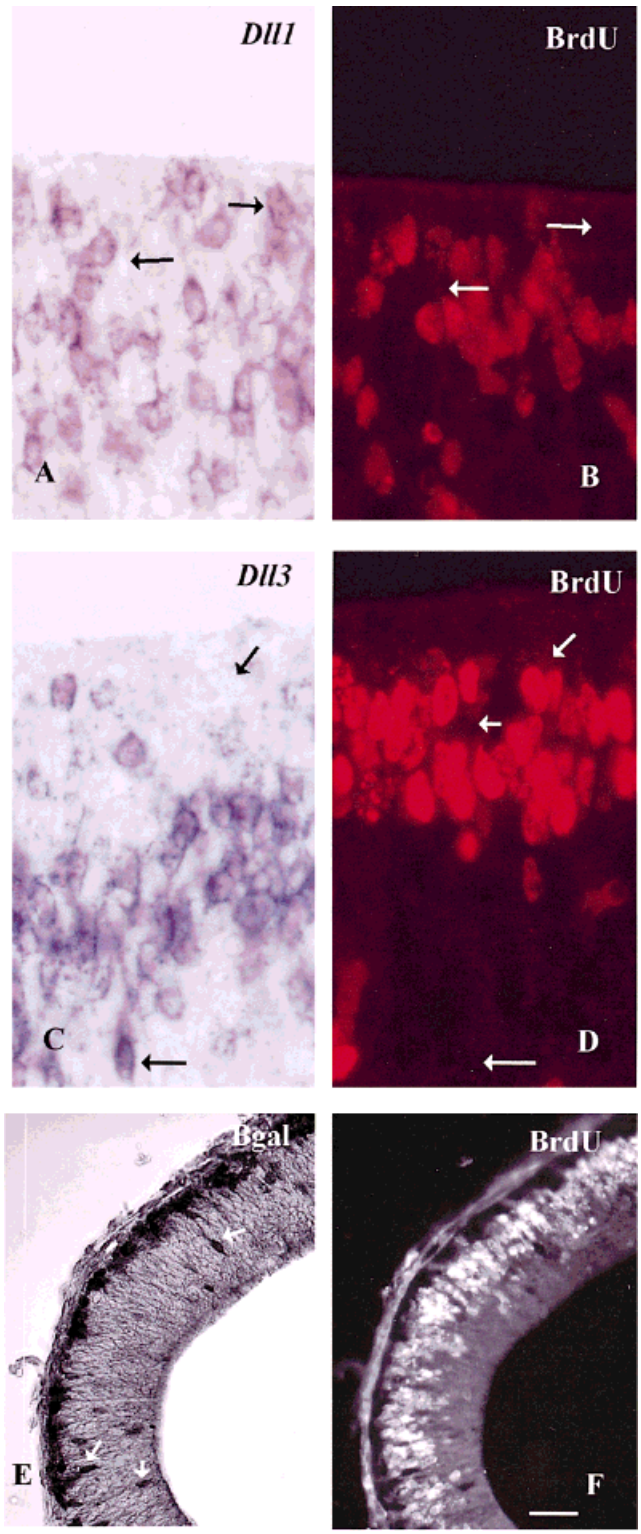

Fig. 6. Sagital sections through the ventricular system of E14.5 pc BrdU-treated mice. In situ hybridization detects *mDll1* mRNA in the VZ (**A**); in the same section, immunohistochemistry shows that BrdU incorporation occurs in some cells (**B**) that appear red, but no BrdU incorporation occurs with *mDll1* expression. *mDll3* mRNA is detected in cells adjacent to the VZ (**C**), and neighboring cells incorporate BrdU (**D**), but no doubly labelled cells can be detected. Sagital sections through the lateral ventricle and cortex of E12.5 pc BrdU-treated Dll1-LacZ transgenic animals. β-Galactosidase-expressing cells can be detected by immunohistochemistry in the MZ/CP (**E**). BrdU incorporation occurs in the neuroepithelium (**F**) but not in the β-galactosidase-positive cells. Arrows show migrating cells.

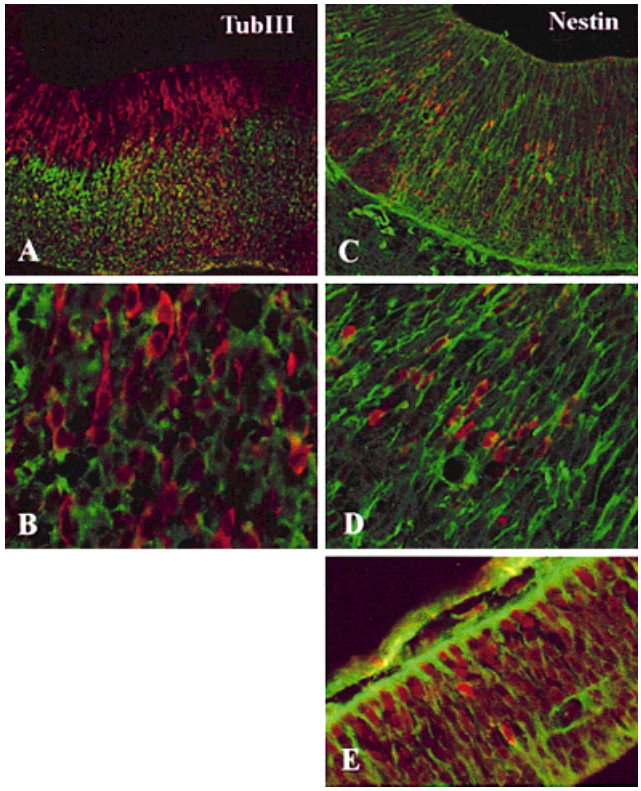

Fig. 7. Sagital sections through E12.5 pc Dll1-lacZ transgenic mouse brain. β-Galactosidase expression is shown in red and tubulin in green in the floor of the fourth ventricle (**A,B**). β-Galactosidase expression is shown in red and nestin in green in the fourth ventricle floor (**C,D**) and in the cortex (**E**). No double staining is apparent in any photograph.

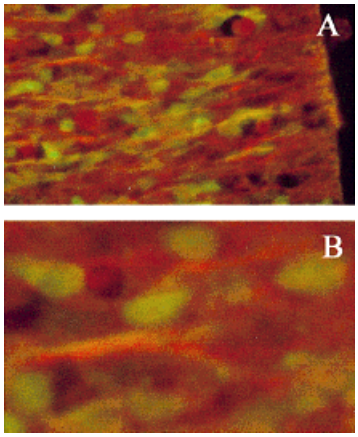

Fig. 8. Sagital sections through E12.5 pc Dll1-lacZ transgenic mouse brain. β-Galactosidase expression is shown in green and RC2 in red (**A,B**). A detail of A is shown in B. The RC2 staining resembles the nestin filamentous staining seen in Figure 7.

time when olfactory bulb and cortical interneuron production is active (Altman, 1969; Hatten, 1999) could be late-generated neurons. Nevertheless, we could not detect *mDll1* expression by in situ hybridization using digoxigenin-labelled probes in the adult brain (data not shown), where neurogenesis is known to persist (Lois and Alvarez-Buylla, 1993; Weiss et al., 1996). This could be due to basic differences between embryonic and adult neural progenitors, to changes in their environment established during maturation (Morrison et al., 1997), or to the expression of other Notch ligands in the adult brain. Moreover, we found that *mDll3* also plays a role during embryonic neurogenesis. Dunwoodie et al. (1997) suggested that a wave of *mDll3* expression followed *mDll1* expression and that the progression from *mDll1* to *mDll3* expression represents an increasing commitment to terminal differentiation of distinct neuronal types. Our results show that during embryonic development the timing of *mDll3* and *mDll1* expression in the brain are closely linked. The pattern of expression is also remarkably similar, although *mDll3* mRNA is detected not in the VZ but deeper in the cerebral structure. In particular, we show that this happens in the developing cortex. Nevertheless, it is not clear from the double in situ hybridization data (Fig. 5) whether it is the same cell population that progresses from expressing *mDll1* to expressing *mDll3* or whether two different populations express these genes separately in the mouse cortex. The comparison between the *mDll3* mRNA cortical expression pattern at E12.5 pc and the Dll1-lacZ expression in the transgenic mouse supports the former hypothesis, insofar as β-galactosidase expression and *mDll3* mRNA are detectable in the same layer (Fig. 4B,C). Postnatally, no *mDll3* mRNA was observed in the cortex (data not shown), suggesting that, if *mDll3* plays a role in neurogenesis, it does so only during early and midgestation. Unlike the case with *mDll1*, which seems to be directly involved in determining the neurogenic fate of a cell at the VZ level (Henrique et al., 1997), the time and place of *mDll3* expression suggest its involvement in a later (and yet unknown) event. In fact, cells express *mDll3* after migrating away from the VZ. Since a population of cells outside the VZ divides and gives birth to neurons in the process (Ishii et al., 2000), *mDll3* could be expressed during this second wave of cell generation. However, no *mDll3*-expressing cells were seen to incorporate BrdU. Wood et al. (1992) showed that a transient population is present in the subplate and MZ of the mouse developing cortex and that this transient population is formed by the earliest-generated cortical cells. *mDll1* and *mDll3* expression patterns suggest that these genes might be involved in the generation of such a transient subplate.

mDll1 Could Play a Role During Gliogenesis

Although the cells born from E11.5 pc onwards are mainly neurons, a specific type of cell, the radial glial cell, is present throughout the neurogenesis period (Hatten, 1999). These cells provide the primary pathway for directed migration. Recent evidence has shown a role for Notch1 activation in promoting radial glia identity in the forebrain (Gaiano et al., 2000). Morrison et al. (2000) have shown that transient Notch activation initiates a switch from neurogenesis to gliogenesis in neural crest stem cells. Therefore, the possibility exists that the second wave of *mDll1* expression that we observe in the developing cortex could be involved in glial cell generation. Our results support this hypothesis by showing that *mDll1* and *mDll3* mRNAs are found in the adequate layers of the cortex, at the right time to affect the radial scaffold behavior. Moreover, the expression of the *lacZ* reporter gene under the control of the *mDll1* promoter shows that cells that have previously expressed mRNA for *mDll1* and that actively express the reporter gene in the developing cortex (as detected by immunohistochemistry) are in close contact with radial glial cells, as defined by nestin or RC2 staining. This result strongly supports the hypothesis that Delta1 is the Notch1 ligand responsible for interacting and/or promoting radial glial identity. Our in situ hybridization data also show a decrease in *mDll1* mRNA at the time when radial glial cells are known to start to transform into protoplasmic astrocytes, as described by Voigt (1989) and Takahashi et al. (1990). This transformation and its timing could then be a consequence of the down-regulation of *mDll1* expression. Nevertheless, it is difficult to distinguish between two hypotheses: first, that *mDll1* signalling could be actively keeping radial glial cells in an undifferentiated state; we would then predict that the disappearance of the ligand would lead to total differentiation; and, second, that the *mDll1* signalling could be actively inducing neural stem cells to become radial glia, as suggested by Gaiano et al. (2000).

mDll1 and *mDll3* Expression and Cortical Laminar Organization

We found that the expression pattern for *mDll1* accompanies the initial laminar organization of the cortex.

Two layers of *mDll1* mRNA expression are seen in some areas of the developing cortex at days E13.5 and E14.5 pc, one adjacent to the VZ and a peripheral one that probably corresponds to the intermediate zone (IZ) outer region or to the preplate. The layer closest to the VZ could reflect the postmitotic neuronal determination role established for the spinal cord by Henrique et al. (1997). A similar function could be suggested for the more external layer, in that mitosis leading to neural cell generation occurs away from the VZ (Ishii et al, 2000): *mDll1* could then be expressed again, just after cell division. Nevertheless, in the Dll1-lacZ transgenic mouse, cortical β-galactosidase-positive cells do not incorporate BrdU, and neither do the *mDll1* mRNA-expressing cells found away from the cortical VZ at E14.5 pc (data not shown), making this hypothesis unlikely. Another possibility is that the external positive layer could be related to an unknown function of the Notch/Delta signalling system, unrelated to the generation of postmitotic neurons. This latter hypothesis takes into account the recent work on the role of Notch in postmitotic cells. Notch1 activation has been shown to regulate the capacity of cortical neurons to extend and elaborate neurites during development and in the adult (Berezovska et al., 1999; Franklin et al., 1999; Sestan et al., 1999; Redmond et al., 2000), a mechanism likely to be at work while cells are migrating and/or have not reached their final position, or when their cell fate has not been fully determined.

At the same embryonic ages, *mDll3* is expressed in a more external layer, coinciding with the cortical plate/subplate which is fully formed by E14.5 pc. This peripheral and transient position of the *mDll3*-positive cells suggests that this *Delta* homologue is involved not in early neuronal determination but in an event occurring later, during precursor migration or maturation. In fact, although mitosis is known to occur in progenitors away from the VZ (Ishii et al., 2000), cells expressing *mDll3* mRNA do not incorporate BrdU.

We conclude that *mDll1* and *mDll3* are involved in cortical neurogenesis and in the early formation of the future multilayered cortex. First, our in situ hybridization results (showing concentrical layers in the developing cortex) support the view of Dunwoodie et al. (1997), which indicated that the sequential expression of Dll3 after Dll1 could represent an increasing of commitment towards the neuronal fate. Second, the expression of the β-galactosidase reporter gene in cells that resemble Cajal-Retzius cells supports the suggestion that *mDll1* is involved in the generation of postmitotic neurons from very early stages, in regions of the CNS other than the spinal cord. Third, the laminar expression of *mDll1* and *mDll3* in the developing cortex suggests that, apart from a role in neuronal cell fate determination, these genes could be essential for cortical development through other mechanisms. For example, there could be a role for *mDll1* and *mDll3* in glial development. In light of data presented by other authors, the expression of *mDll1* and *mDll3* away from the VZ in nondividing cells could suggest an effect on the scaffold cells (radial glial cells), which are known to express Notch1. Our results, which show the β-galactosidase-positive cells in close contact with radial glial cells, support the suggestion that Dll1 could have an effect on the latter population. Such an effect could be explained by two hypotheses. First, Notch activation could lead the radial glial cells to differentiate toward a glial fate, as in the neural crest (Morrison et al., 2000), through a transient signal. Alternatively, activation could keep these cells in an undifferentiated state, while neurons migrate. The first hypothesis could require Dll1 to be present only transiently, at the beginning of gliogenesis, as has been described for neural crest glial cells (Morrison et al., 2000). We favor the second hypothesis, which could explain how the decrease in neurogenesis at the end of gestation, and the resulting decrease in *mDll1* expression, would deprive radial glial cells of an essential signal and lead them to differentiate into astrocytes, which could still retain some stem cell capacity, as suggested by Doetsch et al. (1999). Further studies will be necessary to clarify the role of this signalling system in the developing cortex and in particular in glial biology.

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