INTRODUCTION

During the development of the central nervous system, distinct regions must be specified and delineated from one another. Most insights into the mechanisms that underlie such spatial organisation have been gained in the developing hindbrain, which is subdivided into segmental units called rhombomeres (Lumsden and Krumlauf, 1996). Rhombomeres are first specified by distinct expression of transcription factors, such as Krox20, and then by patterned expression of cell surface molecules such as cadherin 6 or members of the ephrin-family (Inoue et al., 1997; Xu et al., 1999). Eph-receptors and their respective ligands are distributed in alternating rhombomeres, and their binding is involved in the separation of cells from adjacent rhombomeres (Xu et al., 1999).

In the developing forebrain, gene expression territories coincide with regions of restricted cell mixing and migration (Figdor and Stern, 1993; Fishell et al., 1993; Götz et al., 1996), consistent with the suggestion that the CNS develops as distinct territories called neuromeres or prosomeres (Puuelles and Rubenstein, 1993). On the other hand, prominent cell migration between brain regions has been observed in mouse embryos, challenging the concept of strict cell isolation (DeCarlos et al., 1996; Anderson et al., 1997; Tamamaki et al., 1997; Sussel et al., 1999; Wichterle et al., 1999). For example, a considerable proportion of GABAergic cells in the cerebral cortex, the dorsal telencephalon, originates in the basal ganglia, called ganglionic eminences (GE), during development (DeDiego et al., 1994; Anderson et al., 1997; Tamamaki et al., 1997; Sussel et al., 1999; Wichterle et al., 1999). The lateral ganglionic eminence (LGE) gives rise to the striatum and is the ventral neighbour of the cortex, whereas the medial ganglionic eminence (MGE) mostly generates the globus pallidus and septum (Deacon et al., 1994; Olsson et al., 1995). Interestingly, recent data suggest that at least some cells migrate from the MGE into the cortex, crossing the entire telencephalon from ventral to dorsal (Sussel et al., 1999; Wichterle et al., 1999).

The differing migration results could be explained by the labelling of different cell types in previous studies (Lumsden and Gulisano, 1997). Since migration analysis performed in the cortex mostly assessed precursor cells (Fishell et al., 1993; Neyt et al., 1997), it is not known whether or not neurons from this region migrate ventrally. Thus, it may be that precursor cells from adjacent regions are restricted in their migration, whereas neurons may be able to cross from both sides of the boundary (Lumsden and Gulisano, 1997). Alternatively, the cortico-striatal boundary may exhibit a true asymmetry that allows only ventro-dorsal migration, while restricting dorso-ventral cell migration.

In order to elucidate the molecular mechanisms responsible...
for dorso-ventral patterning and cell migration in the telencephalon, we analysed the Pax6 mutant Small eye (Sey; Hill et al., 1991; Stoykova et al., 1996, 1997). Several transcription factors expressed in the ventral telencephalon, such as Dlx1/2 (Anderson et al., 1997), Mash1 (Casarosa et al., 1999) and Nkx2.1 (Sussel et al., 1999), are necessary for cell migration from the GE into the cortex. In this study we therefore analysed how the mutation of the dorsally restricted transcription factor Pax6 affects cell migration along the dorso-ventral axis. It has previously been shown that the Dlx1 expression gradually invades the cortical region in the Pax6 mutant (Stoykova et al., 1996). Hence, we were interested to determine whether this could be due to an enhanced ventro-dorsal cell migration, or whether the loss of Pax6 leads to an upregulation of Dlx in the cortex. To this end, we developed a new labelling technique to analyse cell migration by the use of focal injections of adeno-virus encoding the green fluorescent protein (GFP).

MATERIALS AND METHODS

Animals

In this study, we used wild-type and homozygous Sey/Sey littermates, obtained from crossings of heterozygous mice that carry the Sey allele on a C57BL/6J × DBA/2J background. This naturally occurring point mutation in the Pax6 gene leads to the expression of a truncated non-functional protein (Hill et al., 1991). The day of vaginal plug was taken as embryonic day 0 (E0).

EGFP adenovirus

The cDNA for the red-shifted EGFP (GFPmut1) was obtained by HindIII/NotI digest of the pEGFP-N1 vector (Clontech Laboratories). The cDNA was purified and cloned into the pXCJL-CMV-BGH vector. Recombinant replication-deficient virus Ad5CMVGFP was obtained by cotransfection of pXCJL-CMV-GFP and pJM17 (pXCJL-CMV-BGH and pJM17 were kindly provided by Claude Gravel, Université Laval, Quebec, Canada) into 293 cells (Microbix), with subsequent homologous recombination (Graham and Prevec, 1991). The virus was purified after two rounds of plaque purification in a caesium chloride density gradient. Virus titres, estimated by plaque assay (Graham and Prevec, 1991), were in the range of 10^10-10^11 pfu/ml.

Slice preparation and injections

Embryos (E13-17) were removed by cesarean section and decapitated. Brains were isolated in ice-cold Hank’s balanced salt solution (HBSS), buffered with 10 mM Hepes. Coronal sections of the telencephalon were cut at 250-300 μm thickness using a tissue chopper (McIlwain). The slices were collected in HBSS/Hepes and transferred to Millipore inserts (Millicell- CM 0.4 μm) in 6-well plates, where they were cultured in 1.5 ml Dulbecco’s modified medium (DMEM) with 10% foetal calf serum (FCS) for 2 days at 37°C and 5% CO2. The injections were performed immediately after preparation of the slices: EGFP adenovirus was pressure-injected focally onto the surface of the slice by a Pneumatic PicoPump PV820 (WPI) through a glass pipette with a 10 μm opening diameter. About 10-100 cells were infected in an area of 200-400 μm. In some control experiments, culture medium containing 2-8 μg/ml cytochalasin-D was added immediately after the injections, in order to inhibit cell migration.

Migration analysis

In order to follow the migration of green fluorescent cells, the infected slices were photographed at three time points after the infection (at 16/17, 25/26 and 44/45 hours), using a fluorescence microscope (Zeiss Axioiophot) with 10× magnification. The distribution of green fluorescent cells was drawn from these photos for each time point, and the three drawings were superimposed (Fig. 5A). The maximal distance of migration was measured as the distance between the injection site (defined as the 16 hours area) and the furthest cell after 25 and 45 hours. The mean distance of migration was calculated as the average of the distances of all migrating cells in a respective slice. When some cells already exceeded the rim of the injection site after 16 hours, we used their location as a reference point to measure the distance. Values are given as means ± s.e.m.

Dissociated cell cultures

The cerebral cortex and the GE of E14 mice were dissociated as previously described (Götz et al., 1995). Infection with 0.5–1 μl GFP-adenovirus per coverslip was performed 1 hour after plating of the cells. Cultures were fixed after 1 day and immunostained as described below. Neurons were detected by a monoclonal antibody (mAb) directed against β-III-tubulin (IgG2b, 1:100, Sigma), and precursor cells by a mAb against nestin (IgG1, 1:4, Developmental Studies Hybridoma Bank).

Immunohistochemistry

Forebrains of wild-type and Sey/Sey littermates (E13-17) were dissected as described above. For calbindin and calretinin stainings, brains were fixed in 2% paraformaldehyde (PFA) in PBS at 4°C for 6 hours. For GABA stainings, brains were fixed in 4% PFA with 0.2% glutaraldehyde at 4°C for 8 hours. Brains were embedded in 3% agarose in PBS, and 100 μm thick coronal sections were cut on a vibratome. Primary rabbit polyclonal antibodies directed against calbindin or calretinin (Swant) were diluted 1:500, and rabbit polyclonal anti-GABA antibodies (Sigma) were diluted 1:1000 in PBS with 0.5% Triton X-100 and 10% normal goat serum, and incubated overnight at 4°C. The secondary antibody goat-anti-rabbit-Cy3 (Dianova) was diluted 1:200 in the same solution as the primary antibody, and incubated for 30 minutes at room temperature. Washings were performed in PBS and the sections mounted in Aqua Poly/Mount (Polysciences Inc). Positive cells were counted at different rostro-caudal levels of the telencephalon in 8-16 sections. For some experiments, all sections of an entire telencephalon were counted to exclude differences in the sectioning plane. Using a 40× objective, all immunopositive cells in a single field of view were counted in the lateral cortex, immediately ventral to the sulcus (Fig. 7). All cells in the cortical plate of the dorsal cortex were counted from the sulcus to the fold of the medial cortex.

RESULTS

Technical considerations regarding the use of adeno-virus injection to analyse cell migration

In order to analyse cell migration, we performed focal injections of adeno-virus vectors containing GFP (see Materials and Methods). We first examined whether all cell types in the telencephalon were equally well infected by the GFP-adenovirus. When dissociated cell cultures of embryonic day 14 (E14) cortex and GE were infected with the GFP-adenovirus, 44% of the GFP-fluorescent cells were β-III-tubulin immunoreactive neurons and 43% were nestin-positive precursor cells after 1 day in vitro. These proportions correspond well to the relative proportions of neurons and precursor cells at this time, showing that all the cell types present were equally well infected.

Adeno-viral vectors were injected into either the cerebral cortex or LGE in coronal slices from the telencephalon of E14
mice. Both the size of the injections and the number of infected cells were highly reproducible in our experiments. The size of injection ranged between 200 and 400 µm diameter (see also Figs 1-4). Since the increase in area of dense GFP signal ceased around 16 hours after the injection, we defined this area as the injection site. The injections usually spanned proliferative and differentiating cell layers in both cortical and LGE injections. After 25 hours, cells were detected outside the injection site, and their number and distance increased after 45 hours (see Figs 1, 3-5; Table 1A,B).

In order to ascertain whether the apparent spread of GFP-expressing cells was due to migration or to passive diffusion of adenoviral particles, we blocked cell migration by the addition of cytochalasin-D, which inhibits actin polymerisation (Cooper, 1987). Cytochalasin-D was applied at 2-8 µg/ml immediately after the injection with GFP-adenovirus. Under these conditions, no GFP-expressing cell could be found outside the injection spot up to 45 hours later (Fig. 2). Thus, the spread of infective virus particles seems to be very restricted in this preparation, indicating that the fluorescent cells outside the injection site, as observed under normal conditions, are migrating. Indeed, these cells exhibit a morphology that is typical for migrating cells, with a small ovoid cell soma and a longer leading process (see Fig. 1C; Fishell et al., 1993; Anderson et al., 1997; Tamamaki et al., 1997; O’Rourke et al., 1997). Moreover, the mean distance of cells from the injection site increased from 140 to 220 µm between 25 and 45 hours (LGE injection; Table 1C), further supporting the notion that these cells are migrating. GFP-expressing cells migrated over a mean distance of 220 µm in 29 hours (from 16-45 hours), i.e. at a mean speed of 8 µm per hour. This is relatively slow, but within the range of migrational speed obtained in previous studies (O’Rourke et al., 1997).

The use of focal GFP-adenovirus injections showed several advantages compared with the application of DiI to analyse cell migration. For the quantitative comparison between wild-type and mutant mice, DiI injections were too variable, at least under our conditions. In particular, the number of infected cells and the size of the injection site proved to be very reproducible in GFP-adenovirus injections. In contrast to prominent fibre labelling by DiI-application, we detected hardly any retrograde-labelled cells after adenoviral injection. Last but not least, GFP is considerably less phototoxic and the use of co-expression constructs may allow the migration of genetically manipulated cells in a wild-type environment to be imaged.

### Cell migration across the cortico-striatal boundary

The main interest of this study was the analysis of migration across the cortico-striatal boundary. It was crucial to inject the adenovirus at comparable distances on either side of the boundary, in the cortex and LGE. Here, it is important to realise that LGE injections also label cells migrating through this region from further ventral positions, e.g. the MGE (Sussel et al., 1999; Wichterle et al., 1999). Since the radial glia fascicle that indicates the cortico-striatal boundary spans the region from the sulcus to further ventral positions (Fig. 1), we used the sulcus as a suitable ‘landmark’. From there, the cortico-striatal boundary extends in a latero-ventral direction, as visible in phase contrast microscopy (Fig. 1). The position of the boundary was confirmed by staining for the radial glia fascicle, which also persisted during the 2 days in vitro (data not shown). The distance of the injection site from the boundary was 364 µm (±36, n=19) in the LGE and 222 µm (±39, n=12)

| Tables 1. Quantitative analysis of migration in slices injected with GFP-adenovirus into the cortex or LGE |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **(A) Cortex injections**       | **(B) LGE injections**          | **(C) Mean distances of migration after 45 hours (µm)** |
| Proportion of slices with cells in the LGE 45 hours | Mean number of cells/slice in the LGE 45 hours | Maximum distance of migration within the cortex (µm) 25 hours 45 hours | Maximum distance of migration to the LGE (µm) 45 hours |
| Wild type 23% (n=26) | 0.8 | 331±8 (n=14) 526±82 (n=13) | 322±52 (n=3) |
| Small eye 35% (n=26) | 1.3 | 227±57 (n=14) 529±76 (n=15) | 336±128 (n=3) |
| Proportion of slices with cells in the cortex | Mean number of cells/slice in the cortex | Maximum distance of migration within the LGE (µm) 25 hours 45 hours | Maximum distance of migration to the cortex (µm) 45 hours |
| Wild type 22% (n=27) 89% | 1 13 | 238±34 (n=19) 525±40 (n=19) | 928±65 (n=18) |
| Small eye 57% (n=26) 85% | 3 21 | 303±59 (n=13) 571±48 (n=19) | 997±53 (n=16) |
| LGE injections | Cortex injections | LGE injections | Cortex injections |
| Wild type | 220±10 (n=182) 645±28 (n=73) | 228±15 (n=125) 202±54 (n=5) |
| Small eye | 306±10 (n=301) 606±13 (n=264) | 238±15 (n=125) 288±78 (n=4) |

(A,B) n = the number of slices analysed; (C) n = the number of cells analysed. Values are means ± s.e.m.

For details, see Materials and Methods.

Note the comparable migration of cortical cells in wild type and Sey/Sey littermates and the low number of cortical cells that migrate into the LGE. In contrast, the number of LGE cells migrating into the cortex is increased (1.6-fold) in the telencephalic slices from Sey/Sey littermates.
in cortical injections. Since cells labelled in the LGE migrated a maximum distance of 928 μm, and cells labelled in the cortex a maximum of 526 μm (Table 1A,B), at least some cells should be able to cross the boundary from both sides. The quantification of migration distance showed that 57% of cortical and 39% of GE cells migrated over a distance exceeding that to the boundary. We therefore consider this preparation to be an appropriate system for examining the migrational restriction between cortex and GE.

When GFP-adenovirus was injected at the cortical side of the boundary, fluorescent cells were observed to migrate radially and laterally within the cortex, but hardly any cell crossed the boundary from both sides. The quantification of migration distance showed that 57% of cortical and 39% of GE cells migrated over a distance exceeding that to the boundary. We therefore consider this preparation to be an appropriate system for examining the migrational restriction between cortex and GE.

Fig. 1. GFP-adenovirus injection in telencephalic slices. (A-C) Phase-contrast micrographs of frontal slices of embryonic day 14 telencephalon. The slice was photographed under both phase contrast and fluorescence at a 5x magnification 16 hours (A), 26 hours (B) and 41 hours (C) after the injection of adenovirus encoding the green fluorescent protein (GFP) into the LGE. The LGE and the cortex can be discerned in phase contrast and are indicated in A (medial is to the left, lateral to the right, ventral down and dorsal up). The cortico-striatal boundary is indicated as a dotted line. The green fluorescent cells are visible as white dots in A-C as seen in the corresponding fluorescence micrographs shown under B and C, and indicated by black frames in B and C. C is a fluorescence micrograph taken at higher magnification in the lateral cortex and depicting four green fluorescent cells that have migrated from the LGE into the cortex after 41 hours.

In contrast, a substantial number of cells were observed to migrate in the opposite direction, from the GE into the cortex, consistent with previous observations (Anderson et al., 1997; Tamamaki et al., 1997; Sussel et al., 1999; Wichterle et al., 1999). Fig. 4A depicts an example of cells labelled in the LGE with some cells in the cortex after 43 hours in vitro. Hardly any cells had crossed the boundary to the cortex after 25 hours, but a mean of 13 cells per slice had migrated into the cortex in almost every culture (89%, Table 1B) 45 hours after injection into the LGE. Most of the cells that migrated from the GE were found in the lateral cortex (see Fig. 1C, 4A), and some were also detected in more dorsal areas of the cortex (arrows in Fig. 4A). Interestingly, for cells migrating into the cortex, the maximum distance of migration from the injection site was almost double that for those migrating within the LGE (Table 1B). This is in contrast to the behaviour of cortical cells that migrated less far towards and across the boundary than in other directions. Thus, there is a marked difference in the behaviour of cells around the boundary, depending on their side of origin.

In order to examine the cells crossing the boundary more
closely, we quantified cells moving towards and away from the boundary, as depicted in Fig. 5A. For example, the LGE was divided into two segments by a line through the injection site parallel to the boundary (Fig. 5A). The proportion of cells migrating towards (56%) and away (44%, n=257) from the boundary were similar. Moreover, the mean distance of migration was comparable for cells migrating in both directions (197±14, n=72; 235±14, n=112). In fact, half of the cells migrating towards the boundary crossed it into the cortex. As depicted in Fig. 5B, there is an obvious trough in the number of cells that migrate as far as the boundary. Few cells are found in the region of the boundary. This paucity of cells is reminiscent of that of TUJ1-positive neurons in the boundary region previously observed by Neyt et al. (1997). This observation suggests a certain degree of migrational restriction also for cells from the GE.

Migration in the Pax-6 mutant

After injection into the Sey/Sey cortex (Fig. 3B), cells migrated for distances comparable to those observed in the wild-type cortex (Table 1A,C). Nevertheless, we noted some differences in the distribution of green fluorescent cells in slices from Sey/Sey compared with these from wild type. In the Pax6 mutant cortex, cells rarely migrated in radial columns as described above for the wild type, but appeared to migrate in a less organised fashion (Fig. 3). The less radially directed migration within the Sey/Sey cortex might account for the decrease in the net radial movement observed in BrdU-birthdating studies (Caric et al., 1997). These defects are likely related to alterations in the radial glia cells of Pax6 mutant cortex (Götz et al., 1998). As in the wild type, very few cortical cells migrated into the LGE (Table 1A). The distance of the injection site from the boundary in Sey/Sey cortex (277±47 μm,
n=12) was comparable to the wild-type cortex (222±47 μm, n=12), as was the distance of migration (Table 1A,C). Despite a slight increase in the total number of cells infected in the Sey/Sey cortex (85±16, n=13) compared to the wild-type cortex (65±8, n=12), the number of cells crossing into the LGE was similarly low (Table 1A). These results indicate that cell migration is also restricted in the Pax6 mutant cortex.

In contrast, injections in the LGE of E14 Sey/Sey mice showed a significant increase of cells migrating into the cortex as compared to wild-type littermates (Table 1B). As observed in wild-type LGE injections, cells mostly migrated to the lateral part of the cortex. An example showing a massive migration of cells from the LGE into the Sey/Sey mutant cortex is depicted in Fig. 4B. Increased migration into the cortex was already
observed after 27 hours in 57% of all slices from Sey/Sey, as compared with 22% in wild-type slices. Although very few in number (3 cells per slice) this amount increased up to 43 hours after injection, when a mean of 21 cells per slice were found in the mutant cortex, as compared to 13 cells per slice in wild-type slices (Table 1B). Since, however, the total number of cells labelled in the Sey/Sey LGE (100±12, \(N=18\)) was higher than in wild-type LGE injections (82±8, \(N=23\)), we also quantified the proportion of labelled cells that crossed into the cortex. Indeed, in Sey/Sey slices, 46% of all migrating GE cells (n=263, analysed in nine slices) crossed the boundary into the cortex, whereas this was the case for only 28% of wild-type GE cells. This represents a 1.6-fold increase in cell migration from the GE into the cortex in Sey/Sey telencephalic slices. We also took care that the injections were not closer to the boundary in Sey/Sey slices (383±35 \(\mu m\), \(n=14\), Sey/Sey LGE; 364±36 \(\mu m\), \(n=19\), wild-type LGE) or that GE cells in Sey/Sey slices did not migrate faster than in wild type (Table 1B,C). We analysed the latter aspect more closely by quantification of the mean migrational distance for cells migrating within the GE either towards the cortex (279±12 \(\mu m\), \(n=158\)) or in other directions (335±15 \(\mu m\), \(n=144\)). Taken together, these results suggest that GE cells are free to move into the Pax6 mutant cortex. In wild type, cells from the GE were restricted to some extent in their migration into the cortex. This is evident from the continuous distribution of cells in the boundary region of the Pax6 mutant, compared to the prominent trough observed in wild-type slices (Fig. 5B/C). Hence, these data demonstrate that Pax6 is required to restrict the migration of cells from the GE into the cortex.

**Tangential migration in vivo**

It has previously been shown that the cells migrating from the GE into the cortex contain Dlx, GAD 67, GABA, calbindin and calretinin (DeDiego et al., 1994; Anderson et al., 1997; Lavdas et al., 1999; Sussel et al., 1999). This prompts the prediction that the enhanced ventro-dorsal cell migration in Sey mice should be reflected by an increase of GABA-immunoreactive cells in the cortex. Indeed, the lateral cortex of Pax6 mutants contains a dense accumulation of calbindin-positive cells (Figs 6, 7). A quantification of GABA-, calbindin- and calretinin-immunoreactive cells revealed that about twice as many immunoreactive cells were located in the lateral cortex of Pax6 mutants as compared to wild-type littermates (Fig. 7). This increase is very comparable to the value obtained in our migrational analysis in slice preparations. Interestingly, we also noted the complete absence of calretinin- and calbindin-positive fibers in the intermediate zone of the cortex in the Pax6 mutant (Fig. 6C,F). This is most likely due to the lack of thalamocortical projections in the Pax6 mutant (Kawano et al., 1999).

Since GABAergic cells are also generated in the cortex, the increase of GABAergic cells in Pax6 mutant mice could also be explained by a misspecification of cortical neurons. Therefore, we quantified the number of GABAergic cells in the cortical plate of the dorsal cortex, i.e. a different cortical region of the same section (Figs 6, 7). Since similar numbers of GABA/-calbindin- and calretinin-positive cells were found in the cortical plate of the dorsal cortex of wild-type and Sey/Sey mice at E15, their specification does not seem to be impaired in the Pax6 mutant. These results suggest that the increase of GABAergic cells in the lateral cortex of Sey/Sey mice is due to an enhanced migration from the GE.

When we analysed sections at later stages, at E17, we still observed the higher number of calbindin-positive cells in the lateral cortex of Sey/Sey mice as compared to wild type (2.2×; Fig. 7). Interestingly, the number of calbindin-positive cells at this stage was also increased in the cortical plate of the dorsal cortex in Sey/Sey mice, although to a lesser extent (1.4×). Thus, the increase of immunoreactive cells in Pax6 mutant mice seems to spread from the lateral to the dorsal cortex. These
findings suggest that the ongoing cell migration from the LGE into the cortex in Pax6 mutant mice leads to an ever-increasing number of GABAergic cells in the cortex.

**DISCUSSION**

**Asymmetric cell migration between the GE and the cortex**

The migration analysis performed in this study demonstrated a different behaviour of cells on both sides of the corticostriatal boundary. While many cells labelled in the LGE invaded the adjacent cortex within the 2-day observation period, few (if any) cortical cells migrated into the adjacent LGE. Moreover, cortical cells only migrated for a short distance into the LGE, whereas GE cells continued to migrate in the cortex for several hundred μm. Since both neurons and precursor cells were labelled on either side of the boundary, this study now reveals a true asymmetry of this boundary, in
that cells can cross from ventral to dorsal, but not in the opposite direction.

Even though ventrally derived cells cross the boundary into the cortex, we show here that in wild-type animals there is a migrational barrier for GE cells invading the cortex. A trough in the number of cells migrating as far as the boundary indicates that some cells from the GE stop at the cortico-striatal boundary. Moreover, the cell migration from the GE into the cortex is increased in the Pax6 mutant, further supporting some degree of restriction under normal conditions. Thus, the migration of both ventral and dorsal cells is restricted at the cortico-striatal boundary, however, in a disproportionate manner.

The lack of Pax6-function leads to the loss of restricted migration of ventral cells but does not affect the restriction of cortical cells within dorsal territories. Thus, the asymmetry of ventro-dorsal cell migration is maintained, showing that distinct mechanisms restrict cells in the ventral and dorsal parts of the developing telencephalon. Before discussing the nature of these mechanisms, we turn first to the nature of cells migrating from the GE into the cortex.

Cells that enter the cortex from ventral positions express Dlx, Lhx6, GAD67, GABA, calbindin and calretinin (DeDiego et al., 1994; Anderson et al., 1997; Lavdas et al., 1999; Meyer et al., 1998; Sussel et al., 1999). Some of these cells originate in the MGE and migrate through the entire telencephalon from its most ventral parts into dorsal regions (Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999). Interestingly, MGE cells expressing Nkx2.1 seem to migrate dorsally into the LGE, but then not to enter the cortex (Sussel et al., 1999). In contrast, MGE cells expressing Lhx6 apparently spread far into the cortex (Lavdas et al., 1999; Sussel et al., 1999). Whether or not cells originating in the LGE also migrate into the cortex is not clear. In our migration analysis we cannot discriminate whether we label cells originating in the LGE or MGE.

The analysis of mice deficient in different transcription factors suggests some degree of cell diversity in the ventro-dorsal cell migration. The deletions of Dlx1/2, Nkx2.1 and Mash1 affect distinct populations of cells migrating into the cortex (Anderson et al., 1997; Sussel et al., 1999; Casarosa et al., 1999). The absence of Nkx2.1, a transcription factor expressed in the MGE, leads to an almost complete absence of calbindin-positive cells in the cortex around birth, suggesting that these cells fail to migrate from the MGE (Sussel et al., 1999). However, 50% of GAD67-positive cells and the calretinin-positive cells in the marginal zone of the cortex are unaffected by the absence of Nkx2.1. The mutation of Dlx1/2 genes that are expressed in the LGE and MGE causes a loss of most GAD67-positive cells in the cortex (Anderson et al., 1997). The loss of the basic-helix-loop-helix transcription factor Mash1, expressed in the LGE and MGE, mostly affects GAD67-positive cells in the marginal zone of the cortex (Casarosa et al., 1999). These intriguing observations allow two important conclusions to be drawn. First, ventral transcription factors are required for the migration of ventral cells into dorsal territories. This could most easily be explained by a lack of differentiation of ventral cell types in the absence of the respective transcription factors. Second, distinct cell populations migrate from the GE into the cortex and require distinct transcription factors.

Interestingly, the migration of all of these populations is enhanced by the absence of functional Pax6 in the cortex. This mutation causes a similar increase in the number of GABA-, calbindin- and calretinin-positive cells. This increase is observed first in the lateral then the dorsal cortex. Moreover, the number of calretinin-positive cells in layer 1 of the Pax6 mutant cortex is double that in wild-type cortex (M. G. and A. Stoykova, unpublished observations). Since these cells also migrate from ventral positions (Lavdas et al., 1999; Meyer et al., 1998), their increase in the Pax6 mutant is also consistent with an enhanced ventro-dorsal cell migration. Hence, these data suggest that many, if not all cells that encounter the Pax6 mutant cortex can now enter it. Indeed, this is consistent with our migration analysis in telencephalic slices. The distribution of all labelled GE cells that reach the boundary in the Pax6 mutant shows no indication of any migrational inhibition, in contrast to the data obtained in wild type. Thus, the way into the cortex seems to be completely open in the absence of Pax6.

Mechanisms restricting cell migration in the telencephalon

Which mechanisms could then be responsible for the migrational restriction of cells from the GE? Since the migration of GE cells into the cortex is free in the absence of functional Pax6, the cues that restrict GE cells should be lost in this mutant. We have previously detected several changes in the Pax6 mutant with regard to the delineation between the cortex and GE that are schematically depicted in Fig. 8 (Stoykova et al., 1996, 1997). The radial glia fascicle at the cortico-striatal boundary region and also the cortex-specific expression of R-cadherin (Matsunami and Takeichi, 1995) are...
both absent in the Pax6 mutant *Sey*. Thus, the absence of the mechanical obstacle formed by the radial glia fascicle may ease migration from the GE. Alternatively, the loss of R-cadherin on cortical cells may permit the intermingling of GE cells with cortical cells that is less favoured in the wild type (Götz et al., 1996; Stoykova et al., 1997). Needless to say, other as yet uncharacterized molecules may well contribute to the specific migrational restriction of GE cells. The Pax6 mutant should serve as an excellent model for investigating which molecules co-operate at the cortico-striatal boundary to restrict the migration of ventral cells.

Perhaps one of the most interesting findings in this study is that the Pax6 mutation affects the migration of GE, but not cortical cells, across the cortico-striatal boundary. Thus, the changes in R-cadherin and the boundary region in the Pax6 mutant have no effect on the migration of cortical cells that remain confined in their cortical territory. This is reminiscent of the observation that restricted cell migration between adjacent rhombomeres is also maintained in the absence of boundary structures (Guthrie et al., 1993; Nittenberg et al., 1997).

Previous studies by Neyt et al. (1997) have proposed a short-range inhibitory action of cues in the boundary region on migrating cortical cells. Time-lapse video microscopy showed that cortical cells slow down and eventually stop migrating when they approach the boundary. Our observation that the few cortical cells that enter or cross the boundary migrate for much shorter distances than those outside this region corroborates the notion of an inhibitory or repulsive function of this region in wild-type mice. Since we observed the same migrational behaviour in Pax6 mutant mice, it is possible that these inhibitory molecules are still present in the boundary region. This would, however, be relatively surprising, since to date all molecules specific for the boundary region were found to be absent in the Pax6 mutant *Small Eye* (Stoykova et al., 1996, 1997; own unpublished results). Beyond the boundary region, molecules in the GE may be responsible for the maintained restriction of cortical cells. Indeed, the results obtained in the Pax6 mutants are consistent with a crucial role of the adjacent region in restricting cell migration. The recent evidence that slit is a repellent for migrating neurons suggests it is a good candidate for such a role (Zhu et al., 1999).

In this regard, transcription factors in the developing striatum are of interest as potential regulators of molecules that restrict cortical cells from invading this region. It would therefore be interesting to analyse the cortical migration of mice deficient in transcription factors that are specifically expressed in the GE, such as Dlx1/2 and Mash1 (Anderson et al., 1997; Casarosa et al., 1999). If the adjacent ventral territory is instrumental in mediating migrational restriction, cortical cells could then be free to spread into the GE in some of these mutants.

**Dorso-ventral patterning in the telencephalon**

The importance of transcription factors expressed specifically in dorsal or ventral regions of the CNS has been confirmed by mutant analysis of these genes. The loss of the ventral transcription factor Nkx2.1 causes the spread of further dorsally restricted transcription factors into ventral territories (Sussel et al., 1999). Conversely, the mutation of Pax6 that is mostly expressed in the dorsal part of the telencephalon leads to the spread of some transcription factors from ventral to dorsal positions (Stoykova et al., 1997). These data suggest two possible and by no means mutually exclusive mechanisms for dorso-ventral patterning. First, the loss of Pax6 in cortical precursor cells may lead to the upregulation of Dlx in cortical cells and thereby instruct a ventral phenotype. If this were the case we might even suspect that cortical cells then intermingle with cells in the GE, with which they would share at least some phenotypic aspects. Second, Dlx-positive cells in the Pax6 mutant cortex might originate at ventral positions and now invade a formerly restrictive territory. In this case, ventral to dorsal migration would be enhanced, but not vice versa. Our experiments provide strong evidence for the latter scenario. Taken together, these results therefore indicate an important contribution of migrational restriction to dorso-ventral patterning in the telencephalon.

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