Dlx2 Progenitor Migration in Wild Type and *Nkx2.1* Mutant Telencephalon

Susana Nery, Joshua G. Corbin and Gord Fishell

Developmental Genetics Program and the Department of Cell Biology, The Skirball Institute of Biomolecular Medicine, New York University Medical Center, 540 First Avenue, New York, NY 10016, USA

The transcription factor Dlx2 is expressed widely throughout the ventral telencephalon. We have examined the *in vitro* and *in vivo* migration of Dlx*2* progenitors originating from the different ganglionic eminences of both wild type and *Nkx2.1* mutant animals. By examining the expression of *tauLacZ* targeted into the *Dlx2* locus we were able to visualize the distribution of cells expressing this gene at both embryonic and postnatal stages. This analysis suggested that Dlx*2*-expressing cells traverse a number of characteristic migratory routes to populate both cortical and subcortical regions. We also examined how these patterns of migration were affected in *Nkx2.1* mutant animals. In these mutants, the early but not late populations of Dlx*2*-expressing cells originating in the ventral telencephalon that migrate to the cortex are lost. This recovery may be, at least in part, a result of the late migration of Dlx2 progenitors from the caudal ganglionic eminences (CGE), which, based on our previous work, does not appear to require *Nkx2.1* gene function.

Introduction

The *Dlx* homeobox genes are required for the differentiation of the basal ganglia and craniofacial morphogenesis (Qiu *et al*., 1995; Anderson *et al*., 1997a,b). There are six *Dlx* genes in the mouse that are expressed in several tissues including the nervous system (Liu *et al*., 1997). *Dlx1*, *2*, *5* and *6* are expressed in the forebrain, including the telencephalon (Liu *et al*., 1997). Within the developing basal telencephalon, *Dlx* gene expression is found in numerous structures, including the medial, lateral and caudal ganglionic eminences (MGE, LGE and CGE, respectively), as well as the septal region (Eisenstat *et al*., 1999; Nery *et al*., 2002). Although *Dlx1*, *2*, *5* and *6* are expressed in the same telencephalic structures, they are expressed at different differentiation stages (Liu *et al*., 1997; Eisenstat *et al*., 1999). *Dlx1* and *2* gene expression commences as progenitors transit from the ventricular zone (VZ) into the subventricular zone (SVZ) and is extinguished as cells differentiate, while *Dlx5* and *6* are expressed in more mature cells (Eisenstat *et al*., 1999).

Numerous lines of evidence have established that Dlx2-expressing cells or their progeny are highly migratory and disperse tangentially to populate the cerebral cortex, olfactory bulb and hippocampus, where they differentiate into interneurons and perhaps other neural populations [reviewed in (Corbin *et al*., 2001; Marin and Rubenstein, 2001)]. Direct assessment of the requirement for *Dlx* genes in progenitors has been done through the examination of loss of function mutants. Single mutants of *Dlx*1, *Dlx*2 or *Dlx*5 have almost no forebrain defects (Qiu *et al*., 1995, 1997), however, both *Dlx1/2* and *Dlx5/6* mutants have marked abnormalities (Anderson *et al*., 1997b; Robledo *et al*., 2002). In *Dlx1/2* compound mutants the development of the large population of basal telencephalic cells that undergo long-range tangential migration throughout dorsoventral extent of the telencephalon is arrested (Anderson *et al*., 1997b). Specifically, *Dlx1/2* double mutants exhibit an 80% reduction in cortical interneurons (Anderson *et al*., 1997a). These animals die perinatally, confounding the analysis of the ultimate fate of cells in which *Dlx1/2* is normally expressed. The best assessment of the fate of *Dlx*-expressing cells has come from a recent analysis that used a transgenic approach in which LacZ was expressed directly by a *Dlx5/6* promoter element. This study indicated that the vast majority of LacZ-expressing cells in the cortex adopted a GABAergic interneuronal identity (Stuhmer *et al*., 2002b). Consistent with this observation, recent gain of function experiments have demonstrated that overexpression of *Dlx2* or *Dlx5* can induce telencephalic progenitors to express GAD65 and GAD67, markers indicative of GABAergic interneuronal identity (Stuhmer *et al*., 2002a).

To investigate the migratory patterns of Dlx2-expressing cells and how these patterns of dispersion are affected in *Nkx2.1* null animals, in which the MGE is converted to an LGE fate (Sussel *et al*., 1999), we have created a mouse line in which the *tauLacZ* gene has been inserted into the *Dlx2* locus. In addition to revealing the locations and populations of cells that express Dlx2, the robustness of the βgal protein allows tangential migration to be inferred. The distribution of the βgal protein revealed numerous putative cell migratory pathways, suggesting that streams of tangentially migrating cells originating from the ventral telencephalon are diverse. This analysis revealed streams of cells migrating to the developing cerebral cortex, olfactory bulb and ventral lateral telencephalon. In addition, small numbers of tauLacZ cells were observed in both the cortical gray and white matter as well as the hippocampus in postnatal ages. By crossing this reporter line onto an *Nkx2.1* null background, we were able to show that the distribution of the βgal was dramatically altered at early embryonic times (E12–E14) and specifically affects the streams of Dlx2 cells originating from the MGE or CGE. By contrast, the normal pattern of βgal seen in the *Dlx2–tauLacZ* heterozygotes is largely restored in *Nkx2.1* null mutants by E18.

Materials and Methods

Animal Use and Generation of Nkx2.1/Dlx2–tauLacZ Mice

Swiss Webster (Taconic, Albany, NY) or CD1 (Charles River, Wilmington, MA) mice used in these studies were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York University School of Medicine. The day when the sperm plug was detected was considered E0.5.

Generation of *Dlx2–tauLacZ* mice has been previously described (Corbin *et al*., 2000; Nery *et al*., 2002). As with previous *Dlx2* null alleles (Qui *et al*., 1995), no phenotype is seen when these mice are maintained as heterozygotes. When bred to homozygosity the phenotype of *Dlx2–tauLacZ* mice appeared indistinguishable from that seen in homozygotes of a previously published *Dlx2* loss of function allele (Qui *et al*., 1995). For genotyping, either whole embryo bodies or, for postnatal animals, a toe clip was subjected to X-gal staining as previously described

(Corbin *et al*., 2000). *Nkx2.1* animals were a kind gift of S. Kimura (Kimura *et al*., 1996). PCR was used to genotype *Nkx2.1* heterozygous and homozygous mice as described in Nery *et al*. (Nery *et al*., 2001). To generate *Nkx2.1/Dlx2–tauLacZ* lines, *Dlx2–tauLacZ* and *Nkx2.1* heterozygotes were intercrossed. Double heterozygous animals were then crossed to *Nkx2.1* heterozygotes and embryos genotyped as described above.

Tissue Preparation and Histology

For section *in situ* hybridization, immunofluorescence or X-gal staining, heads of *Dlx2–tauLacZ* or *Nkx2.1/Dlx2–tauLacZ* embryos younger than E15.5 were fixed by immersion in 4% paraformaldehyde (PFA) for 2–3 h at 4°C, and older embryos, P23 and adult animals were transcardially perfused with 4% PFA and their brains were postfixed for 3–4 h or overnight at 4°C. Samples were then cryoprotected in 30% sucrose in PBS, embedded in Histoprep (Fisher Scientific, Pittsburgh, PA), frozen and sectioned serially at 20–50 μm using a cryostat.

In Situ *Hybridization and X-Gal Staining*

Section *in situ* hybridizations were carried out as described previously (Schaeren-Wiemers and Gerfin-Moser, 1993; Wilkinson and Nieto, 1993) using non-radioactive *Dlx2* DIG-labeled probes. X-gal staining was performed as previously described (Corbin *et al*., 2000).

Tissue Dissection and Culture Conditions for Matrigel Explants

Ventricular zone (VZ) and subventricular zone (SVZ) from MGE, LGE, CGE and dorsolateral cortex were dissected from E13.5 embryos, as described by Wichterle *et al*. (Wichterle *et al*., 1999). Explants were embedded in matrigel (Becton Dickinson, Bedford, MA) and cultured for 4 and 9 days in Neurobasal medium (Gibco, Gaithersburg, MD) supplemented with Pen/Strep (Gibco, 1:100), B27 supplement (Gibco, 1:50) and glutamine (Gibco, 1:100). In long-term cultures, the medium was changed every 3 days. Explants were then X-gal stained.

Results

Dlx2-expressing Ventral Telencephalic Cells Migrate to the Cortex and Hippocampus

In animals where the *tauLacZ* reporter is targeted to the *Dlx2* locus, the pattern of LacZ expression strongly resembles that seen by *Dlx2 in situ* hybridization (Fig. 1*A*,*B*; Corbin *et al*., 2000; Nery *et al*., 2002) and is indistinguishable from the pattern of Dlx2 immunoreactivity (Porteus *et al*., 1994) (J. Kohtz and G. Fishell, unpublished observations). Having examined a *Dlx2–tauLacZ* heterozygous allele in numerous contexts, all evidence suggests that tauLacZ expression in these animals reflects the normal population of Dlx2-expressing cells. Hence for simplicity, cells expressing Dlx2 and cells expressing a tauLacZ reporter, which has been targeted to the *Dlx2* locus, will be referred to interchangeably. For the purpose of this study we focused on the expression in the ganglionic eminences and the cortex.

At E12.5, Dlx2-expressing cells visualized as a result of the expression of the tauLacZ reporter were observed in the MGE, LGE and CGE but none were detected in the cortex (Fig. 1*B–D*). Examination of the LacZ expression pattern in these animals revealed that the migration of Dlx2 cells to the cortex begins around E13.5 (data not shown). In addition, at E14.5, a stream of Dlx2-expressing cells was observed to emanate from both the MGE (Fig. 1*E*,*F*) and CGE [Fig. 1*G*,*H* and Nery *et al*. (Nery *et al*., 2002)]. These streams appeared to consist of cells migrating to the cortex and hippocampus via the SVZ (Fig. 1*E–H*). As development proceeds, more cells are seen in the cortical SVZ, while in the ventral regions of the telencephalon Dlx2-expressing cells are largely restricted to the VZ and SVZ. At E15.5, Dlx2-expressing cells in the cortical VZ (Fig. 2*H*) were also detected. At E16.5 more cells were seen in the cortex within

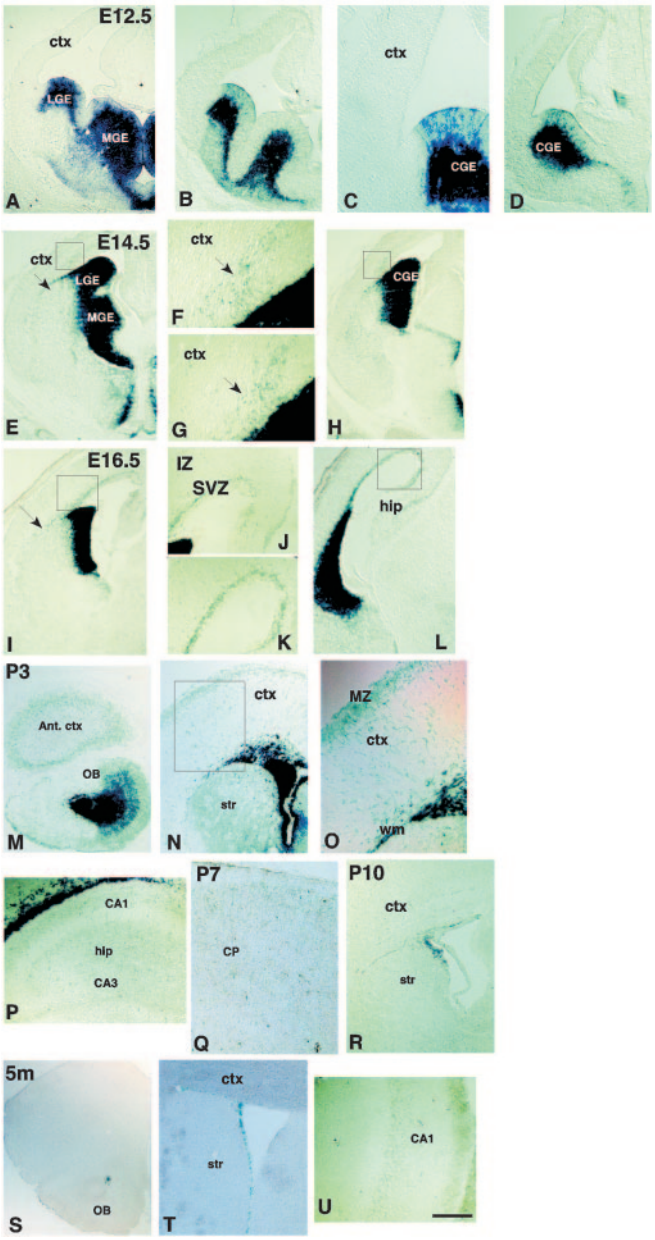

Figure 1. Expression of *Dlx2*-LacZ within the telencephalon: *in situ* hybridization and X-Gal stained coronal sections at different embryonic and postnatal ages. At E12.5, Dlx2 is expressed in the ventral telencephalon and was visualized either by *in situ* hybridization (*A*) or X-gal staining (*B–D*). At this age, no Dlx2 cells were observed migrating to the cortex. At E14.5 (*E–H*), streams of cells are seen emanating from the basal ganglia into the cortex (arrowheads) and toward the ventrolateral telencephalon (arrow in *E*). *F* and *G* are high power views of the boxed areas shown in *E* and *H*, respectively. At E16.5 (*I–L*) and P3 (*M–P*) more cells are observed directed towards and within the cortical SVZ, hippocampus, ventrolateral telencephalon (arrow in *I*), as well as in the MZ and white matter. *J* and *K* are high power views of the boxed areas shown in *I* and *L*, respectively. Cells are also seen in the olfactory bulb (*M*). *O* shows a high power view of the boxed area shown in *N*. At P7 (*Q*) Dlx2-expressing cells are seen in the cortical plate but not in the MZ. From P10 (*R*) and onwards through to adulthood (5 months *S–T*), Dlx2-expressing cells are only detected in the SVZ, white matter and RMS. Some weakly expressing Dlx2 cells are also detected in the hippocampus at both P3 (*P*) and in the adult (*U*), this is especially true in the CA1 region. Ant. Ctx, anterior cortex; CGE, caudal ganglionic eminence; CP, cortical plate; Ctx, cortex; Hip, hippocampus; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; MZ, marginal zone; OB, olfactory bulb; Str, striatum; SVZ, subventricular zone; WM, white matter. Scale bar in *U* represents 300 μm in *A*, *B*, *D*, *E*, *I*, *M*, *R* and *S*; 200 μm in *H* and *L*; 150 μm in *N*, *P*, *Q*, *T* and *U*; 100 μm in *C*, *F*, *G*, *J*, *K* and *O*.

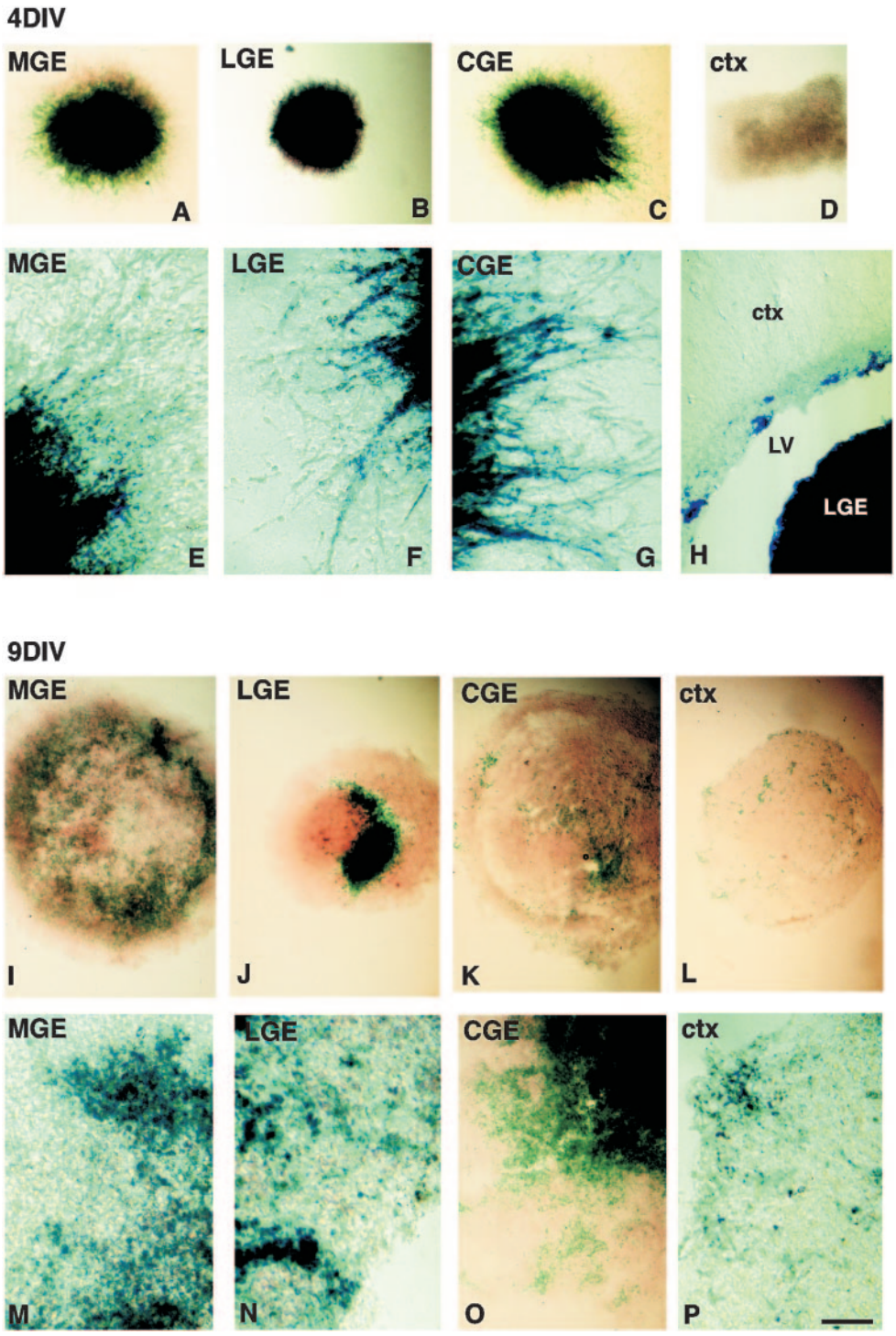

Figure 2. Dlx2-expressing cells are highly migratory at E13.5 *in vitro* and are a subpopulation of migrating cells. (*A–G*) *In vitro* explants cultured in matrigel for 4 days reveals cells from the MGE (*A*, *E*) and CGE (*C*, *G*) migrate extensively out of explants. In contrast, LGE (*B*, *F*) explants exhibit less migration, and cortical cells do not migrate away from the explant (*D*). X-gal staining of the explants reveals that Dlx2-expressing cells are migratory *in vitro* and represent a subpopulation of the cells that have migratory ability. After 9 days, the core of the MGE (*I*, *M*), LGE (*J*, *N*) and CGE (*K*, *O*) explants downregulated Dlx2 expression. On the other hand, cortical (*L*, *P*) explants contain βgal positive cells after long term culture. After prolonged X-gal histochemical staining, Dlx2 positive cells are also observed in the cortical ventricular zone as shown in an E15.5 coronal section (*H*). LV, lateral ventricle. Scale bar in *P* represents 300 μm in *A–D*; 200 μm in *I–L*; 150 μm in *E–H*; 100 μm in *M–N*.

three locations, the SVZ, scattered in the cortical plate and in the MZ (Fig. 1*I–L*). In addition, Dlx2 positive cells appeared to be migrating along the lateral migratory stream to the ventro-lateral telencephalon (Fig. 1*E*, *I*). At E18.5 the number of cortical cells expressing Dlx2 seems to be higher than at E16.5, and there was a great number of βgal cells in the cortical white matter (data not shown).

Postnatally, Dlx2-expressing cells were detected in the

cortical plate and cortical white matter (Fig. 1*N*,*O*). In addition, Dlx2-expressing cells were observed in the SVZ of the ventral telencephalon at early postnatal days (Fig. 1*N*). At P3, as at E18.5, many cells were seen in the cortical MZ, and in the cortical plate and white matter (Fig. 1*N*,*O*). Cells were also detected in the hippocampus (Fig. 1*P*). Ventrally there was a high level of expression in the SVZ and lower levels in the mantle and also in the olfactory bulb (Fig. 1*M*,*N*). By P7 there was a significant decrease in the number of Dlx2-expressing cells both in ventral and dorsal regions. Dorsally, positive cells were no longer detected in the MZ although they were still present in the cortical plate and white matter (Fig. 1*Q* and data not shown). Ventrally, βgal positive cells were seen in the SVZ but few were observed in the mantle of the basal ganglia (data not shown). At P10 the expression of Dlx2 was confined to cells in the SVZ, the white matter and the rostral migratory stream. There were no Dlx2-expressing cells in the cortical plate (Fig. 1*R* and data not shown). In the adult the few remaining Dlx2-expressing cells were mainly restricted to the SVZ (Fig. 1*T*). While the number of Dlx2-expressing cells decreases in the young and in the mature adult, Dlx2 positive cells were seen in similar locations to that seen in P10 animals (Fig. 1*S*,*T*). Interestingly cells expressing low levels of βgal were detected in the hippocampus mainly concentrated in the CA1 region (Fig. 1*U*).

Our results support previous studies that suggest that Dlx2 cells give rise to cells that migrate to the cortex. Furthermore, the sensitivity of LacZ histochemistry allowed for a better visualization of the position of Dlx2-expressing cells. In general, Dlx2 is highly expressed in regions where tangential migration has been shown to occur but is downregulated in regions such as the cortical mantle. These results support the notion that this gene is downregulated in these cells as they differentiate.

Dlx2-expressing Cells Are Highly Migratory In Vitro

The streams of βgal positive cells in the cortical SVZ support the previously suggested idea that these cells tangentially migrate to the cortex. Indeed, numerous studies have demonstrated that a substantial portion of cortical interneurons appear to be derived from the Dlx2-expressing population (Anderson *et al*., 1997b, 1999). To further investigate the migratory ability of these cells, we have taken explants from animals heterozygous for the *Dlx2–tauLacZ* transgene and cultured them in an *in vitro* matrigel system. MGE, LGE, CGE and cortical explants were dissected from E13.5 *Dlx2–tauLacZ* heterozygotes and cultured for 1–9 days *in vitro* (DIV).

At 4 and 9 DIV, explants were stained for βgal. At 4 DIV, the core of the MGE, LGE and CGE explants is Dlx2 positive, while the cortical explants do not express Dlx2 (Fig. 2*A–D*). As described previously, MGE (Wichterle *et al*., 1999; Nery *et al*., 2001, 2002) and CGE (Nery *et al*., 2001, 2002) cells migrated extensively out of the explant while LGE cells showed minimal migration. Notably, a subpopulation of migratory cells was found to be Dlx2 positive (Fig. 2*E–G*). After 9 days in culture, cells in MGE, LGE and CGE explants started to downregulate Dlx2, suggesting that the cells were differentiating (Fig. 2*I–K* and *M–O*). On the other hand, at 9 DIV some Dlx2 cells were detected in the cortical explants suggesting that not all Dlx2-expressing cells seen dorsally originate in ventral regions (Fig. 2*L*,*P*). This result is in agreement with the existence of Dlx2-expressing cells in the cortical VZ at E15.5 (Fig. 2*H*) that are probably born in the cortex. Notably, this population does not express high levels of Dlx2, as tauLacZ histochemistry is only observed after prolonged staining. Despite this, the staining is cellular and does not appear to be background. As suggested by recent work in humans (Letinic *et al*., 2002), these cells may comprise a population of Dlx2-expressing interneurons distinct from those that reach the cortex via the SVZ from the GE. However, one cannot rule out that the Dlx2 cells observed in the cortical explants are cells that ectopically induced expression of this gene after long periods of *in vitro* culturing. In summary, our results show that Dlx2-expressing cells are highly migratory *in vitro* and comprise a subpopulation of the cells that tangentially migrate from the ventral telencephalon to the cortex.

Dlx2-expressing Cells Migrate to the Cortex via the SVZ

Lhx6-expressing cells are also considered to be a source of tangential migrating cells from the basal ganglia to the hippocampus (Lavdas *et al*., 1999). Immunostainings of *Dlx2–tauLacZ* transgenics at E16.5 for βgal and Lhx6 showed that in ventral regions these two populations partially overlap (Fig. 3*A*). However, while Dlx2 is expressed in the VZ and SVZ, Lhx6 is expressed only within the MGE and is restricted to the SVZ and mantle (Porteus *et al*., 1992; Grigoriou *et al*., 1998). In the cortex and hippocampus, cells that potentially migrated from ventral regions expressed either Dlx2 or Lhx6 but not both, suggesting that these may be distinct tangentially migrating populations (Fig. 3*B* and data not shown). In addition, the distribution of Dlx2 and Lhx6 positive cells is quite different. While Dlx2 cells are found mainly in a tight stream within the SVZ, Lhx6 cells are seen in a wider distribution that extends throughout the cortical plate. This is also supported by the previous results *in vitro*, where only a subpopulation of cells migrating out of the explants were found to be Dlx2 positive.

Dlx2-expressing cells migrating to the cortex were first observed in the SVZ. Immunofluorescence analysis at E16.5 and E18.5 showed that βgal-expressing cells do not overlap with neurofilament-145 expressing cortico-fugal fibers (NF) (Fig. 3*C*), in accordance with recent findings suggesting that tangentially migrating cells in rodents and humans also travel in the SVZ, but not the intermediate zone (Wichterle *et al*., 2001; Letinic *et al*., 2002; Nery *et al*., 2002). Later in development, around E16.5, a stream of Dlx2-expressing cells was also detected in the MZ. These cells did not overlap with the Cajal–Retzius marker CR50 (Fig. 3*D*), suggesting that Dlx2 cells do not give rise to this cell population.

We also addressed the question of whether Dlx2 cells divide while migrating. Our analysis with anti-phospho-histone 3 (PH3) and Ki67 antibodies showed that cells migrating to the cortex were only rarely mitotically active. By contrast, some Dlx2-expressing cells in the rostral migratory stream expressed mitotic markers (Fig. 3*E–G*).

Dlx2-expressing Cells do not Express Markers of Differentiated Interneurons or Oligodendrocytes

Analysis of the *Dlx1/2* KO revealed that 20% of cortical GABA-expressing cells persist in these mutants (Anderson *et al*., 1997a). Recently, it has been suggested that Dlx2-expressing cells also give rise to oligodendrocytes (He *et al*., 2001). Together these results suggest that the populations of cells that migrate to the cerebral cortex are heterogeneous. However, since expression of Dlx2 is extinguished prior to the terminal differentiation of cells expressing this marker, definitive proof of the fate of these cells has been lacking. Given the robustness of the LacZ expression in cells expressing Dlx2, we hoped to address this issue through double immunostainings at different embryonic and postnatal ages for βgal and interneuronal (GABA, GAD67 and Calbindin) (Fig. 4*A–E*) or oligodendrocytic (O1,

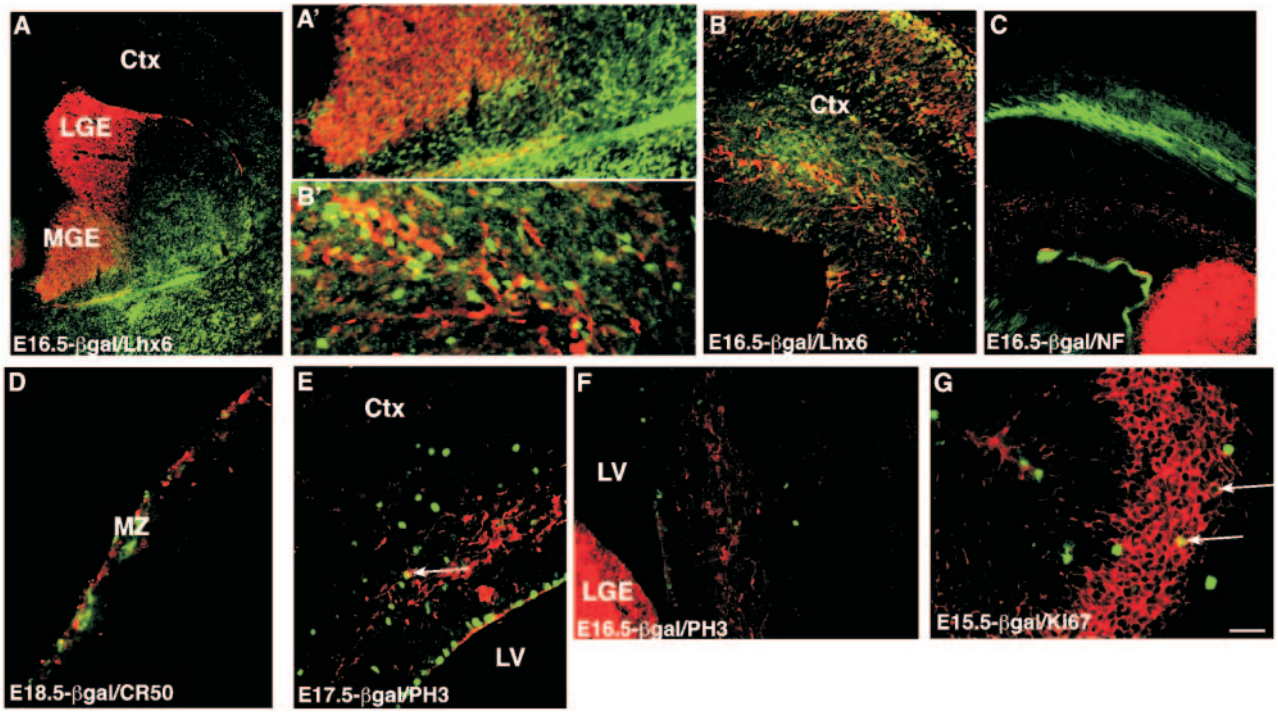

Figure 3. Dlx2-expressing cells are a subpopulation of the cells that migrate to the cortex. Immunostaining of coronal sections at E16.5 reveals that Lhx6 (green) and Dlx2 (red) expressing cells do not overlap in regions outside of the MGE (*A*, *B*, boxed areas in *A*, *B* are shown at higher power in *A′* and *B′*). At E16.5, Dlx2-expressing cells (red) do not migrate via the intermediate zone, as visualized by neurofilament-145 antibody staining (green) (*C*). At E18.5, cells in the cortical MZ do not co-express βgal (red) and CR50 (green) (*D*). At E17.5, Dlx2-expressing cells are not mitotically active as they do not overlap with PH3 (green) (*E*, *F*). In contrast, a few Dlx2 positive cells (red) in the termination of the RMS in the olfactory bulb are dividing at E15.5, as indicated by double labeling with Ki67 (green) (*G*). Scale bar in *G* represents 300 μm in *A*; 150 μm in *B*, *C* and *F*; 100 μm in *D* and *E*; 50 μm in *G*.

MBP) (Fig. 4*F–H*) markers. We found a close association between cells expressing Dlx2 and these cell type specific markers in the cortex and white matter (Fig. 4*A–H*). In general, however, we were not able to observe double labeling of these markers and Dlx2 expression, as the residual LacZ staining is lost prior to the expression of differentiation markers. The only exception was the detection in the piriform cortex of Dlx2-expressing cells that were also positive for calbindin (Fig. 4*D*).

Dlx2–tauLacZ Reporter Expression Reveals Migration Defects in Nkx2.1 Mutants

It is known that interneurons arise in the ventral forebrain and tangentially migrate to the cortex [reviewed in (Corbin *et al.*, 2001; Marin and Rubenstein, 2001)]. As evidenced by the 40% loss of interneurons in *Nkx2.1* mutant animals, the MGE is a major source for these cells (Sussel *et al.*, 1999).

To further study migration defects at different stages in development in the *Nkx2.1* mutant, we used an *in vivo* approach by crossing *Nkx2.1*$^{-/+}$ and *Nkx2.1*$^{-/+}$*; Dlx2–tauLacZ*$^{-/+}$ mice, to produce *Nkx2.1* null mice carrying the *Dlx2–tauLacZ* marker. We compared these animals to wild type controls at E13.5 and E15.5. Although there is a loss of the Dlx2 positive stream in the *Nkx2.1* mutants at E13.5 (Fig. 5*A–D*), this population of cells has partially recovered by E15.5 (Fig. 5*E–H*). In accordance with this late recovery in tangential migration, we detected cells in the cortical SVZ and cortical plate of E18.5 mutant animals (Fig. 5*I–L*). These findings could either represent a recovery late in development of the defects caused by the loss of *Nkx2.1* gene function or that the Dlx2 population seen during late development arises from ventral regions that do not require *Nkx2.1* for their normal development. Regardless of which, these results are also consistent with the idea that there may be compensation in the early defect on oligodendrocyte generation previously reported (Nery *et al.*, 2001). Unfortunately due to perinatal lethality the question of whether normal numbers of oligodendrocytes or interneurons are generated in *Nkx2.1* mutants is presently unclear.

Discussion

The *Dlx* family of genes has long been recognized as key mediators of various aspects of development within the limb, face and brain (Qiu *et al.*, 1995; Anderson *et al.*, 1997b; Thomas *et al.*, 1997; Robledo *et al.*, 2002). Although loss of function analysis of *Dlx1/2* compound mutants (Anderson *et al.*, 1997a,b) has previously revealed that Dlx1 and 2 have an overlapping role in the maturation of cells in the brain, a number of outstanding questions concerning the populations of cells that express Dlx2 have remained. In this study we have explored the migration of populations of Dlx2-expressing cells in various regions within the telencephalon in both wild type and *Nkx2.1* mutant animals. Using a transgenic allele in which the reporter gene *tauLacZ* has been knocked into the *Dlx2* locus, we found evidence in support of previous data suggesting that these cells arise from the MGE and CGE [reviewed in (Corbin *et al.*, 2001; Marin and Rubenstein, 2001); see also (Nery *et al.*, 2002)]. Furthermore, we showed that a population of Dlx2-expressing cells appeared to originate directly within the cortex. In addition, examination of these *Dlx2–tauLacZ* animals demonstrated that the paths of tangential migration utilized by these cells are diverse. Loss

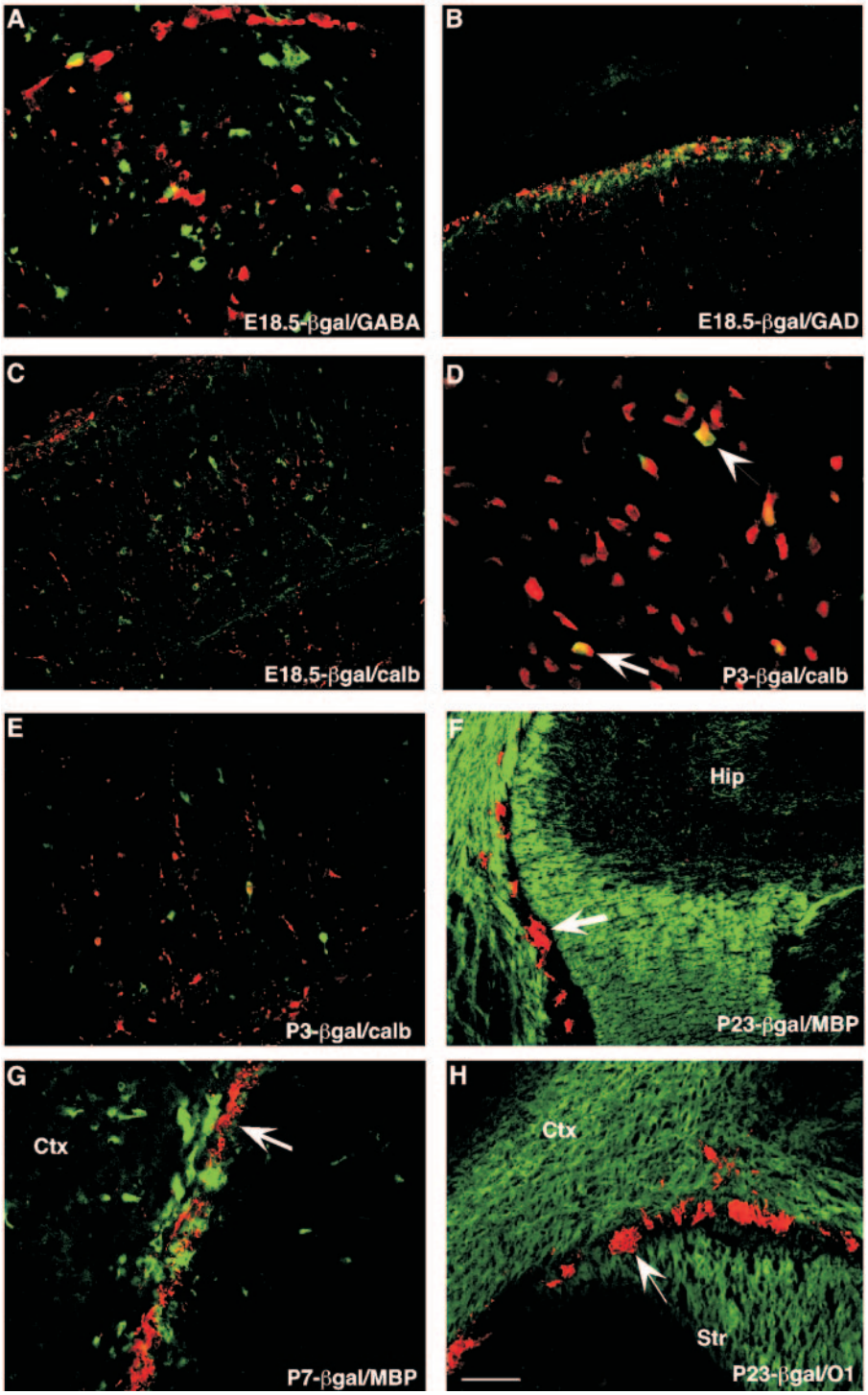

Figure 4. βgal positive cells are in close association with interneurons and oligodendrocytes. Immunostainings of E18.5, P3, P7 and P23 coronal sections from *Dlx2–tauLacZ* KI mice. At E18.5, while we observe Dlx2 positive cells throughout the cortex (red), they do not overlap with the interneuronal markers (green) GABA (*A*), GAD67 (*B*) or calbindin (*C*, *E*). The only exception is the existence of cells that co-express βgal and calbindin in the piriform cortex at P3 (arrowheads) (*D*). Both at P7 and P23 we observe Dlx2 positive cells in the white matter, but double immunostaining for βgal (red) and MBP (green) (*F*, *G*) or O1 (green) (*H*) reveal that, although Dlx2 positive cells (arrowheads) are in close association with oligodendrocytes, the two markers do not overlap. Scale bar in *H* represents 100 μm in *B*, *C*, *F* and *H*; 70 μm in *E*; 50 μm in *A* and *G*; 30 μm in *D*.

of *Nkx2.1* gene function appears to selectively affect a subpopulation of Dlx2-expressing cells. Notably, while migration to the olfactory bulb and within ventral regions of the telencephalon appears relatively normal in *Nkx2.1* mutants, early but not late migration of Dlx2-expressing progenitors is lost in *Nkx2.1* mutants. Together, our results suggest that Dlx2-expressing cells from different regions, while sharing in their ability to undergo long-range tangential migration, vary in

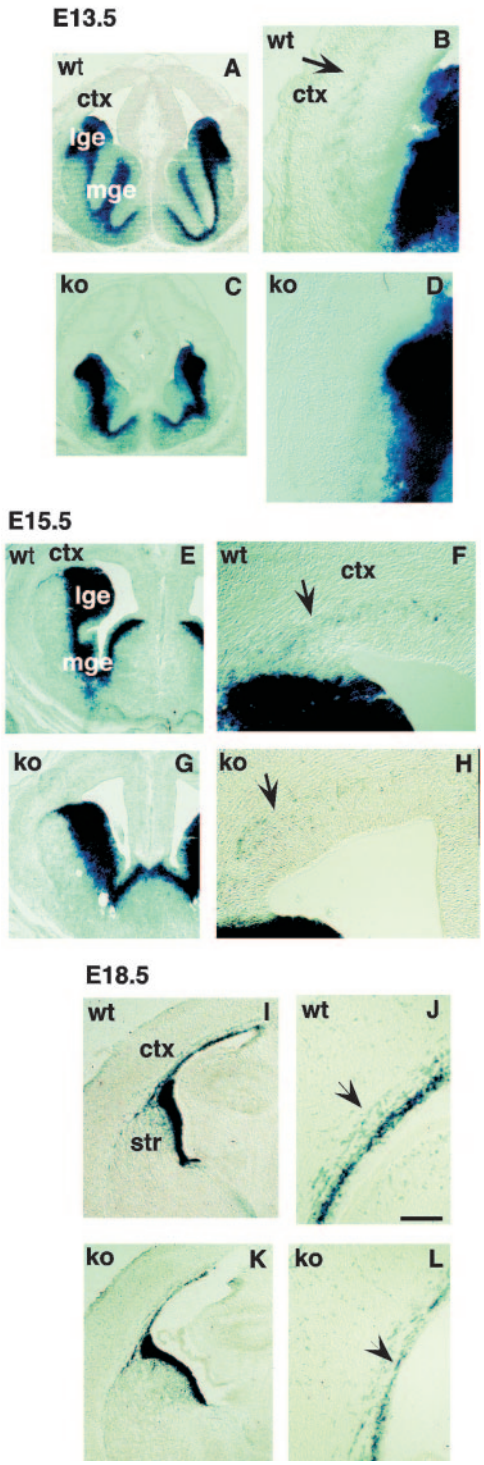

Figure 5. The mutant MGE exhibits early but not late defects in tangentially migrating Dlx2 populations. The early migratory defects in *Nkx2.1* mutant mice appear to recover by perinatal times, as assessed by X-gal staining of E13.5, E15.5 and E18.5 coronal sections of *Dlx-tauLacZ* control and *Nkx2.1* null embryos. Arrowheads show ventral to dorsal migrating streams of Dlx2 positive cells. At E13.5, there is a stream of Dlx2 positive cells that migrating from the ventral telencephalon to the cortex (*A*, *B*). This stream of Dlx2 positive cells is not observed in *Nkx2.1* null embryos at this age (*C*, *D*). At E15.5, Dlx2 positive cells begin to be observed in the cortex of the *Nkx2.1* null animals (*G*, *H*) but in a lower number than control brains (*E*, *F*). By E18.5, cortical Dlx2 staining has a similar distribution in control (*I*, *J*) and *Nkx2.1* null mutants (*K*, *L*), although the absolute numbers of Dlx2 positive cells appears to be diminished in mutant animals. Scale bar in *J* represents 500 μm in *A* and *C*; 300 μm *E* and *G*; 200 μm in *I* and *K*; 100 μm in *B*, *D*, *F*, *H*, *J* and *L*.

their patterns of dispersion and in their dependence on *Nkx2.1* gene function.

The Origins of Dlx2-expressing Cells in the Telencephalon

Within the telencephalon, the pattern of Dlx2 expression changes dramatically as development proceeds (Porteus *et al.*, 1991). Initially Dlx2 is expressed exclusively in ventral regions of the telencephalon. Around E13.5, Dlx2 cells are seen in streams emanating towards the cortex from different regions of the ventral telencephalon. Over the next few days of development, Dlx2 cells accumulate within the cortex, the olfactory bulb and the hippocampus. Numerous lines of evidence suggest that these streams of Dlx2-expressing cells represent populations of neurons undergoing long-range migrations (Valverde *et al.*, 1995, Anderson *et al.*, 1997a). Aside from the pattern of Dlx2 expression, analyses of null alleles in which various regionally expressed transcription factors including *Gsh2*, *Nkx2.1* and the *Dlx* genes themselves have been ablated support this conclusion (Anderson *et al.*, 1997b; Sussel *et al.*, 1999; Corbin *et al.*, 2000; Toresson *et al.*, 2000; Yun *et al.*, 2001). While direct evidence that tangential migration of ventral cells occurs has come from *in vitro* and *in vivo* cell-tracking experiments (Wichterle *et al.*, 2001), the sources of Dlx2 positive cells within the ventral telencephalon was unclear. By using an *in vitro* migration assay we were able to show that a subpopulation of the robust migration emanating from both the CGE and MGE are cells that express Dlx2 [see also (Nery *et al.*, 2002)]. Notably, Dlx2 cells in the LGE show much less tendency to migrate, suggesting that the migratory character of all Dlx2 cells is not equivalent. In addition, we were surprised to discover that cortical explants from *Dlx2–tauLacZ* heterozygotes appear to initiate limited expression of Dlx2 after 9 days culture *in vitro.* Combined with our observation that Dlx2 expression is observed in the deepest regions of the cortical ventricular zone, where tangential migration is excluded, this suggests that not all cortical Dlx2-expressing cells originate in ventral regions of the telencephalon. This is consistent with recent findings by the Rakic laboratory suggesting that in humans a substantial population of cortical interneurons arises within the cortex itself (Letinic *et al.*, 2002). This suggests that there may also be a population of cortically-derived interneurons in non-primates.

In Nkx2.1 Mutants, Dlx2 Cells Are Absent in the Cortex during Early but Not Late Telencephalic Neurogenesis

Fate mapping experiments have suggested that the MGE and CGE are the major sources for cortical interneurons (Wichterle *et al.*, 2001; Nery *et al.*, 2002). In accordance with this idea, previous work has shown that 40% of GABAergic cortical neurons are lost in animals mutant for *Nkx2.1*, a gene expressed throughout the MGE (Sussel *et al.*, 1999) but only the rostral-most CGE (Nery *et al.*, 2002). Notably however, 80% of cortical interneurons are absent in *Dlx1/2* compound mutants (Anderson *et al.*, 1997a,b). One possibility is that cortical interneurons also arise from *Nkx2.1*-negative regions, such as the LGE (Anderson *et al.*, 2001) or CGE (Nery *et al.*, 2002). Alternatively, there may be a late recovery in the ability of *Nkx2.1* regions to generate interneurons. To explore these possibilities, we used the *Dlx2–tauLacZ* transgenic to examine the effect the loss of *Nkx2.1* gene function has on the dorsally migrating population of Dlx2 cells. Although the normal stream of Dlx2 positive cells in the cortical SVZ is absent at E13.5, some recovery of this population is observed at E15.5 and E18.5. At least in part, this late generated population appears to arise from

the CGE, suggesting that *Nkx2.1* is required for early but not late CGE-derived interneurons. Similarly, at late times in development the appearance of a stream of Dlx2-expressing cells at more rostral regions is observed. In this latter case it is impossible to know if this recovery in the ventral to dorsal dispersion of Dlx2 cells results from cells which stem from the LGE (Anderson *et al*., 2001) or a recovery of the MGE in *Nkx2.1* mutants to produce Dlx2-expressing cells during later development (Wichterle *et al*., 2001).

Concluding Remarks

In this paper we have explored the dispersion of Dlx2 cells derived from different regions of the telencephalon in both wild-type and *Nkx2.1* mutant animals. We find evidence to suggest that Dlx2 positive cells arise form a variety of different telencephalic regions, including perhaps the cortex itself. Both the divergent dispersion patterns of migration of Dlx2-positive cells, as well as the selective effect that loss of *Nkx2.1* gene function has on early but not late populations of Dlx2 cells suggest that this population is not homogeneous. A number of fundamental challenges remain with regard to the role of *Dlx* genes within the telencephalon. Foremost of these will be to compare the fate of *Dlx*-expressing cells originating in the different regions of the telencephalon and to investigate the downstream targets of the various members of this family. It will be interesting to determine from this analysis whether *Dlx* gene function is conserved within different populations within the telencephalon, or rather whether the function of this gene family is context dependent.

Notes

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Address correspondence to Gord Fishell, Developmental Genetics Program and the Department of Cell Biology, The Skirball Institute of Biomolecular Medicine, New York University Medical Center, 540 First Avenue, New York, NY 10016, USA. Email: fishell@saturn.med.nyu.edu.

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