# Combinatorial function of the homeodomain proteins Nkx2.1 and Gsh2 in ventral telencephalic patterning

**Joshua G. Corbin[1], Michael Rutlin[1], Nicholas Gaiano[2] and Gord Fishell[1,*]**

[1]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
[2]Department of Neurology, Institute for Cell Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA
*Author for correspondence (e-mail: fishell@saturn.med.nyu.edu)

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## Summary

**Regional patterning of the mammalian telencephalon requires the function of three homeodomain-containing transcription factors, Pax6, Gsh2 and Nkx2.1. These factors are required for the development of the dorsal, lateral and medial domains of the telencephalon, respectively. Previous work has indicated that two of the genes encoding these factors, *Pax6* and *Gsh2*, cross-repress one another in the formation of the border between dorsal and lateral region of the telencephalon. Here, we examine whether similar interactions are responsible for the establishment of other boundaries of telencephalic gene expression. Surprisingly, despite the fact that, at specific times in development, both Pax6 and Gsh2 maintain a complementary pattern of expression with Nkx2.1, in neither case are these boundaries maintained through a similar cross-repressive mechanism. Rather, as revealed by analysis of double-mutant mice, Nkx2.1 and Gsh2 act cooperatively in many aspects to pattern the ventral telencephalon. By contrast, as indicated by both loss- and gain-of-function analysis, Gsh2 expression in the medial ganglionic eminence after E10.5 may negatively regulate Nkx2.1 dependent specification of oligodendrocytes. Therefore, both integrative and antagonistic interactions between homeodomain-containing transcription factors contribute to the patterning of the telencephalon.**

Supplemental data available online

Key words: Telencephalon, Mouse, Patterning, *Pax6*, *Gsh2*, *Nkx2.1*

## Introduction

Development of the vertebrate telencephalon is a complex process that is dependent on the coordinate interactions of extrinsic and intrinsic cues to regulate the growth, patterning, fate specification and migration of cells (reviewed by Wilson and Rubenstein, 2000; Schuurmans and Guillemot, 2002; Rallu et al., 2002b; Campbell, 2003). Foremost among the extrinsic cues that act to establish dorsal (pallial) and ventral (subpallial) domains within the neuraxis is sonic hedgehog (Shh) (reviewed by Ho and Scott, 2002). Although in the telencephalon it is now clear that cues other than Shh must contribute to establishing regional identity (Rallu et al., 2002a), extrinsic signals appear to converge in their induction of a common set of transcription factors. These intrinsic determinants apparently form a transcriptional code, which acts directly to establish regional identity within the telencephalon. Among the factors that contribute to this code are Pax6, Gsh2 and Nkx2.1 (Titf1 – Mouse Genome Informatics), three homeodomain containing transcription factors (reviewed by Wilson and Rubenstein, 2000; Schuurmans and Guillemot, 2002; Rallu et al., 2002b; Campbell, 2003). Recent analysis indicates that all domains of the telencephalon require at least one of these proteins for the proper development of regional pattern.

In the E10.0 mouse telencephalon the expression of Pax6, Gsh2 and Nkx2.1 is complementary, although, as shown here, this situation exists only transiently. Nonetheless, this pattern of gene expression provides a reliable indication of where these genes are required for regional patterning. For example, Pax6, whose expression at E10.0 is restricted to the dorsal telencephalon (pallium), regulates many aspects of cortical development, including specification of progenitor populations (Stoykova et al., 1996; Caric et al., 1997; Götz et al., 1998; Heins et al., 2002). Similarly, Nkx2.1 and Gsh2, whose expression at this stage is confined to the medial and lateral telencephalic domains, respectively, are required for the proper patterning of each of these regions. In *Nkx2.1*$^{-/-}$ mutants, the medial ganglionic eminence (MGE) acquires a lateral ganglionic eminence (LGE) character (Sussel et al., 1999). Moreover, the loss of *Gsh2* results in the ectopic expression of cortical genes throughout much of the LGE (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001).

Both the means by which these three genes regulate one another's expression and their role in establishing regional telencephalic pattern are of considerable interest. In this regard, it is notable that the *Drosophila* gene *vnd* (an ortholog of *Nkx2.1*) functions to repress expansion of lateral fate into the ventral domain of the *Drosophila* nerve cord (Chu et al., 1998; McDonald et al., 1998). Furthermore, the *Drosophila ind* gene (an ortholog of *Gsh2*) is essential for repressing dorsal character within the lateral domain of the fly nerve cord (Weiss et al., 1998). Recent work indicates that similar mechanisms may regulate telencephalic patterning in mice. For example,

the primary function of *Pax6* and *Gsh2*, with regard to regional patterning of the telencephalon, is to cross-repress one another. This is evident both from the complementary expansion of *Gsh2* into the normal *Pax6* domain in *Pax6*$^{-/-}$ mutants (Toresson et al., 2000; Yun et al., 2001), and from the expansion of *Pax6* into the normal territory of *Gsh2* expression in *Gsh2*$^{-/-}$ mice (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Importantly, telencephalic patterning is largely normal in *Gsh2*$^{-/-}$*;Pax6*$^{-/-}$ double mutants (Toresson et al., 2000).

In this study we have analyzed how the complementary patterns of Pax6, Gsh2 and Nkx2.1 expression observed at E10.0 are generated. Interestingly, we find that prior to the onset of Gsh2 expression in the telencephalon, Nkx2.1 expression transiently abuts the Pax6 expression domain at E9.5. Prior to the present study, it was not known whether Nkx2.1 functions to repress either Pax6 or Gsh2 at the stages of development in which their expression patterns are complementary, although such interactions might have been predicted based on the interactions of the orthologs of these genes in the *Drosophila* nerve cord (Chu et al., 1998; McDonald et al., 1998; Weiss et al., 1998). Surprisingly, we find that these genes do not function cross-repressively to establish distinct progenitor domains within the telencephalon. Furthermore, unlike *Gsh2*$^{-/-}$*;Pax6*$^{-/-}$ double mutants, in which the defects observed in the single mutants are rescued in the double mutant, regional patterning is further perturbed in double-mutant mice lacking both *Nkx2.1* and *Gsh2* gene function. Indeed, in many aspects, the phenotype observed in *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ double mutants resembles that observed in *Shh*$^{-/-}$ animals. This indicates that these two genes are primary downstream effectors of the extrinsic signals that act to establish ventral identity in the telencephalon. Hence, although a small ventral telencephalic domain persists in *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ double mutants, it is clear that patterning in this region is largely dependent on the combined function of these genes.

## Materials and methods

### Animals, virus preparation, and injection

All animals used in these studies were maintained according to protocols approved by the Institutional Animal Care and Use Committee at the NYU School of Medicine. Wild-type, heterozygous and homozygous *Gsh2* (Szucsik et al., 1997), *Nkx2.1* (Sussel et al., 1999), *Pax6* (*Sey*) (Hill et al., 1991) and *Shh* (Chiang et al., 1996) mutant embryos were obtained from intercrosses of *Gsh2*$^{+/-}$, *Nkx2.1*$^{+/-}$, *Pax6* (*Sey*)$^{+/-}$ or *Shh*$^{+/-}$ mice. Double *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ mutant mice were obtained by intercrosses of *Gsh2*$^{+/-}$ and *Nkx2.1*$^{+/-}$ mice. For staging of embryos, midday of the vaginal plug was considered as embryonic day 0.5 (E0.5). For viral injection studies, Swiss Webster mice (Taconic Farms, Germantown, New York) were used. Virus preparation and ultrasound surgery were both performed as previously described (Gaiano et al., 1999). CLEG (CLE virus expressing GSH2) was injected into the developing forebrain at titers of 2 to $5\times10^8$ cfu/ml.

### Genotyping

The *Nkx2.1* and *Gsh2* alleles were genotyped by PCR using primers previously described (Nery et al., 2001; Szucsik et al., 1997), with the exception that the PCR reaction was enhanced by the use of GC-rich PCR reagents (Roche). *Pax6* (*Sey*) mice were identified based on eye morphology (Hill et al., 1991) and confirmed by PCR as previously described (Grindley et al., 1995). *Shh* mutants were readily identified based on their severe holoprosencephalic phenotype (Chiang et al., 1996). All genomic DNA for PCR was isolated using a QIAamp Genomic DNA isolation kit (Qiagen).

### RNA in situ hybridization

Whole heads (E9.5-E12.5) or isolated brains (E18.5) were fixed at 4°C in 4% paraformaldehyde for 1-4 hours, rinsed in phosphate-buffered saline (PBS), cryoprotected overnight in 30% sucrose in PBS and embedded in HistoPrep (Fisher Scientific). Embedded tissues were sectioned on a cryostat between 12-20 μm. Section RNA in situ hybridization was performed as described (Schaeren-Wiemers and Gerfin-Moser, 1993; Wilkinson and Nieto, 1993) using non-radioactive DIG-labelled probes. The following probes were used in this study: *Gad67* (Behar et al., 1994), *Mash1* (Guillemot and Joyner, 1993), *Ebf1* (Garel et al., 1997), *Ngn2* (Gradwohl et al., 1996), *Gsh1* (Valerius et al., 1995), *Lhx6* (Grigoriou et al., 1998), *Olig2* (Lu et al., 2000), *Pdgrfa* (Mercola et al., 1990) and *Plp/DM20* (Timsit et al., 1995). Localization of *Dlx2* expression was achieved by X-gal staining (Corbin et al., 2000) of *Dlx2-tau-lacZ* heterozygous animals (Corbin et al., 2000; Nery et al., 2002; Nery et al., 2003), or by in situ hybridization using a probe to *Dlx2* mRNA (Porteus et al., 1991).

### Immunohistochemistry

Tissue was processed for immunohistochemistry as described above. The following antibodies were used for immunofluorescence: mouse anti-Pax6 (1:1000, gift of A. Kawakami), rabbit anti-Pax6 (1:1000, Covance, CA, USA), mouse anti-Nkx2.1 (1:2000, DAKO, CA, USA), rabbit anti-Nkx2.1 (1:150, Biopat, Italy), mouse anti-5E1 (anti-Shh, 1:2000, Developmental Studies Hybridoma Bank, IA, USA), rabbit anti-Crbp1 (1:400, gift of U. Eriksson, Stockholm, Sweden), rabbit anti-Gsh2 (1:2000, gift of K. Campbell, Cincinnati, OH, USA), rabbit anti-GABA (1:1000, Sigma, MO, USA), rabbit anti-Calbindin (1:1000, Calbiochem, CA, USA), rabbit anti-PLAP (1:100, Accurate Chemical, NY, USA). Secondary antibodies used were: FITC-conjugated donkey anti-rabbit, Cy3-conjugated donkey anti-rabbit, Cy3-conjugated donkey anti-mouse, FITC-conjugated donkey anti-mouse (all from Jackson ImmunoResearch, West Grove, PA, USA). Sections were washed in PBS, blocked for 1 hour with PBS containing 10% donkey serum and 0.2% Triton X-100. Sections were incubated in primary antibodies diluted in block (with 10% serum) overnight at 4°C, then washed three times in PBS and incubated with secondary antibodies diluted in PBS containing 1% donkey serum and 0.2% Triton X-100 for 1-2 hours at room temperature in the dark. Fluorescent images were obtained using either a cooled-CCD camera (Princeton Instruments) and Meta-morph software (Universal Imaging, West Chester, Pennsylvania) or a confocal microscope (Leica).

## Results

### Expression of Shh, Nkx2.1, Gsh2 and Pax6 during early telencephalic development

Of the four genes we examined, *Pax6* is expressed earliest and is first detected at 5 somites throughout the anterior neural plate (Inoue et al., 2000; Bell et al., 2001). By 8 somites, expression of *Pax6* in the telencephalon is excluded from the ventral-most regions. *Nkx2.1* expression is initially observed slightly later at 10-12 somites, followed shortly by the nested expression of *Shh* in the ventral telencephalon (Shimamura et al., 1995; Sussel et al., 1999). *Gsh2* expression is the last to be observed, beginning at about E10.0 (Corbin et al., 2000). Currently, how the expression of these genes changes relative to one another during early telencephalic development has not been well characterized. We therefore performed an analysis

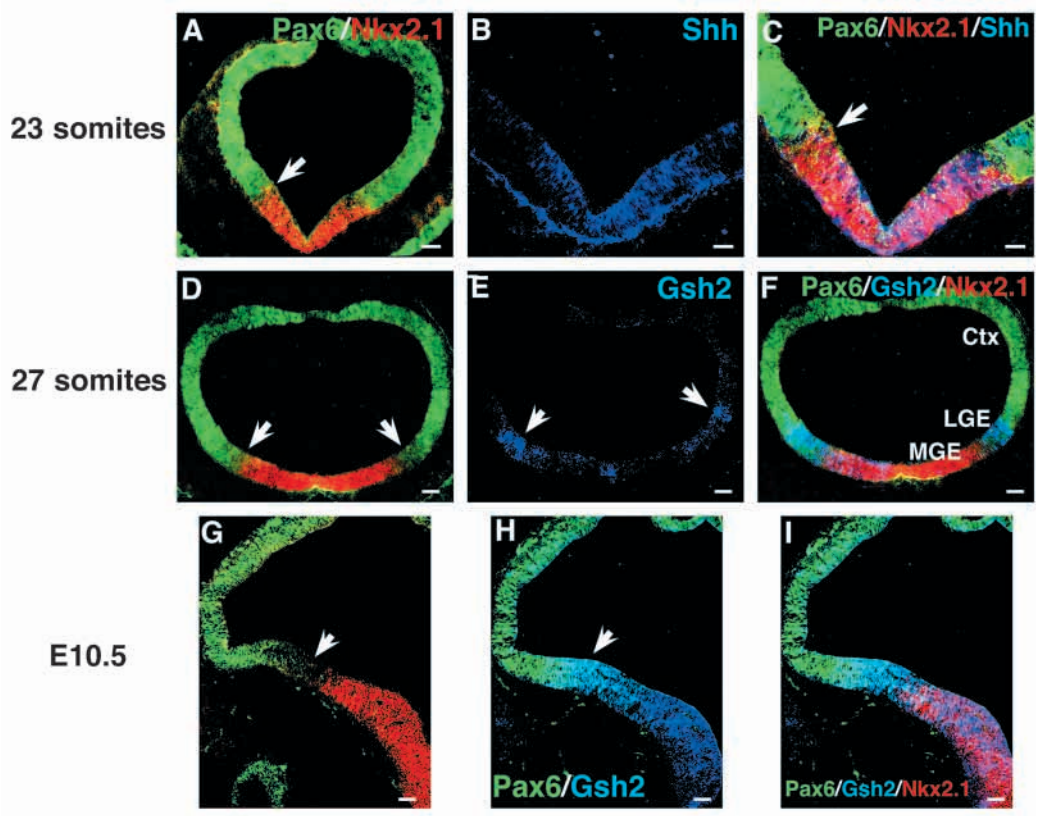



**Fig. 1.** Expression of patterning genes between E9.5 and E10.5. (A) As shown in coronal sections, at 23 somites (~E9.5), expression of Pax6 (green) and Nkx2.1 (red) forms a boundary in the ventral telencephalon (arrow). (B) Higher power view shows Shh (blue) expression in the ventral-most telencephalon. (C) The border of Pax6 and Nkx2.1 expression, and nested Shh expression, is also shown. (D) By 27 somites (~E10.0), a slight gap (arrows) appears between Pax6 (green) and Nkx2.1 (red) expression; (E,F) Gsh2 is (blue) expressed laterally (arrows in E). (G) By E10.5, the gap in the expression of Pax6 (green) and Nkx2.1 (red) expands (arrow). (H,I) Gsh2 (blue) is expressed in this gap (I) and is partially overlapping (white) with Pax6 expression (green; arrow; H). (I) At this stage, expression of Gsh2 (blue) is also no longer restricted to the lateral domain, but expands more ventrally into the Nkx2.1-positive (red) domain. Ctx, cerebral cortex; LGE, presumptive future lateral ganglionic eminence; MGE, presumptive future medial ganglionic eminence. Scale bars: 50 μm (A,D,E,F); 25 μm (B,C).

of the expression patterns of the proteins encoded by these genes.

At 23 somites (~E9.5), before the onset of Gsh2 expression, Nkx2.1 and Pax6 expression is complementary and forms a clear boundary in the subpallium (Fig. 1A,C). At this stage, Shh is expressed in the ventral-most telencephalon nested within the Nkx2.1 positive domain (Fig. 1B,C; see Fig. S1A at http://dev.biologists.org/supplemental/). By 27 somites (~E10.0), a small gap appears between Nkx2.1 and Pax6 expression in the lateral telencephalon (Fig. 1D). At this stage, Gsh2 expression is first observed in this lateral region in a pattern complementary to Pax6, and largely complementary to Nkx2.1 expression (Fig. 1E,F). By E10.5, a larger gap between Nkx2.1 and Pax6 expression is observed (Fig. 1G), and Gsh2 expression expands ventrally into the Nkx2.1 positive domain (Fig. 1H,I). At E10.5, expression of Gsh2 and Pax6 partially overlaps (Fig. 1H) (Toresson et al., 2000); however, by E12.5 this border is more sharply defined (Rallu et al., 2002a) (data not shown).

By 36-38 somites (E10.25-E10.5), Gsh2-expressing cells are no longer restricted laterally, and begin to appear more medially in a posterior to anterior gradient (Fig. 2A-F). At anterior levels, Gsh2 expression remains restricted to the ventrolateral region (Fig. 2A). Further posterior, at the level of the septum (Fig. 2B) and anterior MGE (Fig. 2C), Gsh2 expression remains mostly laterally restricted, but a few Gsh2-positive cells are observed more ventrally (Fig. 2B). However, in the more caudal MGE and LGE more Gsh2-positive cells are present within the Nkx2.1 positive domain (Fig. 2D,E). At the most posterior region of the telencephalon, Gsh2 is highly expressed in the caudal ganglionic eminence (CGE) (Fig. 2F). At this level, Nkx2.1 expression is only observed in the diencephalon. In summary, these data reveal that between E9.5 and E10.5 expression of Nkx2.1, Gsh2 and Pax6 in the telencephalon is highly dynamic, and during a brief period (~1/2 day) these three proteins are expressed in a complementary pattern.

### At developmental stages of complementary expression, neither Pax6 nor Gsh2 are cross-repressive with Nkx2.1

The complementary expression of Nkx2.1 and Pax6 at 23 somites, and of Gsh2 and Nkx2.1 at 27 somites, suggests that these proteins may be acting cross-repressively to generate distinct domains within the telencephalon. To explore this possibility, we analyzed mice lacking the genes encoding these proteins to determine whether there were changes in gene expression consistent with such cross-repressive interactions (i.e. expansion of gene expression across boundaries). In the absence of *Pax6* gene function (in *Sey/Sey* mutant mice), expression of Nkx2.1 does not expand dorsally (Fig. 3A,B). Conversely, in *Nkx2.1*$^{-/-}$ mutant mice, expression of Pax6 does not expand ventrally (Fig. 3C,D). Hence, at ~E9.5, when the expression of Pax6 and Nkx2.1 form a boundary, Pax6 and Nkx2.1 do not cross repress one another. Notably, *Nkx2.1*$^{-/-}$ mutants display a loss of telencephalic Shh (Sussel et al., 1999) (see Fig. S1A,B at http://dev.biologists.org/supplemental/), indicating that telencephalic Shh is not required to repress

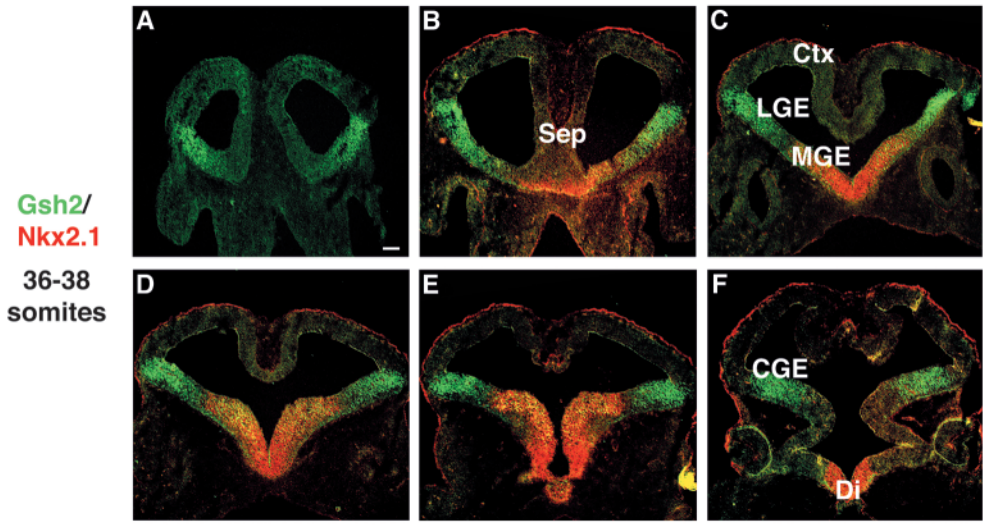
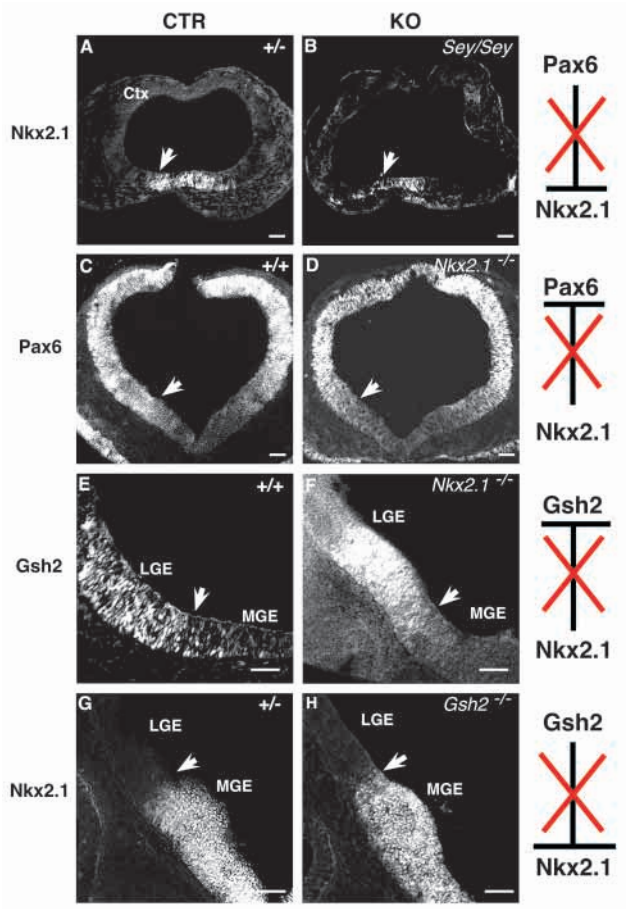



**Fig. 2.** Telencephalic expression of Gsh2 and Nkx2.1 at 36-38 somites. Expression of Gsh2 (green) and Nkx2.1 (red) on coronal telencephalic sections is shown from anterior (A) to posterior (F). (A) In the anterior telencephalon, Gsh2 is expressed in the lateral domain; Nkx2.1 is not expressed at this level. (B) The most anterior expression of Nkx2.1 is at the level of the septum, where Gsh2 expression is non-overlapping with Nkx2.1 expression. (C-E) More posteriorly, some Gsh2-positive cells (yellow) begin to be observed within the Nkx2.1-positive domain. (F) At the most posterior level of the telencephalon, Gsh2 is highly expressed in the region of the presumptive CGE, whereas Nkx2.1 is expressed only in the diencephalon. CGE, presumptive future caudal ganglionic eminence; Di, diencephalon; Sep, septum. Scale bar: 50 μm (A-F).

Pax6 expression from the subpallium. Thus, Shh-dependent repression of Pax6 in the subpallium (Chiang et al., 1996) is mediated by non-telencephalic sources of Shh (M. Fuccillo and G.F., unpublished). Moreover, Pax6 does not regulate the domain of ventral telencephalic Shh expression because the expression of Shh is unaltered in *Sey/Sey* mutant mice (see Fig. S1C,D at http://dev.biologists.org/supplemental/).




Examination of potential interactions between *Gsh2* and *Nkx2.1* prior to when Gsh2 expression partially expands into the Nkx2.1 domain also provided no evidence for cross-repression between these genes (or gene products). In *Nkx2.1*$^{-/-}$ mice, Gsh2 expression does not expand (Fig. 3E,F). Conversely, in *Gsh2*$^{-/-}$ mice, Nkx2.1 expression remains restricted to its normal ventral domain (Fig. 3G,H). Interestingly however, retroviral-mediated ectopic expression of Gsh2 at in the MGE at E9.5, is sufficient to repress Nkx2.1 expression (Fig. 4A-F). Notably, Gsh2-expressing retrovirus was delivered at E9.5, a time that is before Gsh2-expressing cells are normally observed in the MGE (~E10.5). Thus, Gsh2 gain- and loss-of-function experiments reveal that although Gsh2 is sufficient to repress Nkx2.1 expression in the medial domain prior to its normal onset of expression, it is not necessary to repress Nkx2.1 expression in the lateral domain. Previous studies have also revealed that *Nkx2.1* expression does not expand dorsally in the absence of either *Gsh1* alone, or in the combined absence of both *Gsh1* and *Gsh2*, indicating that neither of the known Gsh genes repress *Nkx2.1* gene function in the LGE (Toresson and Campbell, 2001). Hence, although Pax6 and Gsh2 function to cross-repress one another (Toresson et al., 2000; Yun et al., 2001) (data not shown) during the developmental periods when Pax6 and Nkx2.1, and Nkx2.1 and Gsh2, maintain complementary patterns of expression, they do not function cross-repressively.

**Fig. 3.** Analysis of potential cross-repressive interactions in the early telencephalon. (A-H) Immunohistochemical analysis of Nkx2.1, Gsh2 and Pax6 expression on coronal sections in *Pax6*$^{-/-}$ (*Sey/Sey*), *Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ mutant mice, respectively. Arrows mark the ventral or dorsal limit of expression of each protein. (A,B) At 22 somites, when expression of Pax6 and Nkx2.1 form a distinct border, Nkx2.1 expression does not expand dorsally in *Sey/Sey* mutants. (C,D) At this same stage, Pax6 expression also does not expand ventrally in *Nkx2.1*$^{-/-}$ mutants. (E,F) Between 26 and 30 somites, in *Nkx2.1*$^{-/-}$ mutants, Gsh2 expression in the lateral domain does not expand ventrally into the presumptive MGE region. (G,H) Conversely, in *Gsh2*$^{-/-}$ mutants at 31-32 somites, Nkx2.1 expression remains restricted ventrally. Scale bars: 50 μm (A-H).

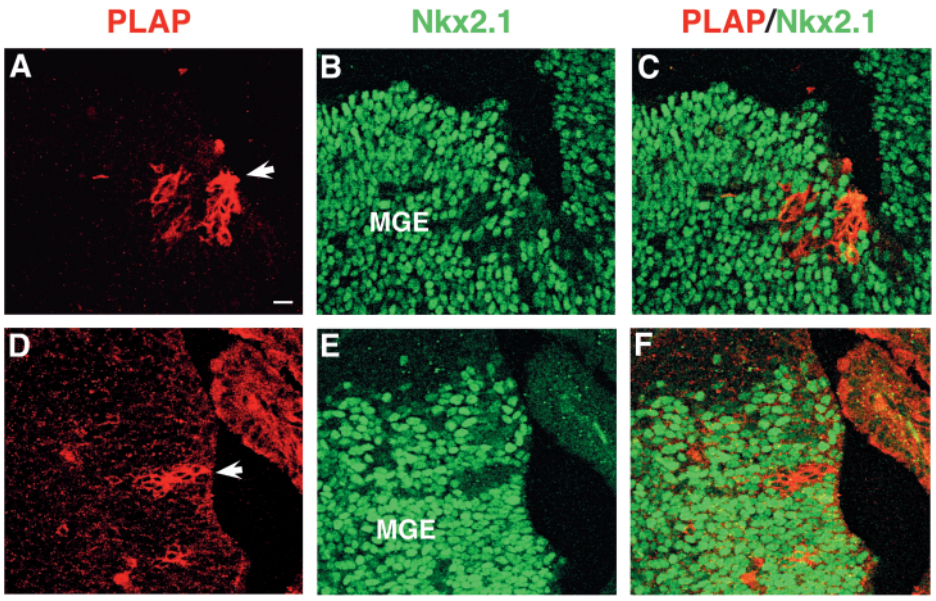



**Fig. 4.** Retroviral expression of Gsh2 represses Nkx2.1 expression in the MGE. Gsh2-expressing retroviruses were delivered to the developing telencephalon at E9.5 and analyzed at E14.5. Expression of bi-cistronic retroviral inserts was assayed by PLAP reporter expression. (A,D) Retrovirally infected, PLAP-expressing cells (red) are shown in the MGE (arrows) in coronal sections. (B,E) Nkx2.1 expression (green) in the MGE is repressed in clusters of retroviral expression. (C,F) Overlay of PLAP and Nkx2.1 shows a direct correspondence between retrovirally infected cells (red) and repression of Nkx2.1 expression (green). Scale bar in A: 25 μm (A-F).

### Comparison of ventral telencephalic patterning defects in *Shh*$^{-/-}$ single and *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ compound mutant mice

*Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ mutant mice display defective patterning of the MGE and LGE, respectively. In *Nkx2.1*$^{-/-}$ mutant mice there is a conversion of the MGE to an LGE fate (Sussel et al., 1999). Conversely, the loss of *Gsh2* in mice results in severe patterning defects in the LGE. In *Gsh2*$^{-/-}$ mutants, there is a loss in the expression of pan-ventral telencephalic genes (e.g. *Dlx1/2*, *Mash1*), in all but the ventral-most domain of the LGE (Suczick et al., 1997; Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001), that is combined with an expansion of pallial markers, such as *Pax6* and *Ngn2*. These results indicate that *Nkx2.1* and *Gsh2* may act in combination to pattern both the MGE and LGE. To investigate the effect of the combined loss of these genes on early ventral telencephalic patterning, *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ compound mutant mice were generated and the expression of a variety of subpallial markers (*Dlx2*, *Mash1*, *Gsh1*, *Lhx6*) was analyzed. As loss or reduction of *Nkx2.1* and *Gsh2* gene expression are two of the more prominent abnormalities seen in the telencephalon of *Shh*$^{-/-}$ mutants (Corbin et al., 2000; Pabst et al., 2000; Rallu et al., 2002a), patterning defects in *Shh*$^{-/-}$ mutant mice were also compared with that of double *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutant mice.

At E12.5, the patterning genes *Dlx2* (Fig. 5A) and *Mash1* (Fig. 5F) are expressed pan-ventrally. In the absence of *Gsh2*, expression of both *Dlx2* and *Mash1* is lost in the dorsal-most two thirds of the LGE (Fig. 5B,G) (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). In *Nkx2.1*$^{-/-}$ mutant mice, the domain of *Dlx2* and *Mash1* expression is unaffected (Fig. 5C,H) (Sussel et al., 1999). In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double-mutant mice, *Dlx2* and *Mash1* expression is significantly reduced (Fig. 5D,I). A similar persistence of *Dlx2* and *Mash1* expression is observed in the ventral-most region of the mutant telencephalon of less severely affected *Shh* mutant mice (Fig. 5E,J) (Rallu et al., 2002a) (K. Campbell, personal communication).

*Gsh1*, the other known member of the Gsh family, is expressed in the MGE and the ventral-most LGE (Fig. 5K) (Toresson and Campbell, 2001). Functioning in combination with *Gsh2*, *Gsh1* plays a key role in both patterning of the LGE and generation of early born striatal cells (Toresson and Campbell, 2001). In *Gsh2*$^{-/-}$ mutant mice, *Gsh1* expression expands dorsally into the LGE (Fig. 5L) (Toresson and Campbell, 2001). In *Nkx2.1*$^{-/-}$ mutant mice, a small domain of *Gsh1* expression persists in the subpallium (Fig. 5M). A similar level of *Gsh1* expression is also observed in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutant mice (Fig. 5N). By contrast, *Gsh1* expression is completely lost in all *Shh*$^{-/-}$ mutants analyzed (*n*=9), regardless of their severity (Fig. 5O). At E12.5, *Lhx6*, a LIM homeodomain-containing transcription factor gene, is expressed in the MGE and MGE-derived cells that migrate to the LGE and cortex (Fig. 5P) (Lavdas et al., 1999). Expression of *Lhx6* is unaffected in *Gsh2*$^{-/-}$ mutants (Fig. 5Q), but is completely lost in *Nkx2.1*$^{-/-}$ mutants (Fig. 5R) (Sussel et al., 1999). In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (Fig. 5S), and in all *Shh*$^{-/-}$ mutants analyzed (Fig. 5T) (K. Campbell, personal communication), *Lhx6* expression is also lost. Taken together, these analyses reveal that the loss of both *Nkx2.1* and *Gsh2* results in more severe patterning defects than the loss of either *Nkx2.1* or *Gsh2* alone. Furthermore, specific changes in subpallial gene expression (*Dlx2*, *Mash1*, *Lhx6*) mimic that found in *Shh*$^{-/-}$ mutant mice.

### *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutant mice display combined phenotypes of both *Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ single mutants

To further compare the phenotype observed in *Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ single versus compound mutants, we analyzed the expression of LGE (Crbp1, *Ebf1*) and pallial (*Ngn2*, Pax6) specific markers in mice bearing these genotypes. Expression of the pallial markers *Ngn2* and Pax6 normally extends just across the cortical-striatal sulcus into the dorsolateral LGE (Fig. 6A,E,M). In *Gsh2*$^{-/-}$ mutants, expression of both *Ngn2* and Pax6 extends ectopically into all but the ventral third of the LGE (Fig. 6B,F,N) (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Expression of *Ngn2* and Pax6 in *Nkx2.1*$^{-/-}$ mutants (Fig. 6C,G,O) resembles that observed in control embryos. In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (Fig.

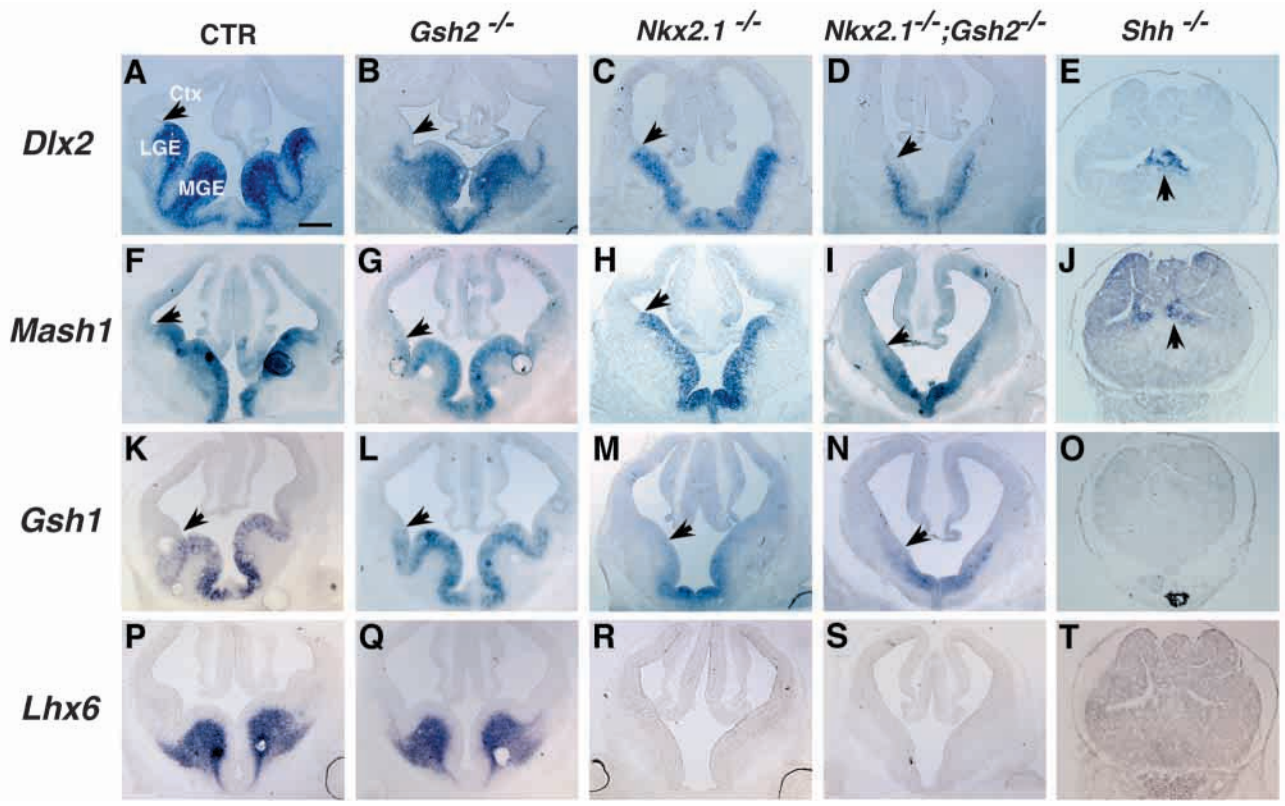



**Fig. 5.** Comparison of ventral patterning defects in *Nkx2.1*$^{-/-}$, *Gsh2*$^{-/-}$ and *Shh*$^{-/-}$ single mutants, and *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants. At E12.5, *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double-mutant mice (D,I,N,S) display a notable decrease in the size of ventral telencephalic structures and a reduction in the expression of *Dlx2*, *Mash1*, *Gsh1* and *Lhx6* compared with controls (A,F,K,P). Comparison with single *Gsh2*$^{-/-}$ mutants (B,G,L,Q) and single *Nkx2.1*$^{-/-}$ mutants (C,H,M,R) is also shown. Arrows (A-N) show the dorsal limits of *Dlx2*, *Mash1* and *Gsh1* gene expression. In less severely affected *Shh*$^{-/-}$ mutants, expression of *Dlx2* and *Mash1* (arrows) persists in the ventral telencephalon (E,J). By contrast, expression of *Gsh1* and *Lhx6* is never detected in these animals (O,T). Scale bar in A: 200 μm for A-D,F-I,K-N,P-S; 275 μm for E,J,O,T.

6D,H,P) the expansion of *Ngn2* and Pax6 into the subpallium is similar to that observed in *Gsh2*$^{-/-}$ mutants. As in the *Gsh2*$^{-/-}$ single mutants, this expansion extends to the level of remnant subpallial gene expression (Fig. 5D,I,N).

Consistent with the MGE adopting LGE character in *Nkx2.1*$^{-/-}$ mutants (Sussel et al., 1999) (Fig. 6K,O), the LGE-specific marker Crbp1 expands ventrally into the MGE in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (Fig. 6L,P). Similar changes are observed in the expression of the helix-loop-helix transcription factor gene *Ebf1*, an early marker of striatal (LGE-derived) projection neurons (Fig. 6Q) (Garel et al., 1997; Garel et al., 1999). In *Gsh2*$^{-/-}$ mutants, *Ebf1* expression is significantly reduced (Fig. 6R) (Corbin et al., 2000), and in *Nkx2.1*$^{-/-}$ mutants, expression of *Ebf1* expands ventrally (Fig. 6S). Although the level of expression is reduced, *Ebf1* expression also expands ventrally in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (Fig. 6T). Notably, *Ebf1* expression is never observed in *Shh*$^{-/-}$ mutant mice ($n$=9) (data not shown). In summary, these data demonstrate that the defects observed in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutant mice resemble a combination of the phenotypes observed in single *Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ mutants. Similar to *Nkx2.1*$^{-/-}$ mutants, *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants display a conversion of the MGE to an LGE fate, and, similar to *Gsh2* mutants, expression of LGE genes is supplanted by expression of pallial markers throughout the dorsal two-thirds of the LGE.

### Interneuron and oligodendrocyte specification in the absence of *Nkx2.1* and *Gsh2*

Significant numbers of interneurons and oligodendrocytes arise from the subpallium, and populate the pallium via tangential migration (reviewed by Corbin et al., 2001; Marin and Rubenstein, 2001). Furthermore, a number of genes, most prominently *Dlx1/2* and *Mash1*, have been hypothesized to be involved in the specification of both cell types (reviewed by Bertrand et al., 2002). Reduction in the expression of these genes in mutant mice lacking both *Nkx2.1* and *Gsh2* gene function (Fig. 5) indicates that specification of oligodendrocytes and interneurons in ventral regions may also be affected. *Gad67*, the precursor enzyme for formation of GABA (Behar et al., 1994), marks developing ventral interneuron populations, many of which subsequently undergo long range tangential migration to the developing cortex. In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants, *Gad67* expression is significantly reduced (Fig. 7A-D). Interestingly, in less severely affected *Shh*$^{-/-}$ mutants, expression of *Gad67* persists (Fig. 7E). As *Gad67* also marks developing striatal projection neurons, the status of cortical interneurons in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutant mice was determined by the analysis of calbindin and GABA expression at E18.5. At a level similar to the cortical interneuron defect in single *Nkx2.1*$^{-/-}$ mutants (Sussel et al., 1999), generation of cortical interneurons in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants is markedly reduced (Fig. 8A-D).

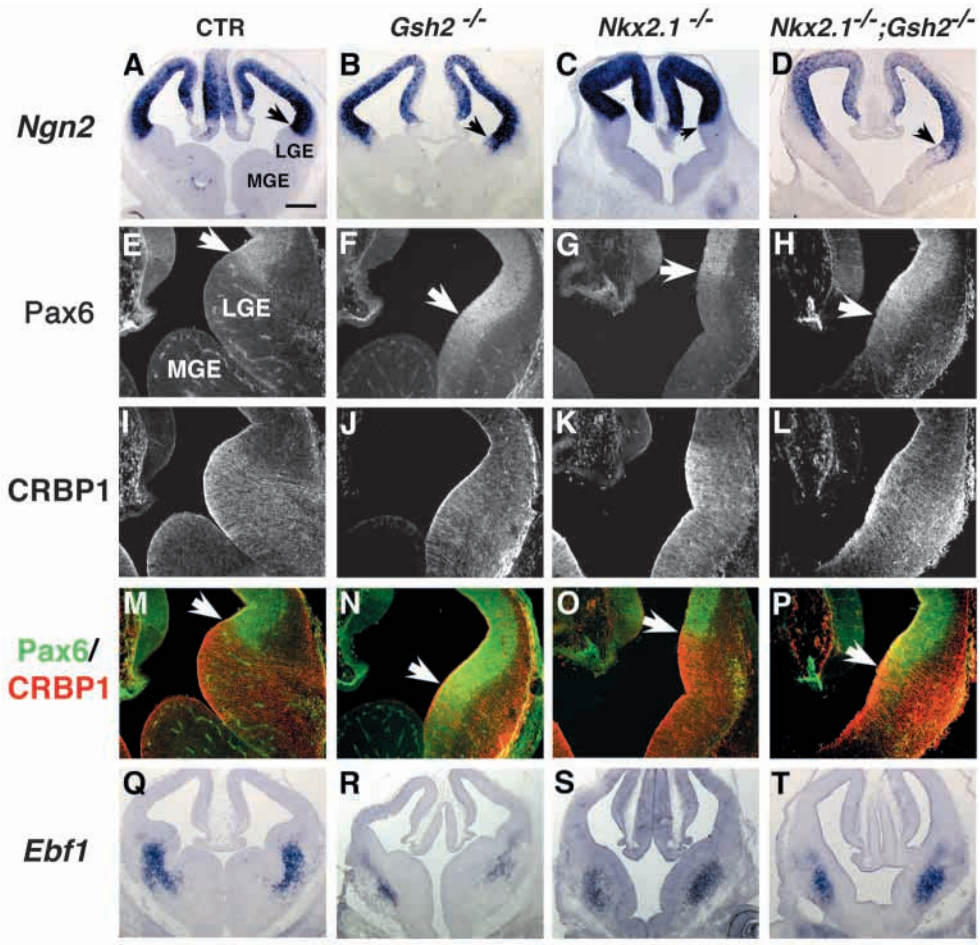



**Fig. 6.** Expansion of dorsal gene expression and reduction of *Ebf1*-positive cells in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mice. In situ hybridization and immunohistochemical analysis on coronal sections at E12.5. *Ngn2* and Pax6 expression normally extends just across the cortical-striatal sulcus into the dorsal LGE (A,E,M), and CRPB1 expression marks LGE radial glia (I). In *Gsh2*$^{-/-}$ mutants, expression of *Ngn2* (B) and Pax6 (F,N) expands ventrally into the CRBP1-positive (J,N) mutant LGE. By contrast, in *Nkx2.1*$^{-/-}$ mutants, *Ngn2* (C) and Pax6 (G,O) expression does not expand into the subpallium, but CRBP1 expression (K,O) expands ventrally into the mutant MGE. Similar to *Gsh2*$^{-/-}$ mutants, *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants display a significant expansion of *Ngn2* (D) and Pax6 (H,P) into the ventral-most telencephalon. Arrows show the ventral-most limit of *Ngn2* (A-D) and Pax6 (E-H,M-P) expression in control and mutant brains. Similar to *Nkx2.1*$^{-/-}$ mutants, CRBP1 expression also expands ventrally *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (L,P). *Ebf1* is expressed in differentiating cells of the LGE (Q). *Ebf1* expression is reduced in the LGE in *Gsh2*$^{-/-}$ mutants (R) and expanded ventrally in *Nkx2.1*$^{-/-}$ mutants (S). In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutants, *Ebf1* expression is both reduced and expanded ventrally (T). Scale bar in A: 200 μm for A-D,Q-T; 100 μm for E-P.

During telencephalic development, oligodendrocyte precursors are marked by the expression of either *Pdgfra* or *Plp/DM20* (Spassky et al., 1998; Perez-Villegas et al., 1999; Nery et al., 2001). Moreover, Olig genes are necessary and sufficient for the generation of oligodendrocytes throughout the neuraxis (reviewed by Marquardt and Pfaff, 2001; Sauvageot and Stiles, 2002). Therefore, the status of oligodendrocytes in single and double *Nkx2.1*$^{-/-}$ and *Gsh2*$^{-/-}$ mutants was examined by the expression of *Olig2*, *Pdgfra* and *Plp/DM20*. *Olig2* is expressed in the MGE, LGE and CGE (Fig. 7F and data not shown). In *Gsh2*$^{-/-}$ mutants, *Olig2* expression is reduced in the LGE (Fig. 7G) to a level similar to the reduction of *Dlx2* and *Mash1* expression (Fig. 5B,G). In *Nkx2.1*$^{-/-}$ mutants, expression of *Olig2* is unaffected (Fig. 7H). In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants, expression of *Olig2* at E12.5, although significantly reduced, persists in the ventral-most telencephalon (Fig. 7I). In addition, consistent with previous observations (Alberta et al., 2001), the expression of *Olig2* is lost in all *Shh*$^{-/-}$ mutant mice examined (Fig. 7J). Expression of *Pdgfra* is observed in the MGE (Fig. 7K), a known source of oligodendrocyte progenitors (Nery et al., 2001; Tekki-Kessaris et al., 2001). In *Gsh2*$^{-/-}$ mutants, the normal population of *Pdgfra*-positive cells in the MGE is unaffected, but *Pdgfra* is ectopically expressed in the VZ of the MGE and in the ventral-most LGE (Fig. 7L). By contrast, *Pdgfra* expression is absent in *Nkx2.1*$^{-/-}$ mutants (Fig. 7M) (Nery et al., 2001; Tekki-Kessaris et al., 2001). In *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants, although the ventral-most population *Pdgfra* is lost, there is strong expression of *Pdgfra* in the lateral domain, similar to that observed in the ventral LGE in *Gsh2*$^{-/-}$ mutants. *Plp/DM20* marks distinct, more caudal populations of subpallial derived oligodendrocytes that arise, in part, from the presumptive amygdaloid region of the caudal ventral telencephalon (Fig. 7P). In contrast to the *Pdgfra*-positive population, the generation of *Plp/DM20*-positive cells is not dependent on *Gsh2* (Fig. 7Q), *Nkx2.1* (Fig. 7R) (Nery et al., 2001), or the combined function of *Nkx2.1* and *Gsh2* (Fig. 7S). However, the expression of both *Pdgfra* and *Plp/DM20*, is lost in *Shh*$^{-/-}$ mutants (Fig. 7O,T). In summary, these data indicate that the loss of both *Nkx2.1* and *Gsh2* gene function has significant effects on the generation of interneurons and oligodendrocytes.

## Discussion

Homeodomain containing proteins play an essential role in patterning of the vertebrate telencephalon. Of these genes, *Nkx2.1*, *Gsh2* and *Pax6* are key regulators of MGE, LGE and cortical development, respectively. We demonstrate that these

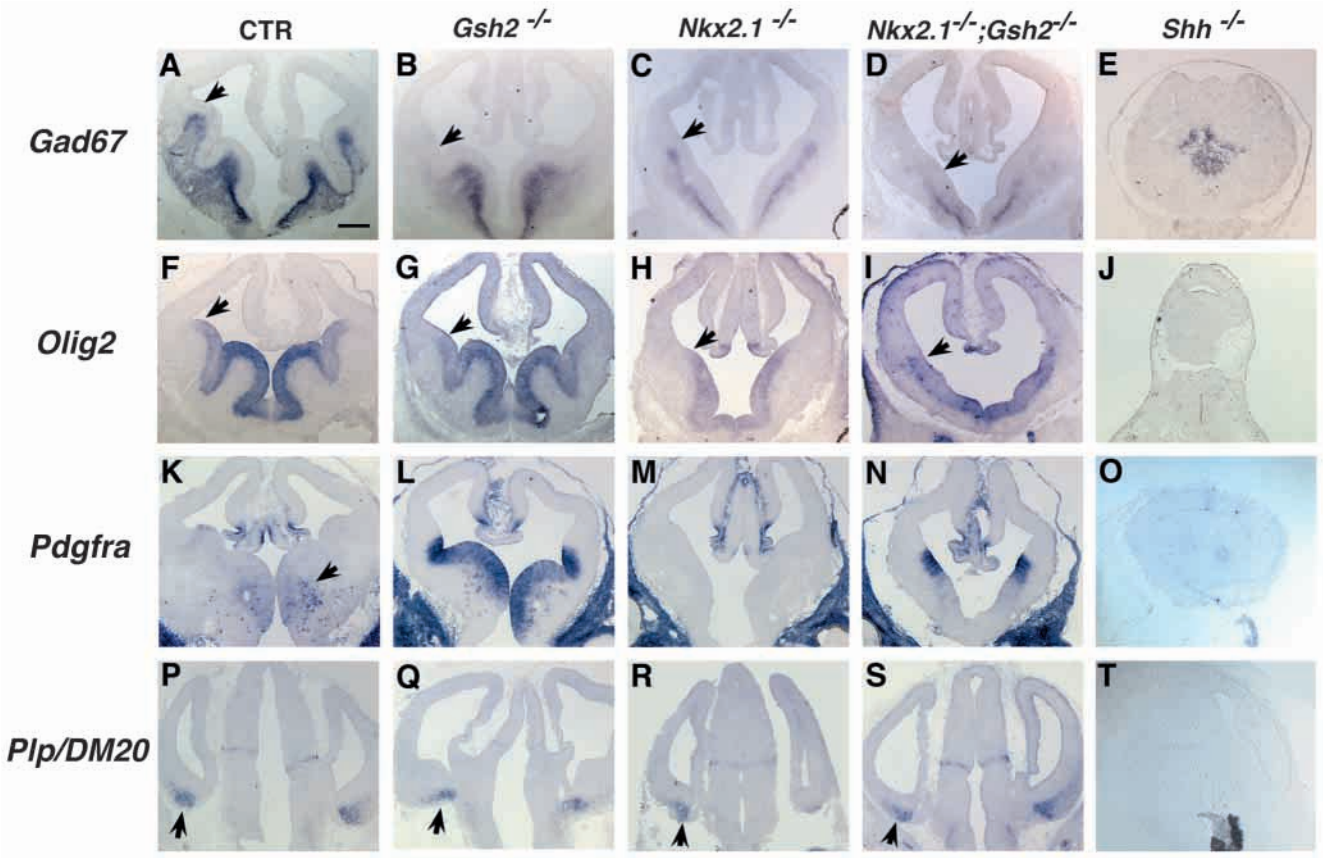



**Fig. 7.** Interneuron and oligodendrocyte specification. The status of developing interneurons and oligodendrocytes was examined on coronal sections at E12.5. *Gad67* marks developing inhibitory neuron populations and is significantly reduced in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double-mutant mice (D) compared with control (A), *Gsh2*$^{-/-}$ (B) or *Nkx2.1*$^{-/-}$ (C) single mutant mice. *Gad67* expression, although reduced and restricted to the ventral midline, persists in *Shh*$^{-/-}$ mutants (E). *Olig2* is expressed in the VZ of the LGE and MGE (F). In *Gsh2*$^{-/-}$ mutants, *Olig2* expression is reduced in the mutant LGE (G). By contrast, *Olig2* expression appears unaffected in *Nkx2.1*$^{-/-}$ mutants (H). *Olig2* expression is reduced in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutants (I), and is absent in *Shh*$^{-/-}$ mutants (J). Arrows show dorsal limit of expression of *Gad67* (A-D) and *Olig2* (F-I) in control and mutant brains. Expression of *Pdgfra* is normally observed in the MGE as punctate staining (K; arrow). In *Gsh2*$^{-/-}$ mutants, *Pdgfra* is ectopically expressed in the VZ of the MGE and the ventral LGE (L), but is completely absent in *Nkx2.1*$^{-/-}$ mutants (M). *Pdgfra* is ectopically expressed in the lateral domain in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants (N), and is absent in *Shh*$^{-/-}$ mutants (O). By contrast, *Plp/DM20* expression (P; arrows) is unaffected in *Gsh2*$^{-/-}$ (Q) or *Nkx2.1*$^{-/-}$ (R) single, or *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double (S), mutants. However, in *Shh*$^{-/-}$ mutants expression of *Plp/DM20* is lost (T). Scale bar in A: 200 μm for A-D,F-I,K-N,P-S; 275 μm for E,J,O,T.

genes undergo dynamic changes in their expression pattern during a brief developmental time period (~E9.5-E10.5). Although both Nkx2.1 and Pax6, and Nkx2.1 and Gsh2 have periods where their expression patterns are largely complementary, we find no evidence to suggest that these domains of gene expression are maintained through cross-repression. However, our gain-of-function data demonstrates that Gsh2 can repress Nkx2.1 when ectopically expressed in the MGE, indicating that redundant factors may prevent Nkx2.1 expansion into the LGE of *Gsh2*$^{-/-}$ mutants. Furthermore, *Nkx2.1* and *Gsh2* compound mutants have a more severe phenotype within the ventral telencephalon than mutants lacking either of these genes individually. This suggests that unlike *Pax6* and *Gsh2*, whose functions appear to be largely antagonistic to one another (Toresson and Campbell, 2000; Corbin et al., 2000; Yun et al., 2001), *Gsh2* and *Nkx2.1* act cooperatively to pattern the ventral telencephalon. Interestingly, our gain- and loss-of-function data also indicate that the developmental lag in the expression of Gsh2 in the medial domain may have important implications for Nkx2.1-dependent early specification of oligodendrocytes.

### Interactions between homeodomain genes in establishing the early telencephalon

In the developing spinal cord, distinct progenitor domains are established via cross-repressive interactions between Shh-induced class II genes (*Nkx2.2*, *Nkx2.9*, *Nkx6.1* and *Olig2*) and Shh-repressed class I genes (*Dbx1*, *Dbx2* and *Pax6*) (reviewed by Marquardt and Pfaff, 2001; Kessaris et al., 2001). To date, with the exception of the bHLH-containing *Olig2* gene, these genes are members of the homeodomain-containing transcription factor family. Similarly, in the telencephalon, the homeodomain containing genes *Pax6* and *Gsh2* function cross-repressively in the establishment of the molecular boundary at the cortico-striatal junction (Toresson et al., 2000; Yun et al., 2001). Despite this, the present findings demonstrate that, at least in the telencephalon, the apposing expression patterns of homeodomain proteins are not always dependent on cross-

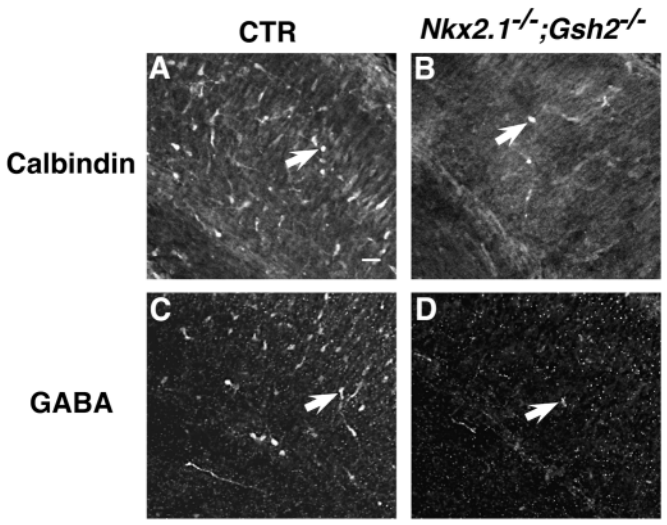



**Fig. 8.** Reduction of cortical interneurons in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutants. E18.5 coronal sections were immunostained for the interneuronal markers Calbindin and GABA. A marked reduction in both Calbindin-positive (A,B) and GABA-positive cortical interneurons (C,D) is observed in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutants (B,D) compared with controls (A,C). Arrows show individual interneurons. Scale bar: 25 μm (A-D).

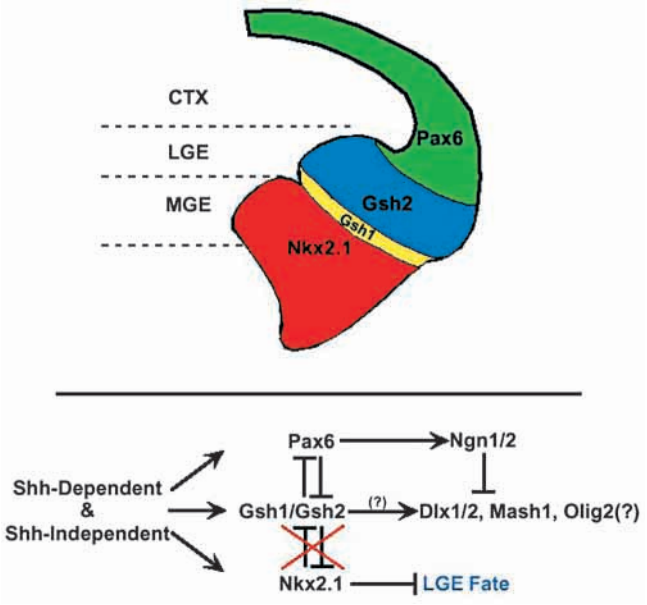



**Fig. 9.** Schematic of homeodomain interactions that pattern the telencephalon. Diagram represents a coronal hemisection of an E12.5 telencephalon showing domains of homeodomain gene expression. The outline of the major genetic interactions governing telencephalic development is incorporated from the results of this study and others (Wilson and Rubenstein, 2000; Schuurmans and Guillemot, 2002; Rallu et al., 2002b; Campbell, 2003). Shh, via repression of the repressive action of *Gli3*, is required for normal ventral patterning. Shh is necessary and sufficient for the expression of *Nkx2.1*, which functions to repress LGE character in the MGE. However, this function of *Nkx2.1* is not mediated through repression of *Gsh1* and/or *Gsh2*. Conversely, *Gsh1* and *Gsh2* are not required to repress *Nkx2.1* expression. By contrast, *Gsh2*, whose expression is regulated both via Shh-dependent and Shh-independent pathways, functions to repress dorsal character in all but the ventral-most one third of the LGE via cross-repression with *Pax6*. Patterning of the ventral-most one third of the LGE is dependent on *Gsh1* gene function, whose expression, similar to *Nkx2.1*, is dependent on Shh. Expression of *Dlx2*, *Mash1* and *Olig2* is mediated either directly through *Gsh1* and *Gsh2* and/or indirectly through *Pax6*. Residual expression of *Dlx2*, *Mash1*, *Olig2* and *Gad67* in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ mutants is hypothesized to be attributable to the persistence of *Gsh1* expression.

repression. In the case of Nkx2.1 and Pax6 this is particularly surprising. In the ventral spinal cord, Nkx (Nkx2.2 and Nkx2.9) and Pax6 function via a cross-repressive mechanism in establishment of the P3 progenitor domain (Briscoe et al., 1999; Briscoe et al., 2000). Indeed, previous studies have indicated that these genes may function cross-repressively in the telencephalon (Sussel et al., 1999; Stoykova et al., 2000), implying that the mechanisms involved in patterning of the telencephalon and spinal cord are analogous (reviewed by Wilson and Rubenstein, 2000; Marin and Rubenstein, 2001). However, this proposal was based, in part, on the observations of the effect of *Nkx2.1* and *Pax6* loss-of-function mutants at later times during development (E11-E13). At this stage, *Nkx2.1*$^{-/-}$ mutants display an expansion of *Pax6* mRNA into the LGE, whereas, in *Pax6*$^{-/-}$ (*Sey/Sey*) mutants, *Nkx2.1*-positive cells are found ectopically in the LGE in a pattern resembling an increase in tangential migration. Notably however, by this time in development, Pax6 and Nkx2.1 expression is separated by the Gsh2 positive domain and they no longer appose each other. Although these studies reveal important later functions of *Nkx2.1* and *Pax6* in the maintenance of regional pattern and/or cell migration pathways, our results indicate that at earlier times during development, when their expression is in apposition, they are not cross-repressive.

The lack of Gsh2 repression by Nkx2.1 is also contrary to predictions based on the genetic interactions utilized to establish neural progenitor domains in *Drosophila* (reviewed by Cornell and Von Ohlen, 2000). The *Drosophila* nerve cord is divided into three distinct neural progenitor domains, dorsal, intermediate and ventral. The ventral domain expresses the homeodomain transcription factor vnd (the Nkx ortholog), which functions in a manner analogous to Nkx2.1 by repressing intermediate character. Despite the similarity in the requirements for these genes, the failure of Gsh2 expression to expand ventrally in *Nkx2.1*$^{-/-}$ mutants demonstrates that the conversion of the MGE to an LGE in *Nkx2.1* mutants is not dependent on *Gsh2*. Furthermore, if Nkx2.1 acts to suppress LGE character in the MGE by blocking Gsh2 function by means other than transcriptional repression, one would predict that the MGE would be rescued in *Nkx2.1*$^{-/-}$;*Gsh2*$^{-/-}$ double mutants. As shown in Fig. 6K,O, this is not the case, as even in the double mutants the MGE appears to adopt LGE character.

### Convergence of Shh-dependent and Shh-independent signaling at the level of *Nkx2.1* and *Gsh2*

Although telencephalic patterning is severely affected by the loss of *Shh*, recent examination of ventral patterning in *Shh*$^{-/-}$ mutants has revealed that specific aspects of ventral patterning

can persist in the absence of *Shh* (Rallu et al., 2002a) (K. Campbell, personal communication). Taken as a whole, these data indicate that the residual ventral patterning in *Shh*$^{-/-}$ mutants is of LGE (i.e. lateral) character. Persistence of pan-ventrally expressed genes in *Shh*$^{-/-}$ mutants, including *Gsh2*, *Dlx2*, *Mash1*, and *Gad67* and Crbp1, which specifically marks LGE radial glia, supports this notion. The observation that Shh-independent processes appear to be required to establish the lateral (LGE) domain of the telencephalon is reminiscent of the specification of the lateral VO and V1 interneuron populations in the absence of *Shh* in the spinal cord (Pierani et al., 1999). Furthermore, although *Gsh2* is a downstream target of *Shh* signaling, as revealed by gain-of-function studies, in *Shh*$^{-/-}$ mutant mice expression of this gene persists, albeit at reduced levels (Rallu et al., 2002a). The most compelling evidence for Shh-independent signaling in the establishment of ventral telencephalic pattern comes from the rescue of ventral patterning seen in *Gli3;Shh* or *Gli3;Smo* mutants, including complete restoration of the normal *Gsh2* and *Nkx2.1* expression domains (Rallu et al., 2002a). Although the nature of this signaling at present remains unclear, Bmp, Fgf, retinoid, Wnt or Nodal signaling all represent promising candidates for mediating Shh-independent signaling within the telencephalon (Rallu et al., 2002b).

The significant reduction in ventral telencephalic patterning in the absence of *Nkx2.1* and *Gsh2* gene function suggests that, regardless of how Shh-dependent and independent mechanisms cooperate in the establishment of ventral telencephalic pattern, their actions must converge at the level of these two homeodomain proteins (Fig. 9). Despite the importance of these genes, we observed persistence of some ventral pattern in the *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ compound mutants. This residual pattern may be attributable to the persistence and expansion of *Gsh1* expression in these animals. In single *Gsh2*$^{-/-}$ mutants, *Dlx2* and *Mash1* expression remains in the ventral most aspect of the LGE (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Strikingly, in compound mutant mice lacking both *Gsh1* and *Gsh2* gene function, this domain of *Dlx2* and *Mash1* expression is completely lost from the entire LGE at E12.5, indicating that *Gsh1* in combination with *Gsh2*, regulates patterning in the entire LGE (Toresson and Campbell, 2001). Furthermore, as the MGE is converted to an LGE fate in the absence of *Nkx2.1* gene function (Sussel et al., 1999), the remnant *Gsh1* expression in *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ double mutants is most probably derived not from the MGE, but from the ventral LGE instead. The observation that the domain of *Dlx2* and *Mash1* expression closely matches that of *Gsh1* expression lends further credence to the hypothesis that *Gsh1* is responsible for the residual ventral patterning observed in *Nkx2.1*$^{-/-}$*;Gsh2*$^{-/-}$ double mutants. Exploration of triple-mutant mice lacking *Nkx2.1*, *Gsh1* and *Gsh2* will be further required to directly address this hypothesis. Therefore, it may be that the function of Nkx and Gsh genes is all that is required to pattern the MGE and LGE, and these genes represent the convergence of Shh-dependent and Shh-independent patterning (Fig. 9).

### Pathways to specification of interneurons and oligodendrocytes

Previous work has indicated that both oligodendrocytes and interneurons are primarily specified in the ventral telencephalon, possibly by the functions of *Dlx1/2* and *Mash1* (reviewed by Bertrand et al., 2002). In the combined absence of both *Nkx2.1* and *Gsh2*, the generation of both of these cell types is altered in profound and distinct ways. Although still present, interneurons in the cerebral cortex of double mutants are markedly reduced and resemble those observed in *Nkx2.1* mutants (Sussel et al., 1999). Recent studies have revealed that the primary source of cortical interneurons is the MGE (Lavdas et al., 1999; Wichterle et al., 1999, 2001; Anderson et al., 2001; Nery et al., 2002). Therefore, because the combined loss of *Nkx2.1* and *Gsh2* results in a conversion of the MGE to an LGE fate similar to that observed in single *Nkx2.1* mutants, it is not surprising that the two mutants resemble each other with regard defects in the generation of interneurons.

Our results also give a novel insight into the function of *Nkx2.1* and *Gsh2* in oligodendrocyte development. Subpallial-derived oligodendrocytes consist of at least two distinct populations: those that express *Pdgfra* and those that express *Plp/DM20* (Spassky et al., 1998; Perez-Villegas et al., 1999; Nery et al., 2001). Our results reveal that only the PDGFRα-positive population is dependent on the function of *Nkx2.1* and *Gsh2*; the *Plp/DM20* population is unaffected by the loss of these genes. As shown here and previously (Nery et al., 2001), the generation of the PDGFRα-population is positively regulated by the function of *Nkx2.1*. Interestingly, this population also appears to be under negative regulation by *Gsh2*, as indicated by the derepression of *Pdgfra* expression in the MGE and LGE VZ in the absence of *Gsh2*. Moreover, ectopic expression of Gsh2 in the medial domain early in development (E9.5), as revealed by retroviral gain-of-function experiments, results in the repression of Nkx2.1, an essential regulator of the generation of PDGFRα-positive oligodendrocytes in the MGE. Therefore, as Gsh2 may repress the generation of PDGFRα-positive oligodendrocytes in the developing MGE, specification of these cells may occur prior to the normal expansion of Gsh2 into the medial domain during development (<E10.5). In contrast to the generation of PDGFRα-positive cells, the specification of the Plp/DM20-positive cells is not dependent on *Nkx2.1* or *Gsh2*. Plp/DM20-positive cells are generated in the presumptive amygdaloid region of the caudal subpallium. We have previously revealed that this region is a unique progenitor zone distinct from the MGE and LGE, and that it is not dependent on the function of *Nkx2.1* and *Gsh2* (Nery et al., 2002). Therefore, other transcription factor(s) must regulate the specification of this population. However, these factors are presumably downstream of Shh, as both PDGFRα- and Plp/DM20-positive oligodendrocyte populations are absent in *Shh*$^{-/-}$ mutants.

Taken together with previous studies, a hierarchy of gene expression for producing interneurons and oligodendrocytes is becoming apparent. Initiating the generation of these cell types in ventral regions are extrinsic cues, including *Shh*. These cues result in the expression of homeodomain genes, including *Nkx2.1* and *Gsh2*, that ensure the expression of pan-ventral transcription factors, such as *Dlx1/2*, *Mash1* and *Olig2*, in the MGE and LGE. These genes, in turn, may act as key effectors in the generation of specific ventral cell types, such as interneurons, and distinct populations of oligodendrocytes.

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