Distinct *cis*-Regulatory Elements from the *Dlx1/Dlx2* Locus Mark Different Progenitor Cell Populations in the Ganglionic Eminences and Different Subtypes of Adult Cortical Interneurons

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Distinct subtypes of cortical GABAergic interneurons provide inhibitory signals that are indispensable for neural network function. The *Dlx* homeobox genes have a central role in regulating their development and function. We have characterized the activity of three *cis*-regulatory sequences involved in forebrain expression of vertebrate *Dlx* genes: upstream regulatory element 2 (URE2), I12b, and I56i. The three regulatory elements display regional and temporal differences in their activities within the lateral ganglionic eminence (LGE), medial ganglionic eminence (MGE), and caudal ganglionic eminence (CGE) and label distinct populations of tangentially migrating neurons at embryonic day 12.5 (E12.5) and E13.5. We provide evidence that the dorsomedial and ventral MGE are distinct sources of tangentially migrating neurons during midgestation. In the adult cortex, URE2 and I12b/I56i are differentially expressed in parvalbumin-, calretinin-, neuropeptide Y-, and neuronal nitric oxide synthase-positive interneurons; I12b and I56i were specifically active in somatostatin-, vasoactive intestinal peptide-, and calbindin-positive interneurons. These data suggest that interneuron subtypes use distinct combinations of *Dlx1/Dlx2* enhancers from the time they are specified through adulthood.

Key words: ganglionic eminence; regionalization; migration; progenitors; enhancers; cerebral cortex; GABAergic neurons; homeobox; mice

Introduction

Two main classes of neocortical neurons constitute the basic functional units on which complex neuronal networks are established during development: (1) glutamatergic neurons, which are largely projection neurons; and (2) GABAergic neurons, which are local circuit neurons (Peters and Saint Marie, 1984; DeFelipe and Farinas, 1992). Inhibitory GABAergic neurons comprise 20–30% of all adult neocortical neurons (Parnavelas et al., 1977; Hendry et al., 1987; Meinecke and Peters, 1987) (for review, see Markram et al., 2004; Wonders and Anderson, 2006). They are subdivided into subtypes based on molecular, morphological, and electrophysiological features (Markram et al., 2004). Interneuron subtypes express one or more of three calcium-binding

proteins [parvalbumin (PV), calretinin (CR), and calbindin (CB)] and four neuropeptides [somatostatin (SOM), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and cholecystokinin (CCK)] (Markram et al., 2004).

In rodents, most interneurons in the neocortex, hippocampus, and olfactory bulb are derived from the embryonic basal ganglia (for review, see Parnavelas, 2000; Corbin et al., 2001; Marin and Rubenstein, 2001). Immature interneurons migrate along distinct routes toward their destinations, where they differentiate. Tangential migration, in which cells migrate orthogonally to the radial axis of the neural tube, is the major direction followed by ventrally derived interneurons to reach the dorsal cortex and hippocampus. Distinct subtypes of interneurons have different spatiotemporal origins (Wichterle et al., 2001; Anderson et al., 2002; Valcanis and Tan, 2003; Xu et al., 2004; Butt et al., 2005). For example, the PV- and SOM-expressing interneurons seem to derive primarily from progenitors located in the medial ganglionic eminence (MGE) between embryonic day 12.5 (E12.5) and E16.5. In contrast, interneurons expressing CR derive exclusively from progenitors found in the caudal ganglionic eminence (CGE) between E14.5 and E16.5 (Xu et al., 2004; Butt et al., 2005).

The mechanisms that specify cortical interneuron subtypes may be explained by yet unknown microenvironments within a

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given progenitor domain created by the combinatorial expression of various molecules, including transcription factors. The environment in which the interneurons migrate and settle may also contribute to their fate determination.

The *Dlx* family comprises six genes organized into three bigene clusters on separate chromosomes. Four of them, *Dlx1*, *Dlx2*, *Dlx5*, and *Dlx6* are sequentially expressed in the subcortical telencephalon starting around embryonic day 9.5 (E9.5) (Liu et al., 1997; Eisenstat et al., 1999). They display overlapping expression patterns in the basal ganglia (subpallium) but with subtle differences [for review, see (Panganiban and Rubenstein, 2002)]. Mutants lacking *Dlx1/Dlx2* function display a block in the differentiation of progenitors in the basal ganglia and lack of migrations to the cerebral cortex, olfactory bulb, and hippocampus (Anderson et al., 1997a,b).

We previously identified *cis*-regulatory elements (CREs) located in the intergenic regions of the *Dlx1/Dlx2* and *Dlx5/Dlx6* loci of vertebrates. These CREs (I56i, I56ii, and I12b) are able to target reporter transgene expression to the subpallial telencephalon and to the diencephalon of transgenic animals (Zerucha et al., 2000; Ghanem et al., 2003).

Here, we compare the spectrum of activity of I12b, I56i, and that of a novel CRE, upstream regulatory element 2 (URE2), located in the 5' flanking region of *Dlx1*. The three CREs display differential activities in the dorsal, medial, and ventral parts of the lateral ganglionic eminence (dLGE, mLGE, and vLGE), MGE (dMGE, mMGE, and vMGE), and CGE (dCGE, mCGE, and vCGE) and mark distinct populations of tangentially migrating neurons. Furthermore, we show that URE2 and I12b/I56i are differentially active in several subtypes of adult cortical interneurons.

Materials and Methods

Transgenic mice. To generate transgenic mice, the enhancer sequences of URE2, I12b, and I56i were subcloned into the p1229/p1230 vectors (Yee and Rigby, 1993) that contain a human β -globin minimal promoter and the *lacZ* reporter gene. The URE2 sequences from human, mouse, and *Takifugu rubripes* were obtained from public databases: human, GenBank accession number NT_005403.16, position 23146508–23147458; mouse, GenBank accession number NT_039207.6, position 12396425–1239757; and *Takifugu rubripes*, GenBank accession number CAAB01001300.1, position 46685–47635. Subclonings were done using either a PCR-based approach or using convenient restriction sites. The p1230-based I12b-alkaline phosphatase construct was generated by replacing the *lacZ* reporter gene with the human placental alkaline phosphatase gene (PLAP) in the p1230-based I12b-*lacZ* plasmid using *Bam*HI sites. The human PLAP sequence was amplified by PCR from the pGT0-PFS plasmid. Transgenic animals were produced and analyzed as described previously (Zerucha et al., 2000).

Morphological analysis of transgenic mice. Embryos from the mating of a transgenic *CD1* male with normal *CD1* females were harvested at various embryonic stages. Pregnant females were killed by cervical dislocation. E11.5 and E12.5 mouse embryos were fixed in 4% paraformaldehyde (PFA) for 1–2 h at room temperature (RT) and then washed in (1×) PBS for 30 min and stained for β -galactosidase activity overnight (O/N) at 28°C in a solution of 1 mg/ml X-gal, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, and 0.02% NP-40 in PBS. Mouse brains were dissected out after staining and soaked in 20% sucrose solution at 4°C O/N. The following day, brains were cryoprotected and frozen using cold isopentane (kept at -80°C). Thirty to 50 μm sections were then cut using a cryostat [Leica (Nussloch, Germany) CM3050 S].

Sectioning and tissue preparation of adult brains. Postnatal day 35 (P35) adult mice were screened by PCR with specific primers for *lacZ* and/or PLAP using genomic DNA extracted from ear tissue. Positive mice were anesthetized with isoflurane and were subjected to cardiac perfusion in 1× PBS followed by 4% cold PFA. Brains were dissected out afterward

and postfixed in 4% PFA for 4 h at RT. Sections of 30–40 μm thickness were cut into cold 0.1 M phosphate buffer (PB) using a vibratome (Vibratome series 1000; Technical Products International, Winnipeg, Manitoba, Canada).

Double immunohistochemistry. Sections of E12.5 and E13.5 mouse brain were generated as described above. Frozen sections were dried for 1 h and then washed three times for 5 min each in 0.1 M PB, pH 7.4, to eliminate residues from tissue protection medium. Sections were incubated in blocking solution: 1% bovine serum albumin, 5% goat serum, and 0.3% Triton-X in 0.1 M PB for at least 1 h at RT. This was followed by incubation with the primary antibody at 4°C O/N. Three 10 min washes in 0.1 M PB were performed the next day. This was followed by incubation with the secondary antibody for 2 h at RT away from light. Sections were washed three times for 10 min each, processed for a second round of immunohistochemical labeling and, finally, mounted in Immuno Fluore mounting media (Fisher, Pittsburgh, PA).

Immunohistochemistry on floating sections of adult mouse tissue was performed as described previously (Cobos et al., 2005). We used the following antibodies: guinea pig anti- β -galactosidase (1:1000; generous gift from Thomas Sargent laboratory), rabbit anti-PLAP (1:100; Serotec, Raleigh, NC), rabbit anti-PV [1:1000; Swant (Bellinzona, Switzerland) Swiss Antibodies], rat anti-SOM (1:100; Millipore, Billerica, MA), rabbit anti-neuronal nitric oxide synthase (nNOS; 1:150; Zymed/Invitrogen, San Francisco, CA), rabbit anti-CR (1:4000; Swant Swiss Antibodies), rabbit anti-NPY (1:4000; ImmunoStar, Hudson, WI), rabbit anti-TH (1:350; Millipore), rabbit anti-GABA (1:4000; Sigma, St. Louis, MO), rabbit anti-VIP (1:200; ImmunoStar), and rabbit pan-DLL antibody (anti-*Dlx*; a gift from G. Boekhoff-Falk, University of Wisconsin, Madison, WI). Secondary antibodies were purchased from Invitrogen (Eugene, OR): goat anti-rabbit Alexa Fluor 488, goat anti-guinea pig Alexa Fluor 594, and goat anti-rat Alexa Fluor 488.

In coimmunolocalization studies, when both signals could be detected in the same cell, regardless of their respective intensities, the cell was counted as being positive for both antigens.

Cell counts and brain sampling. Cell counts were performed on digitized images obtained from randomly selected areas of layers I–VI of the somatosensory and motor cortices using 8–10 coronal sections from different positions along the rostrocaudal axis. Cells expressing β -galactosidase and/or interneuron markers were counted. Quantification was determined from two independent transgenic lines using four brains per line ($n = 4$) and averaged. Data are presented as mean $\pm$ SD.

Organotypic culture. Organotypic slice cultures of embryonic mouse forebrain were performed as described previously (Anderson et al., 1997b). For the transplantation experiment and because of the small size of the tissue, we divided the MGE into two portions instead of three: dMGE/mMGE and vMGE.

Results

Regulation of *Dlx* gene expression in the forebrain by tissue-specific enhancers

The previously identified CREs, I56i and I12b, located in the intergenic regions of *Dlx5/Dlx6* (Zerucha et al., 2000) and *Dlx1/Dlx2* (Ghanem et al., 2003), respectively, show comparable regulatory activities in the subpallial telencephalon and diencephalon when examined in whole-mount transgenic mouse embryos (Ghanem et al., 2003). In a search for additional regulatory elements controlling *Dlx1* and *Dlx2* gene expression in the forebrain, we examined, by “phylogenetic footprinting,” the 5' flanking regions of each gene for conserved noncoding DNA sequences among mouse, human, *Takifugu rubripes*, and zebrafish. We identified two conserved sequences in the 5' flanking region of *Dlx1* that we named URE1 and URE2. The two sequences were found in all of the vertebrate species listed above as well as others. In the mouse, they are located ~ 17 kb (URE1) and ~ 12 kb (URE2) upstream of the *Dlx1* ATG start site (Fig. 1A; supplemental sequence information, available at www.jneurosci.org as supplemental material).

Figure 1. Conserved *cis*-acting regulatory elements in the *Dlx1/Dlx2* locus. **A**, Schematic representation of the *Dlx1/Dlx2* locus of four vertebrate species. Each *Dlx* gene comprises three exons, which are indicated in light blue boxes. Colored squares are conserved noncoding DNA sequences: I12b (blue), URE1 (yellow), and URE2 (red). **B**, Enhancer activity of the mouse URE2 sequence in an E11.5 mouse embryo. URE2 targets *lacZ* reporter gene expression to two domains in the forebrain, the subpallial telencephalon, and diencephalon, as well as other tissues. H, Hyoid arch (second arch); Hy, hypothalamus; L, limbs; PT, prethalamus (ventral thalamus); ST, subpallial telencephalon; St, somites. Scale bar, 1 mm.

We looked for a potential regulatory role played by URE1 and URE2 by generating transgenic mice using a construct comprised of a *lacZ* reporter gene under the control of the human β -globin minimal promoter. Analysis of the β -galactosidase activity in the *URE2-lacZ* transgenic lines at E11.5 showed that URE2 is active in the forebrain of these mice as well as the hyoid arch, the somites, and the apical ectodermal ridge of the limbs (Fig. 1B). Expression of *lacZ* in the forebrain is restricted to the subpallial telencephalon, the prethalamus, and the hypothalamus and was consistent among independently generated transgenic lines. In whole-mount preparations, *URE2-lacZ* reporter activity in the forebrain is reminiscent of the activities of *I12b-lacZ* and *I56i-lacZ* (Ghanem et al., 2003). The other CRE, URE1, showed no activity in the forebrain but is active in other regions of the embryo, notably in the retina, and will not be discussed further here. We also examined the 5' flanking region of *Dlx2* but did not find sequences that were conserved among the five species.

Dlx enhancers identify divisions in the LGE, MGE, and CGE

We compared the expression of *lacZ* driven by URE2, I12b, and I56i in coronal sections of E11.5 and E12.5 telencephalon. Results were confirmed using at least two independent transgenic lines. The *I12b-lacZ* and *I56i-lacZ* transgenic embryos display nearly indistinguishable reporter expression at E11.5 and E12.5 in the subventricular zone (SVZ) and mantle zone (MZ) of the subpallium, the LGE, the MGE, anterior entopeduncular area (AEP), preoptic area (POA), and in the CGE (Fig. 2). *URE2-lacZ* transgenic lines display distinct reporter gene expression in two main regions.

Figure 2. Comparative enhancer activities of URE2, I12b, and I56i in the subpallial telencephalon of transgenic mice. Coronal hemisections showing *lacZ* expression under the control of each enhancer at E11.5 (**A–F**) and E12.5 (**G–L**) in medial and caudal levels. **A, D, G, J**, *URE2-lacZ*; **B, E, H, K**, *I12b-lacZ*; **C, F, I, L**, *I56i-lacZ*. **B, C, E, F**, Insets, Higher-magnification pictures of regions shown in black dashed boxes; arrowheads in the insets mark the beginning of tangential migrations at the level of the dLGE at E11.5. **G, H, I**, Insets, Higher-magnification pictures of the red dashed boxes. **H, I, K, L**, Arrows 1 and 2 show the two streams of migratory cells emerging from the LGE at the level of the corticostriatal boundary (CSB), which coalesce into one stream within the cortex at E12.5: a superficial stream (stream 1) and a deep stream (stream 2). Tangential migration of *URE2-lacZ*-positive cells was not observed at these stages. III, Third ventricle; CP, cortical plate; Hi, hippocampus; Hy, hypothalamus; LV, lateral ventricle; PCx, piriform cortex; SCB, suprachiasmatic band; Th, thalamus; VP, ventral pallidum; VZ, ventricular zone. Scale bar: (in **A–L**, 250 μ m; **B, C, E, F**, insets, 125 μ m; **G, H, I**, insets, 75 μ m).

Figure 3. Activities of three *Dlx* CREs in the MGE and in the cortex near the CSB at E12.5. **A–F**, Double immunohistochemistry showing the expression of *I12b-AP* in green (**A**, **D**), *URE2-lacZ* (**B**), or *I56i-lacZ* in red (**E**). **C**, **F**, Merged pictures of **A**, **B** and **D**, **E**, respectively. Compared with the dMGE, only few radial columns of cells in **B** express *URE2-lacZ* in the SVZ of vMGE (arrowheads), whereas others do not (arrows). In **C**, arrows and arrowheads show radial columns of double-labeled cells (yellow) and single-labeled ones (red or green), respectively. Insets in **B** and **C** are high-magnification pictures of the areas shown in white dashed boxes. **G–L**, Labeling of tangentially migrating cells in the cortex near the CSB of *I12b-AP* mice (**G**, **J**; green), *URE2-lacZ* mice (**H**; red), and *I56i-lacZ* mice (**K**; red) at E12.5. **I**, **L**, Merged pictures of **G**, **H** and **J**, **K**, respectively. As described in Figure 2, migratory cells in **H**, **I**, **K**, and **L** follow superficial (stream 1) and deep (stream 2) streams of migrations at E12.5 that will merge into one along the Mz of the CSB. **H**, There is little *URE2-lacZ* expression in tangentially migrating cells at this age. **I**, **L**, Single-labeled and double-labeled cells are indicated with arrows and arrowheads, respectively. Scale bar: (in **A**) **A–L**, 25 μ m; **B**, **C**, insets, 15 μ m; **G–L**, 12.5 μ m. Symbols are the same as in Figure 2.

(1) *URE2-lacZ* was the only CRE directing expression to the ventricular zone (VZ), in which it is expressed in the LGE, mMGE, and CGE at E11.5 (Fig. 2*A*, *D*). *URE2-lacZ* expression in the VZ weakens by E12.5 in the dLGE and vLGE, the dMGE and vMGE, and the dCGE and vCGE (Fig. 2*G*, *J*); thus, its expression remains strong in the mLGE, mMGE, and mCGE (Fig. 2*G*, *J*). Cells expressing *URE2-lacZ* in the VZ are arranged in radial chains, suggesting that they may be clonally related (Figs. 2*G*, *J*, 3*B*; supplemental Fig. S1*B*, available at www.jneurosci.org as supplemental material). This activity is weak before E11.5 (data not shown) and is maintained at least until E15.5 although with weaker intensity (data not shown). Thus, contrarily to *I12b* and *I56i*, *URE2* is active in subpopulation(s) of VZ progenitor cells between E11.5 and E15.5. Note that, at these ages, cells in the VZ including those expressing *URE2-lacZ* are undifferentiated progenitors and do not express GABA, which is an early differentiation marker that labels committed progenitors mainly found in the subventricular zone (SVZ) and MZ (Ferguson et al., 2005).

(2) *URE2-lacZ* is differentially expressed in subpallial SVZ domains. Only a small number of cells express the *URE2-lacZ* transgene in the vMGE and AEP (Fig. 2*A*, *G*). This markedly contrasts with the stronger expression of *I12b-lacZ* and of *I56i-lacZ* in those regions (Fig. 2*B*, *C*, *H*, *I*, *K*, *L*). Thus, the distinct spatio-temporal activities of *URE2*, compared with *I12b* and *I56i*, provide genetic evidence for three subdivisions of the LGE, MGE, and CGE.

URE2 and *I12b* label distinct populations of tangentially migrating interneurons

To address whether the three CREs (*URE2*, *I12b*, and *I56i*) are active in distinct and/or overlapping subsets of cells of the developing telencephalon, we generated a transgenic mouse line in which the human alkaline phosphatase gene, *AP*, is driven by the *I12b* sequence (*I12b-AP* transgene). We crossed *I12b-AP* mice with *I56i-lacZ* and *URE2-lacZ* lines separately and screened the F1 generations for *lacZ*⁺ and *AP*⁺ double-hemizyote embryos. Then, we compared the expression patterns of the two reporter genes, at E12.5 and E13.5, by double immunohistochemistry on brain sections using antibodies against β -galactosidase and *AP*. First, we compared the activities of *URE2* and *I12b* at E12.5. In the mMGE, *URE2-lacZ* expression is observed in a subset of cells in the VZ that are arranged in radial chains (Fig. 3*B*, inset), whereas *I12b-AP* expression is not detected except in few columns of cells at the VZ/SVZ boundary (Fig. 3*A*).

Both transgenes are expressed in larger subsets of cells in the SVZ, many of which are double labeled, but some are single labeled (Fig. 3*C*, inset).

In the dMGE, vMGE, and AEP, *URE2-lacZ* expression is weaker than in the mMGE; it was weak or absent in the VZ and restricted to some radial columns of cells in the SVZ (Figs. 2*G*, 3*B*, arrows; data not shown). In contrast, *I12b-lacZ* (or *I12b-AP*) expression, although not detected in the VZ, appears homogenous in the SVZ throughout the MGE and AEP (Figs. 2*H*, 3*A*, and data not shown). Similar differences in the profiles of expression of the two transgenes are detected in the LGE at this age (Fig. 2*G*, *H* and data not shown).

The *URE2*- and *I12b*-containing transgenes also show differences in CGE expression (Fig. 2*J–L* and supplemental Fig. S1*A–C*, available at www.jneurosci.org as supplemental material). *URE2-lacZ* expression is present in the VZ of the mCGE but not in the dCGE or vCGE (Fig. 2*J*, supplemental Fig. S1*B*, available at www.jneurosci.org as supplemental material). Furthermore, *URE2-lacZ* expression is weaker in the SVZ and MZ of the vCGE compared with the dCGE and mCGE (Figs. 2*J*, supplemental Fig. S1*B*, available at www.jneurosci.org as supplemental material).

The majority of *URE2-lacZ*- and *I12b-lacZ*-positive cells in the ganglionic eminences express *Dlx* genes between E12.5 and E13.5, as shown by immunolocalization with a pan-Dll antibody (supplemental Fig. S6, available at www.jneurosci.org as supplemental material, and data not shown).

The distinct activities of the *Dlx* CREs within the MGE and CGE prompted us to investigate whether the enhancers are differentially active in interneurons that tangentially migrate to the cortex. Most cortical GABAergic interneurons in mouse are derived from the MGE and CGE (Marin and Rubenstein, 2001, 2003; Nery et al., 2002; Xu et al., 2004; Butt et al., 2005) and express *Dlx* genes (Anderson et al., 1997a; Stuhmer et al., 2002b; Cobos et al., 2005, 2006).

At early stages of the tangential migrations, cells express *I12b-lacZ* much more than they express *URE2-lacZ*. Of the two transgenes, *I12b-lacZ* is the only one expressed in migrating cells at E11.5 (Fig. 2*B,E*, arrowheads in insets). By E12.5, there are two streams of tangentially migrating cells entering the pallium at its boundary with the dLGE and dCGE; these correspond to two migratory routes known to be *Dlx*+ (Lavdas et al., 1999; Anderson et al., 2001; Marin and Rubenstein, 2001; Wichterle et al., 2001). One is a deep stream that traverses the SVZ of the ventral pallium (VP) and lateral pallium (LP) and enters the SVZ/intermediate zone (IZ) of the dorsal pallium (DP) (Fig. 2, stream 1); the second is a superficial stream that traverses the mantle of the VP and LP and continues in the marginal zone (Mz) of the DP (stream 2) (Figs. 2*H–L*, 3*G–I*, arrows 1 and 2). There are approximately three to four times more *I12b-AP*-expressing than *URE2-lacZ*-expressing cells that tangentially migrate from the MGE and CGE at this age (Figs. 3*G–H*, 4*A*, and data not shown). However, the *I12b-AP* and the *I56i-lacZ* transgenes were expressed in the same cells in ~80% of the cases (Fig. 4*C*).

By E13.5, a larger number of tangentially migrating neurons express *URE2-lacZ* in the cortical IZ and Mz (Figs. 5*B,H*, supplemental Figs. S2*B*, S2*H*, available at www.jneurosci.org as supplemental material). Yet, there are still ~2- to 2.5-fold fewer *URE2-lacZ*-expressing cells than *I12b-AP*- (Fig. 4*B*) or *I56i-lacZ*- (Fig. 4*D*) expressing cells. At E14.5, the number of *URE2-lacZ* migrating cells reaches a peak and then starts to decrease at E15.5 (data not shown). The *URE2-lacZ* and *I12b-lacZ* transgenes are expressed in a third migratory pathway that runs through the cortical SVZ, deep to the Mz and IZ between E14.5 and E16.5 (data not shown). β -Galactosidase-positive neurons continue to be detected in the

Figure 4. Differential activities of the *URE2*, *I12b*, and *I56i* CREs in migrating cells. **A–F**, Reporter transgene expression was determined at the level of the CSB at E12.5 (**A**, **C**, **E**) or at the level of the DP at E13.5 (**B**, **D**, **F**). The number of positive cells was determined from two to three sections per mouse of the CSB or DP at the level of the MGE or CGE (total = average from 3 mice). When both immunohistochemical signals were detected for both antigens in the same cell, regardless of signal intensity, the cell was counted as being positive for both antigens. Values are presented as mean $\pm$ SD. **G**, Schematic representation of an E13 coronal hemisection of the mouse telencephalon showing the CSB, the DP, and the two streams (stream 1 and 2) of tangentially migrating cells toward the dorsal cortex. Symbols are the same as in Figure 2.

cortex at P0, P25, and P35 (see Figs. 8, 9, supplemental Fig. S5, available at www.jneurosci.org as supplemental material, and data not shown).

We compared *URE2* and *I12b* activities in tangentially migrating cells using double labeling. Approximately one-half of the tangentially migrating cells expressing *URE2-lacZ* also express

Figure 5. Activities of three *Dlx* CREs in tangentially migrating cells derived from the MGE at E13.5. **A–F**, Double immunohistochemistry showing the expression of *I12b-AP* in green (**A**, **D**) or *URE2-lacZ* (**B**) or *I56i-lacZ* in red (**E**), in the DP. **C**, **F**, Merged pictures of **A**, **B** and **D**, **E**, respectively. Tangentially migrating cells follow the Mz and IZ of the DP at E13.5. **G–L**, Higher-magnification pictures of the boxes shown in **A–F**, respectively. **I**, **L**, Merged pictures of **G**, **H** and **J**, **K**, respectively. There are more migrating cells expressing *URE2-lacZ* mice at this age (**B**, **H**) compared with E12.5 (Fig. 3H). Single-labeled and double-labeled cells in **I** and **L** are indicated with arrows and arrowheads, respectively. Most migrating cells in *I12b-AP/I56i-lacZ* mice are double labeled, whereas a large number of cells in *I12b-AP/URE2-lacZ* mice are single labeled. Scale bar: (in **A**) **A–F**, 25 μ m; **G–L**, 12.5 μ m. Symbols are the same as in Figure 2.

the *I12b-AP* transgene at E12.5 or E13.5 (Figs. 3, 4A,B, supplemental Fig. S1, available at www.jneurosci.org as supplemental material). *URE2-/-I12b+* cells are equally distributed in both stream 1 and stream 2 at E12.5 (Fig. 3I, supplemental Fig. S1I, available at www.jneurosci.org as supplemental material) and in the IZ and Mz at E13.5 (Fig. 5I, supplemental Fig. S2I, available at www.jneurosci.org as supplemental material). In contrast, *URE2+/-I12b-* migrating cells are largely found in stream 1 at E12.5 (Figs. 3I, arrows, 4E, and supplemental Fig. S1I, available at www.jneurosci.org as supplemental material), and IZ at E13.5 (Figs. 4F, 5I, arrows and S2I). In summary, differential activity of CREs from the *Dlx1/Dlx2* locus in tangentially migrating cells suggest the existence of several populations in both the Mz and IZ.

Highly similar activities of *I12b* and *I56i*

We then compared the activities of *I12b* and *I56i* at E12.5. The majority of cells in the SVZ and MZ are double labeled in the MGE and AEP (Fig. 3D–F and data not shown) and in the CGE (supplemental Fig. S1D–F, available at www.jneurosci.org as supplemental material) confirming X-gal staining (Fig. 2) that

suggests activity of the two enhancers in overlapping population(s).

We next compared the expression of *I12b* and *I56i* in tangentially migrating cells at E12.5 and E13.5 as we did for *URE2/I12b* comparisons. The *I12b-AP* and *I56i-lacZ* transgenes are coexpressed in tangentially migrating cells (Figs. 3J–L, 4C,D, 5D–F,J–L, supplemental Figs. S1J–L, S2D–F, and S2J–L, available at www.jneurosci.org as supplemental material). We also detect two populations of single-labeled migrating cells at the levels of the MGE and the CGE. The *I56i+/-I12b-* and the *I56i-/-I12b+* populations accounted for <20% and 12.5% of migrating cells, respectively (Fig. 4C,D). All three cell populations (*I12b+/-I56i+*, *I12b+/-I56i-*, and *I12b-/-I56i+*) are equally distributed between migration stream 1 and 2 at E12.5 and between the Mz and IZ at E13.5 [Figs. 3L, 5L, supplemental Figs. S1L, S2L (arrows), available at www.jneurosci.org as supplemental material]. These results suggest that the *I12b* and *I56i* CRE are mainly active in the same cells and could be responding to similar regulatory factors.

The dMGE/mMGE and the vMGE produce cells that migrate to the cortex

Because the *I12b* and *URE2* CREs are differentially active in the MGE and AEP and may label distinct subpopulations of tangentially migrating neurons derived from these regions, we sought to investigate whether this could be linked to intrinsic properties of the progenitor cells found in these regions in terms of regional specification and migration potential. For this purpose, we performed *in vitro* DiI labeling experiments on brain slice culture taken from wild-type litters at E12.5 and

E13.5. First, we compared the migration potential of neurons derived from progenitors residing in the MGE versus those derived from progenitors found in the AEP and POA, separately. We find no evidence of tangential migrations that reached the cortex from either the AEP or POA (supplemental Figs. S3B,C,F–H, S4B,D,J–L,N,P, available at www.jneurosci.org as supplemental material), but we observe robust tangential migration of neurons from the MGE that enter the cortex (Figs. S3B,D, S4B,C,E–H, available at www.jneurosci.org as supplemental material) ($n = 15$) consistent with previous slice studies (Anderson et al., 1997b, 2001).

Next, we compared the migration potential of neurons derived from progenitors found in the dMGE/mMGE versus vMGE at E12.5 and E13.5. We observe robust tangential migrations to the cortex from both regions (Fig. 6A–D, supplemental Fig. S4B,C,E–H,N,O, available at www.jneurosci.org as supplemental material; data not shown) ($n = 12$). We also performed tissue transplantation assays coupled to DiI experiments at E12.5; the vMGE was dissected out and transplanted into a rostral or a caudal section to replace either the LGE or CGE, respectively.

Then, the tissue transplanted from the vMGE and the one from the remaining dMGE/mMGE were labeled separately with Dil (Fig. 6*E,I,M*). The vMGE transplants show robust tangential migration to the cortex regardless of their new environment (Fig. 6*M–P* and data not shown) ($n = 4$) showing that E12.5 vMGE progenitor cells are specified to produce cells that tangentially migrate to the cortex. In addition, progenitors located in the remaining dMGE/mMGE also retain their ability to migrate to the cortex (Fig. 6*F,G,J,K*). These experiments demonstrate that both the dMGE/mMGE and vMGE are sources of cells that tangentially migrate to the cortex.

URE2 and I12b/I56i mark overlapping and distinct subtypes of adult cortical interneurons

Expression of URE2 and I12b reporter transgenes persists postnatally in subsets of forebrain neurons. We focused on their expression in interneurons of the somatosensory and motor cortices of 5-week-old mice using double immunohistochemistry. We colabeled either *URE2-lacZ*+ or *I12b-lacZ*+ cells with GABA. We find that approximately two-thirds of GABA+ cells ($\sim 66.1 \pm 2.8\%$) were *URE2-lacZ*+, and nearly all of the *URE2-lacZ*+ cells express GABA ($\sim 92.9 \pm 2.1\%$). In contrast, nearly all GABA+ cells are *I12b-lacZ*+ ($\sim 93.8 \pm 2.1\%$) and vice versa ($93.5 \pm 3.4\%$) (Fig. 7 and data not shown).

Next, we determined whether *URE2-lacZ* and *I12b-lacZ* were differentially expressed in subtypes of cortical interneurons. Thus, we colabeled *URE2-lacZ*+ and *I12b-lacZ*+ cells with the following subtype markers: calcium-binding proteins (CR, PV, and CB) and neuropeptides (SOM, NPY, nNOS, and VIP). We find that *URE2-lacZ* is expressed in most PV+ ($89 \pm 1\%$) (Figs. 7, 8), CR+ ($88.4 \pm 0.8\%$) (Figs. 7, 9), NPY+ ($92.8 \pm 3.2\%$) (Fig. 7, supplemental Fig. S5, available at www.jneurosci.org as supplemental material), and nNOS+ ($95.9 \pm 0.1\%$) (Fig. 7, supplemental Fig. S5, available at www.jneurosci.org as supplemental material) interneurons. Likewise, *I12b-lacZ* is also expressed in a large subset of these cells: PV ($85.5 \pm 3.3\%$) (Figs. 7, 8), CR ($79.4 \pm 5.1\%$) (Figs. 7, 9), NPY ($75.7 \pm 5.0\%$) (Fig. 7, supplemental Fig. S5, available at www.jneurosci.org as supplemental material), and nNOS ($27.3 \pm 7.1\%$) (Fig. 7, supplemental Fig. S5, available at www.jneurosci.org as supplemental material).

However, *URE2-lacZ* is expressed in only a very small number of SOM+ ($10.2 \pm 1.2\%$) (Figs. 7, 8), VIP+ ($13.8 \pm 5.4\%$) (Figs. 7, 9), or CB+ ($14.7 \pm 1.1\%$) (Fig. 7) interneurons. In contrast, *I12b-lacZ* is expressed in most of these three interneuron subtypes [SOM, $87.5 \pm 4.7\%$ (Figs. 7, 8); VIP, $85.1 \pm 2.6\%$ (Figs. 7, 9); CB, $85.5 \pm 5.7\%$ (Fig. 7)].

We also double labeled *I56i-lacZ* with the subtype markers mentioned above and found that this transgene is expressed in

Figure 6. The dMGE/mMGE and vMGE are both sources of tangentially migrating cells to the DP at E12.5. *A*, Dil labeling (arrows) of the dMGE/mMGE and vMGE. *A*, Inset, A picture taken after 72 h in culture. *B–D*, Robust migrations toward the cortex are derived from both subdivisions. *E, I*, The left vMGE was dissected and transplanted into a rostral level to replace the LGE (asterisk in *E, I*, and *M*). The left dMGE/mMGE and right vMGE in *E* and *I* as well as the transplanted vMGEs in *M* were labeled with Dil. *E, I, M*, Insets, Pictures taken after 48 h in culture. *F, J, N*, Fluorescent pictures of *E, I, M*, respectively. *G, K, O* and *H, L, P* are higher magnifications of the left and right hemispheres in *E, I, M*, respectively. Hi, Hippocampus. Robust tangential migration to the DP is observed from progenitors located in the dMGE/mMGE (*G, K*) and control vMGE (*H, L*) as well as transplanted vMGE (*O, P*). Scale bar: (in *A*) *A, B, E, F, I, J, M, N*, 100 μ m; *C, D, G, H, K, L, O, P*, 50 μ m. Symbols are the same as in Figure 2.

almost the same percentage of cells with regard to each subtype as *I12b-lacZ* (Figs. 7–9, supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Thus, we conclude that URE2 and I12b/I56i mark common as well as distinct population(s) or subpopulation(s) of cortical interneurons in the adult mouse brain.

Discussion

Multiple enhancers regulate *Dlx1/2* and *Dlx5/6* expression in the LGE, MGE, and CGE

We provide evidence that the overall patterns of *Dlx* expression in the developing and mature brain represent the sum of patterns generated by distinct enhancer elements that may be regulated by distinct mechanisms. This conclusion is based on the detailed examination of *cis*-regulatory elements that control *Dlx* gene expression in the developing telencephalon. Three *Dlx* CREs active in the forebrain, URE2, I12b, and I56i, display partially overlapping activities in the basal ganglia anlage (Figs. 2, 3*A–F*; data not shown). The different activities of URE2, I12b, and I56i cannot be attributed to insertional effects of the transgene or differences in copy number. Patterns of transgene expression at selected time

Figure 7. Percentage of cortical interneurons, classified according to marker gene expression, showing activity of the URE2, I12b, and I56i enhancers. Coexpression is an average from 8 to 10 sections of the somatosensory and motor cortices per mouse at P35 ($n = 4$). When both immunohistochemical signals were detected for both antigens in the same cell, regardless of signal intensity, the cell was counted as being positive for both antigens.

Figure 8. Coexpression of the SOM and PV interneuron markers with URE2-*lacZ*, I12b-*lacZ*, and I56i-*lacZ* in the mouse adult somatosensory cortex at P35. Cells expressing URE2-*lacZ* (A–D, M–P), I12b-*lacZ* (E–H, Q–T), or I56i-*lacZ* (I–L, U–X) are shown in red. Interneurons expressing SOM (A–L) or PV (M–X) are shown in green. D, H, L, P, T, X, Higher magnifications of boxes shown in C, G, K, O, S, W, respectively. O, P, Most PV + interneurons express URE2-*lacZ*. C, D, In contrast, only a few SOM + interneurons express URE2-*lacZ*. The I12b-*lacZ* and I56i-*lacZ* transgenes are expressed in a large number of PV + as well as SOM + interneurons. Double- and single-labeled interneurons are indicated with arrows and arrowheads, respectively. Scale bar: (in A) A–C, E–G, I–K, M–O, Q–S, U–W, 25 μ m; D, H, L, P, T, X, 8.7 μ m.

points were reproducibly obtained from at least two independent integration events and a detailed transgene expression analysis was performed on at least two independent lines for each construct.

The URE2, I12b, and I56i enhancers largely differ in sequence (Ghanem et al., 2003 and this study) and could thus contain binding sites for different combinations of transcription factors. A combination of DNase I footprinting, cotransfection assays, and transgenic experiments performed on the three enhancers (Poitras et al., 2007) (L. Poitras, N. Ghanem, N. Shipley, G. Hatch, and M. Ekker, unpublished observations) support this hypothesis, although some factors such as the DLX proteins themselves could be interacting with multiple enhancers as part of auto-regulatory or cross-regulatory mechanisms (Zerucha et al., 2000; Zhou et al., 2004; Poitras et al., 2007) (Poitras, Ghanem, Shipley, Hatch, and Ekker, unpublished observations).

The differential activities of URE2, I12b, and I56i in subdivisions of the LGE, MGE, and CGE suggest that the dorsal, medial, and ventral parts of these ganglionic eminences may generate

distinct cell types. The *Dlx* progenitors born in these subpallial subdivisions have different molecular properties, which may influence the fate of these progenitors.

URE2 is the only CRE that is strongly active in a subset(s) of radially arranged cells in the VZ of the mLGE, mMGE, and mCGE between E11.5 and E12.5 (Figs. 2, 3, supplemental Fig. S1, available at www.jneurosci.org as supplemental material; data not shown) with patterns resembling the expression of *Dlx1* and *Dlx2* in VZ cells (Porteus et al., 1994; Eisenstat et al., 1999). The strong expression of URE2-*lacZ* in the VZ is consistent with ongoing neurogenesis in that region between E11.5 and E14.5 (Anthony et al., 2004). These URE2-positive cells could be radial glia and/or neuronal progenitors considering that a large subset of radial glia in the ganglionic eminence (>55%) produce neurons between E11.5 and E14.5 (Anthony et al., 2004). By E14.5, most progenitors derived from radial glia enter gliogenesis (Anthony et al., 2004), a time when URE2 activity in the VZ gradually diminishes until E15.5, when β -galactosidase is detected very weakly.

Distinct groups of cells show differential activities of URE2 and I12b in the SVZ and MZ of the LGE, MGE, and CGE at E11.5–E13.5 (Figs. 2–4). I56i and I12b are both active in the LGE, MGE, and CGE in most SVZ cells and a substantial number of MZ cells (Figs. 2, 3D–F, supplemental Fig. S1D–F, available at www.jneurosci.org as supplemental material). Thus, these two CREs are likely to be active in common cell populations at E11.5–E13.5 as well as at later times.

The overlapping activities of the CREs recapitulate similarities in the endogenous *Dlx* gene expression. Previous work provided evidence that similarities in *Dlx* ex-

pression patterns (Liu et al., 1997; Eisenstat et al., 1999) could be explained by cross-regulatory interactions between *Dlx* genes. Thus *Dlx1* and/or *Dlx2* are thought to be upstream regulators of *Dlx5* and *Dlx6*, because *Dlx1/Dlx2* null mice show loss of *Dlx5/Dlx6* expression in most of the telencephalon, except in cells derived from the septal region (Anderson et al., 1997b; Zerucha et al., 2000). The *I56i* enhancer from the *Dlx5/Dlx6* locus is regulated by *DLX1* and/or *DLX2* both *in vitro* and *in vivo* (Zerucha et al., 2000; Stuhmer et al., 2002a; Zhou et al., 2004).

Distinct cell populations tangentially migrate to the cortex between E11.5 and E13.5

Differential spatiotemporal activities of *URE2*, *I12b*, and *I56i* are detected in tangentially migrating cells that are derived from the MGE and CGE between E11.5 and E13.5. *URE2-lacZ* expression in tangentially migrating cells is detected at least 1 d later than for the *I12b-lacZ* or *I56i-lacZ* transgenes. We propose that the *URE2-lacZ*-expressing cells migrating to the cortex are primarily derived from the mMGE and mCGE based on the activity of the enhancer in these progenitor domains at E12.5. Because we do not detect expression of the *URE2-lacZ* transgene in migrating cells at this stage, this suggests that migrating cells at E12.5 originate mainly from regions of low *URE2* activity, namely the dMGE, vMGE, dCGE, and vCGE. At later stages, we suggest that the mMGE contributes tangentially migrating cells, coincident with the increase in *URE2-lacZ* expression. Alternatively, the increase in migrating cells expressing *URE2-lacZ* at later stages could be attributable to increased *URE2* enhancer activity in preexisting cells that did not express the transgene previously.

At E13.5, the majority of cells expressing *URE2-lacZ* but not *I12b-lacZ* (*URE2-lacZ*+/+*I12b-lacZ*−) follow the IZ as their major route of migration.

Together, our data suggest that cells expressing various combinations of *URE2-lacZ*+, *I12b-lacZ*+, and *I56i-lacZ* may correspond to distinct populations of migrating cells, and this may be associated with the distinct fates of these cells once they have reached their destination in the cortex.

Using tissue transplantation coupled to DiI labeling in slice cultures, we provide evidence that the dMGE/mMGE and vMGE are both sources of tangentially migrating cells between E12.5 and E13.5. Alternatively, some of the dMGE/mMGE migrating cells could derive from vMGE and are thus labeled at different positions along their migratory routes.

Dlx CREs are active in distinct subtypes of cortical interneurons in adult mice

Dlx genes are expressed in most mouse neocortical GABAergic interneurons (Stuhmer et al., 2002b; Cobos et al., 2005, 2006).

Figure 9. Coexpression of the CR and VIP interneuron markers with *URE2-lacZ*, *I12b-lacZ*, and *I56i-lacZ* in the mouse adult somatosensory cortex at P35. Cells expressing *URE2-lacZ* (A–D, M–P), *I12b-lacZ* (E–H, Q–T), or *I56i-lacZ* (I–L, U–X) are shown in red. Interneurons expressing CR (A–L) or VIP (M–X) are shown in green. D, H, L, P, T, X, Higher magnifications of boxes shown in C, G, K, O, S, W, respectively. C, D, Most CR + interneurons express *URE2-lacZ*. O, P, In contrast, few VIP + interneurons express *URE2-lacZ*. The *I12b-lacZ* and *I56i-lacZ* transgenes are expressed in a large number of CR + as well as VIP + interneurons. Double- and single-labeled interneurons are indicated with arrows and arrowheads, respectively. Scale bar: (in A) A–C, E–G, I–K, M–O, Q–S, U–W, 25 μm; D, H, L, P, T, X, 8.7 μm.

URE2 and *I12b* are likely to be involved in the regulation of *Dlx1* and *Dlx2* in adult cortical GABAergic neurons, as they are active in 66% and 93% of these cells, respectively (Fig. 7 and data not shown). Most *URE2-lacZ*+/+ (~92%) and *I12b-lacZ*+/+ (~93%) cells in the cortex contain GABA (data not shown).

PV-, SOM-, and CR-positive interneurons constitute distinct subtypes of cortical GABAergic neurons (Markram et al., 2004). *I12b-lacZ* and *I56i-lacZ*, but not *URE2-lacZ*, are expressed in the majority of interneurons expressing SOM, VIP, and CB (Figs. 7–9). In contrast, *URE2-lacZ* is expressed in most PV-, CR-, NPY-, and nNOS-expressing interneurons (>88% of each subtype) (Figs. 7–9, supplemental Fig. S5, available at www.jneurosci.org as supplemental material). *I12b-lacZ* and *I56i-lacZ* are expressed in many but not all interneurons belonging to these subtypes (>75–87% except for nNOS ~27%) (Figs. 7–9, supplemental Fig. S5, available at www.jneurosci.org as supplemental material).

Previous studies showed that PV- and SOM-expressing interneurons derive primarily from progenitors located in the MGE

Figure 10. Proposed model for the origin of adult cortical interneuron subtypes in the embryonic mouse brain. **A, B**, Schematic representation of E12.5 coronal hemisections/sections at the level of the MGE (**A**) and the CGE (**B**). Enhancer activities of *I12b* (red) and *URE2* (blue) in the MGE and CGE are depicted in the left hemispheres in **A** and **B**. Colored arrows in the right hemispheres in **A** and **B** indicate the proposed origin(s) of *Dlx*-progenitor cells that will give rise to various subtypes of cortical GABAergic interneurons. *Dlx*-progenitors that express *URE2-lacZ* and *I12b-lacZ* are represented by blue and red arrows, respectively. Progenitors that express both transgenes are shown in orange. *Dlx*-PV-progenitors are possibly derived from the mMGE whereas *Dlx*-SOM-progenitors could derive from the dMGE and/or the vMGE (see Discussion). *Dlx*-NPY-progenitors and *Dlx*-nNOS-progenitors are probably derived from the mMGE; some of these cells could also form subpopulations of *Dlx*-SOM-progenitors. In the CGE, *Dlx*-VIP-progenitors and *Dlx*-CR-progenitors are probably derived from the mCGE but could also derive from the dCGE (see Discussion). Symbols are the same as in Figure 2. Hi, Hippocampus; Th, thalamus; d, dorsal; m, medial; v, ventral.

between E12.5 and E16.5 (Xu et al., 2004; Butt et al., 2005). Our data support these results but raise the interesting possibility that distinct *Dlx* progenitors originating from MGE subdivisions (i.e., dMGE, mMGE, and/or vMGE) contribute to the various interneuron subtypes. Because most PV- neurons express *URE2-lacZ*, it is possible that this subtype is derived from progenitors in which the *URE2* CRE is active and which are born in the mMGE rather than in the vMGE (Fig. 10A, C). Furthermore, they probably correspond to the *URE2-lacZ*+ cells that undergo robust tangential migrations to the cortex between E13.5 and E14.5. In contrast, SOM interneurons that exclusively express *I12b-lacZ* may derive from progenitors born primarily in the dMGE and/or vMGE (Fig. 10A, C).

URE2-lacZ is only found in ~10% of the SOM+ cells, but in ~92% of the NPY+ cells, suggesting that these two subtype(s) may not be largely overlapping in the mouse somatosensory cortex as it is the case in the rat frontal and visual cortices (Kubota et al., 1994; Freund and Buzsaki, 1996; Gonchar and Burkhalter, 1997). These differences may be species- and/or region-related or may stem from the fact that we pooled cell counts from all six layers, which could have masked some of the differences that are layer- and/or subtype-specific. Thus, our results suggest that at least some NPY-positive neurons could derive from distinct pro-

genitors compared with the SOM-expressing neurons; however, the place of origin of these progenitors in the MGE remains to be determined (Fig. 10A, C).

CR-positive cortical interneurons express *URE2-lacZ* (~88%) and *I12b-lacZ* (~80%), suggesting that they may derive from *URE2-lacZ*+/*I12b-lacZ*+ cells found in the mCGE between E13.5 and E14.5 (Fig. 10B). Arguments that support this conclusion and favor the mCGE over the dCGE and vCGE include the following: (1) *URE2* is only active in the VZ of the mCGE (Fig. 2J, supplemental Fig. S1A–C, available at www.jneurosci.org as supplemental material). (2) Previous studies have shown that interneurons expressing CR are derived exclusively from progenitors found in the dorsal CGE between E13.5 and E15.5 (Xu et al., 2004; Butt et al., 2005); however, we do not know whether this domain includes both the dCGE and mCGE.

In utero transplantation studies performed by Butt et al. (2005) showed that most VIP progenitors are located in the dorsal part of CGE; again, we do not know whether this domain includes both the dCGE and mCGE. VIP+ interneurons mainly express *I12b-lacZ* (~85%). The *URE2-lacZ* transgene is expressed in only ~14% of VIP-expressing cells but in 88% of CR+ neurons. In the rat frontal cortex, the CR and VIP populations are largely overlapping in deep cortical layers and partially overlapping in superficial layers (Kubota et al., 1994), suggesting a common origin for these neurons. This contrasts with the differential activities of *URE2* and *I12b* observed in CR+ and VIP+ interneurons. Although expression

of the reporter transgenes was observed at multiple prenatal and postnatal time points, we cannot formally exclude that, at intermediate time points, especially after birth, the CRE expression either began and/or terminated in certain cell populations. Therefore, the results presented in this study do not constitute direct evidence for the existence of a direct lineage relationship between tangentially migrating cells expressing one or more reporter transgenes and the generation of specific subtype(s) of adult cortical interneurons.

In summary, these studies demonstrate that distinct *Dlx* enhancer elements define molecularly distinct subdivisions of the LGE, MGE, and CGE and distinguish some adult cortical interneuron subtypes. Genetic analyses in mice that alter enhancer function, or use these enhancers to perform formal lineage analyses, will establish the function of the individual *Dlx* enhancer elements.

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