Cerebral Cortex 2006;16:i96-i102
doi:10.1093/cercor/bhk023

Removal of *Pax6* Partially Rescues the Loss of Ventral Structures in *Shh* Null Mice

Marc Fuccillo, Michael Rutlin and Gord Fishell

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

Pax6 **and** ***Gli3*** **are dorsally expressed genes that are known to antagonize sonic hedgehog (Shh) activity. We have previously shown that dorsoventral patterning defects seen in** $Shh^{-/-}$ **mutants are rescued in** $Shh^{-/-};Gli3^{-/-}$ **compound mutants. Here we investigate if the loss of** ***Pax6*** **can also ameliorate defects seen in** $Shh^{-/-}$ **mutants. In support of this notion, we observe that the fusion of the cerebral vesicles seen in** $Shh^{-/-}$ **mutants is partially corrected in E12.5** $Shh^{-/-};Pax6^{-/-}$ **compound mutants. Investigation of pan-ventral markers such as** ***Dlx2*** **also shows that, unlike** $Shh^{-/-}$**, a broad domain of expression of this gene is observed in** $Shh^{-/-};Pax6^{-/-}$ **mice. Interestingly, we observe that while the expression of** ***ER81*** **in the ventral telencephalon is expanded, the expression of** ***Ebf1*** **is lost. This suggests that the rescued ventral domain observed in** $Shh^{-/-};Pax6^{-/-}$ **mice is the dorsal lateral ganglionic eminence region. With regard to dorsal telencephalic patterning, we also observe rescue of the pallial–subpallial boundary, as well as a partial rescue of the dorsal midline. Together, our findings are consistent with** ***Pax6*** **function being required for aspects of** ***Gli3*****-mediated telencephalic patterning.**

Keywords: CGE, dorsoventral patterning, LGE, MGE, midline

Introduction

Studies over the past decade have indicated that sonic hedgehog (Shh) and Pax6 have antagonistic roles in establishing dorsoventral (DV) patterning throughout the neuraxis (Goulding and others 1993; Ericson and others 1997). While numerous studies have indicated that Shh is essential for the establishment of ventral patterning throughout the neuraxis (Roelink and others 1994, 1995; Chiang and others 1996), a large number of papers have demonstrated that *Pax6* is negatively regulated by Shh and has a complementary role in specifying and maintaining dorsal identities in the nervous system (Caric and others 1997; Ericson and others 1997; Warren and others 1999; Kroll and O'Leary 2005). In particular, Pax6 has been implicated in mediating the organization of the cerebral cortex, which comprises the dorsal telencephalon (Stoykova and others 2000).

Despite the notion that the protein encoded by these developmentally significant factors are central to the establishment of DV pattern in the telencephalon, a clear understanding of how *Pax6* and *Shh* functionally interact has been lacking. Understanding how antagonism between these proteins contributes to telencephalic development is confounded by the fact that Shh is secreted protein that acts noncell autonomously (Briscoe and others 2001), whereas Pax6 is a transcription factor whose function is presumably restricted to the cells in which it is expressed (Quinn and others 1996).

In addition to *Pax6*, *Gli3* also appears to be intimately involved in the patterning of the dorsal telencephalon (Theil and others 1999; Tole and others 2000). In mice lacking *Gli3* function, dorsal patterning is perturbed and the cortical hem is absent (Theil and others 1999; Tole and others 2000). Moreover, in both *Gli3* and *Pax6* mutants, dorsal pallial tissue partially adopts ventral character, as evidenced by the ectopic cortical expression of *Dlx2* during development in both these mutant strains (Theil and others 1999; Stoykova and others 2000; Tole and others 2000; Kroll and O'Leary 2005). Whereas *Emx1* and *Emx2* expression is perturbed in *Gli3* mutants (Theil and others 1999; Tole and others 2000), *Ngn2* expression is abnormal in *Pax6* null mice (Stoykova and others 2000). Given the fact that *Emx2* expression is variably lost in *Gli3* mutants, the observation that all dorsal pattern is lost in compound *Emx2;Pax6* null mutants (Muzio and others 2002) further suggests that *Pax6* and *Gli3* cooperate in the establishment of dorsal telencephalic identity.

Recently, an analysis of compound $Shh^{-/-};Gli3^{-/-}$ mutants has shown that these genes genetically interact in the establishment of DV pattern in the telencephalon (Rallu and others 2002). Specifically, whereas the telencephalon is ventralized in *Shh* mutants and dorsalized in *Gli3* mutants, DV patterning is largely restored in compound *Shh;Gli3* null animals. This is consistent with work from other systems demonstrating that one major aspect of Shh signaling is to negatively regulate the processing of Gli3 protein into a repressor fragment (Gli3R) (Litingtung and Chiang 2000; Wang and others 2000).

Taken together, the above data suggest that both *Shh* and *Gli3* appear to genetically interact with *Pax6*. To further explore the genetic interactions between these genes, here we examine the phenotype observed in $Shh^{-/-};Pax6^{-/-}$ mutants. Surprisingly, we find that complete removal of *Pax6* gene function from a $Shh^{-/-}$ background partially rescues multiple aspects of the *Shh* null phenotype. Most notably, the expression of the pan-ventral gene *Dlx2* is partially rescued in these mutants. Interestingly, another defect in the *Shh* mutant, the loss of the dorsal midline, is also partially restored. These observations suggest that like *Gli3*, *Pax6* function antagonizes *Shh* and permits the establishment of some ventral and dorsal telencephalic structures during development.

Materials and Methods

Animal Use and Genotyping of Shh and Pax6 Null Alleles

All mice used in these studies were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York University School of Medicine. The *Shh* null allele (Chiang and others 1996) was maintained on the C57BL/6 background. The Pax6 mutant strain was the small eye deletion (*Sey*), in which a point mutation leads to a truncated nonfunctional protein (Hill and others 1991). *Sey* mice were also maintained on the C57BL/6 background. Mutants were generated by intercrossing $Shh^{+/-};Pax6^{+/-}$

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transheterozygotes. The day when the sperm plug was detected was considered E0.5. Polymerase chain reaction was used to genotype the *Shh* and *Pax6* null alleles as described previously (Hogan and others 1986; Litingtung and Chiang 2000).

In Situ Hybridizations

E12.5 embryos were dissected in cold phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 2–3 h at 4 °C. Embryos were then washed in PBS and cryoprotected in 30% sucrose in PBS. Tissues were embedded in TissueTek, frozen on dry ice, and sectioned serially at 16 μm for in situ hybridization analysis. Section in situ hybridizations were performed as previously described using non-radioactive digoxygenin-labeled probes. The following cDNA probes were employed: *Nkx2.1*, *Lhx6*, *Dlx2*, *ER81*, *Ebf1*, *FoxG1*, *Ngn2*, *Emx2*, *Wnt8b*, *Wnt3a*, *BMP4,* and *Gli3*.

Results

The Holoprosencephaly Observed in $Shh^{-/-}$ Is Partially Rescued in $Shh^{-/-}$;$Pax6^{-/-}$ Mutants

Morphological analysis of E12.5 $Shh^{-/-}$;$Pax6^{-/-}$ mice immediately suggested that removal of *Pax6* from mice already lacking *Shh* partially rescued the dorsalization seen in *Shh* mutants (Fig. 1*A–C*). Other defects, most obviously the syntactic limb abnormality, remain as severe as seen in *Shh* mutants alone (compare Fig. 1*B,C*). At this level of analysis, the most prominent aspects where rescue was observed in $Shh^{-/-}$;$Pax6^{-/-}$ mutants compared with *Shh* null embryos were the reduction of the size of the proboscis and the appearance of paired cerebral hemispheres. However, as might be expected based on the phenotype of $Pax6^{-/-}$ and $Shh^{-/-}$ single mutants, the eye phenotype observed in $Shh^{-/-}$;$Pax6^{-/-}$ mice is in fact more severe than that observed in either of the single mutants. In fact, at least on the basis of morphology, the eyes are absent in $Shh^{-/-}$;$Pax6^{-/-}$ mutants. Finally, although the morphology of the $Shh^{-/-}$;$Pax6^{-/-}$ mutant brain was improved, the overall size of these brains was still considerably reduced compared with that seen in wild-type animals.

We next investigated the extent of ventral patterning in $Shh^{-/-}$;$Pax6^{-/-}$ mutants, as the ventral forebrain was at least morphologically evident in coronal sections. Normally at E12.5, the ventral telencephalon is comprised of 3 prominent eminences, the medial, lateral, and caudal ganglionic eminences (MGE, LGE, and CGE, respectively). Although these 3 eminences could not be morphologically discerned, a large single mass without obvious subdivisions was observed in the ventral telencephalon of $Shh^{-/-}$;$Pax6^{-/-}$ mutants (Fig. 2*C,I,L*).

To determine which aspects of the ventral telencephalon were present in these mutants, we used in situ hybridization for regional molecular markers. The MGE comprises the ventralmost aspect of the telencephalon, and the genes *Nkx2.1* and *Lhx6* provide excellent developmental markers for ventricular zone and subventricular zone MGE progenitors at E12.5 (Grigoriou and others 1998; Sussel and others 1999). Based on the loss of expression of *Nkx2.1* and *Lhx6* in $Shh^{-/-}$;$Pax6^{-/-}$ compound mutants (Fig. 2*A–F*), this structure would appear to be absent in these animals. Consistent with this, the ventral region of low *Gli3* expression seen in both wild-type and *Pax6* mutants is not observed in $Shh^{-/-}$;$Pax6^{-/-}$ mutants (compare Fig. 4*B,C*). However, to confirm that other aspects of the ventral telencephalon are rescued in these compound mutants, we examined coronal tissue from these animals for *Dlx2* expression, which provides a pan-ventral marker for the E12.5 ventral telencephalon. Consistent with the existence of ventral telencephalon in these mutants, a broad domain of *Dlx2* expression was observed (Fig. 2*G–I*).

Other studies have shown that the LGE and CGE can be subdivided based on their molecular markers into dorsal (dLGE/dCGE) and ventral (vLGE/vCGE) domains (Stenman and others 2003; Xu and others 2004; Cobos and others 2005; Sur and Rubenstein 2005). To gain a better understanding of the character of the ventral structures observed in the $Shh^{-/-}$;$Pax6^{-/-}$ mutants, we used markers that are expressed specifically in the dorsal versus ventral aspects of the LGE and CGE, *ER81* and *Ebf1*, respectively. Whereas *Ebf1* is expressed in the mantle region of progenitors originating from the ventral aspect of the LGE and CGE (Fig. 2*M*) (Garel and others 1997; Nery and others 2002; Stenman and others 2003), *ER81* is expressed in the dorsal portion of the LGE (Fig. 2*J*), in the region that contributes nascent neurons to the rostral migratory stream, en route to the olfactory bulb (Stenman and others 2003). Consistent with the dLGE but not the vLGE being present in $Shh^{-/-}$;$Pax6^{-/-}$ mice, we observe that while the domain of *ER81* expression in the E12.5 ventral telencephalon of $Shh^{-/-}$;$Pax6^{-/-}$ is expanded, the domain of *Ebf1* is lost (compare Fig. 2*L,O*).

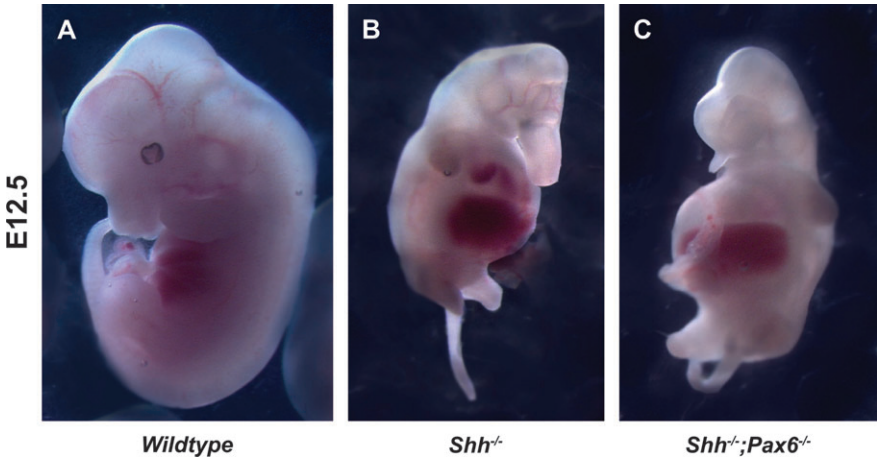

Figure 1. The morphology of the telencephalic vesicles is rescued in $Shh^{-/-}$;$Pax6^{-/-}$ mutants. (*A–C*) Whole mount of E12.5 embryos from wild-type (*A*), $Shh^{-/-}$ (*B*), and $Shh^{-/-}$;$Pax6^{-/-}$ mutants (*C*). Note the reduction in the size of the proboscis and the absence of the eyes in the $Shh^{-/-}$;$Pax6^{-/-}$ mutant.

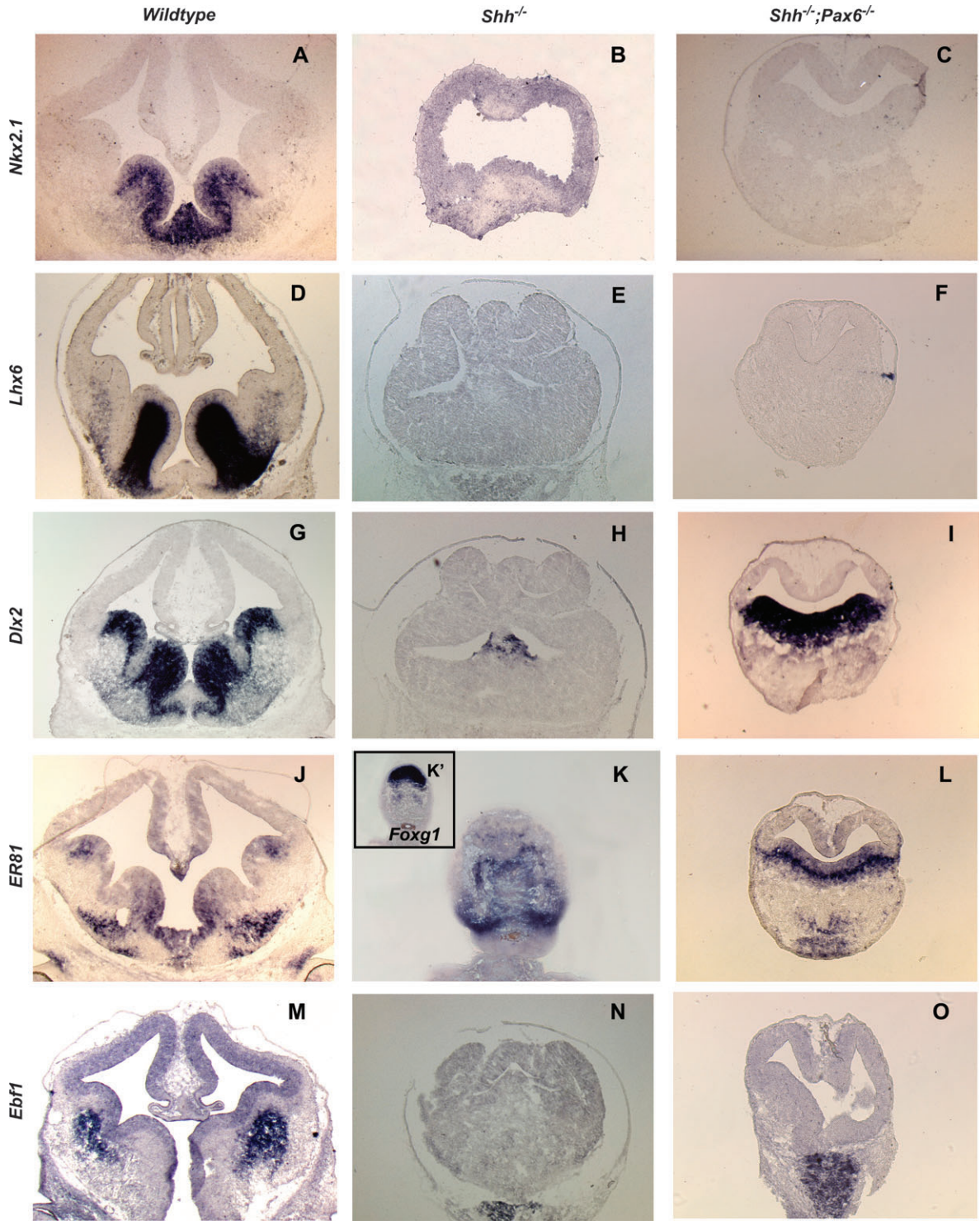

Figure 2. A subdomain of the LGE, but not the MGE, is rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. (*A*–*O*) Coronal sections of E12.5 wild-type (*A*, *D*, *G*, *J*, *M*), *Shh*$^{-/-}$ (*B*,*E*,*H*,*K*,*N*), and *Shh*$^{-/-}$;*Pax6*$^{-/-}$ (*C*, *F*, *I*, *L*, *O*) tissue stained by in situ hybridization for a variety ganglionic eminence transcription factors. Although neither MGE ventricular zone (*A*–*C*) nor mantle (*D*–*F*) markers are rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants, the presence of pan-ventrally expressed *Dlx2* demonstrates the rescue of some ventral ganglionic eminence territory (*G*–*I*). *ER81* expression, which is normally confined to a subdomain of both the MGE and LGE/CGE (*J*), is absent in *Shh*$^{-/-}$ mice (*K*, with inset *K*′ of an adjacent section stained for FoxG1 to delineate telencephalic tissue) and is expressed throughout the rescued ventral tissue of *Shh*$^{-/-}$;*Pax6*$^{-/-}$ double mutants (*L*). *Ebf1*, which is restricted to differentiating neurons of the LGE/CGE (*M*), is not present in either single or double mutants (*N*, *O*).

The Pallial–Subpallial Boundary Is Partially Restored in Shh$^{-/-}$*;Pax6*$^{-/-}$ *Mutant Mice*

Both ventral and dorsal telencephalic structures are absent in *Shh*$^{-/-}$ mice (Chiang and others 1996; Rallu and others 2002; Roessler and Muenke 2003). With regard to dorsal patterning, 3 aspects of the pallium appear to be missing. First, the pallial–subpallial boundary is lost (Rallu and others 2002). Second, the 2 dorsal hemispheres become fused (Cooper and

others 1998). Third, the structures occupying the dorsal midline are absent. All these features appear to be at least partially rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. *Ngn2* and *Emx2* are both expressed by the E12.5 pallium and normally form a sharp expression boundary at the pallial–subpallial boundary. By contrast, in *Shh*$^{-/-}$ mutants, both of these markers are expressed throughout most of the DV extent of the telencephalon (Chiang and others 1996, Rallu and others 2002). The ectopic expression of these markers appears to be suppressed in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants (Fig. 3*A–D*). In addition, unlike *Shh*$^{-/-}$ mutant mice, where the 2 cerebral hemispheres are fused, a clear albeit hypomorphic dorsal midline separates the 2 telencephalic hemispheres of E12.5 *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. To determine what aspects of the dorsal midline are rescued in these compound mutants, we used a number of molecular markers that are expressed in this region. We observe that the expression of *Wnt8b*, which is expressed broadly in dorsal midline tissue of the E12.5 wild-type telencephalon but lost in *Shh*$^{-/-}$ mutants (data not shown), is expressed within the dorsal midline of *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants (compare Fig. 3*E,F*). In contrast, *Wnt3a*, a secreted signaling molecule expressed within the cortical hem, is not rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$

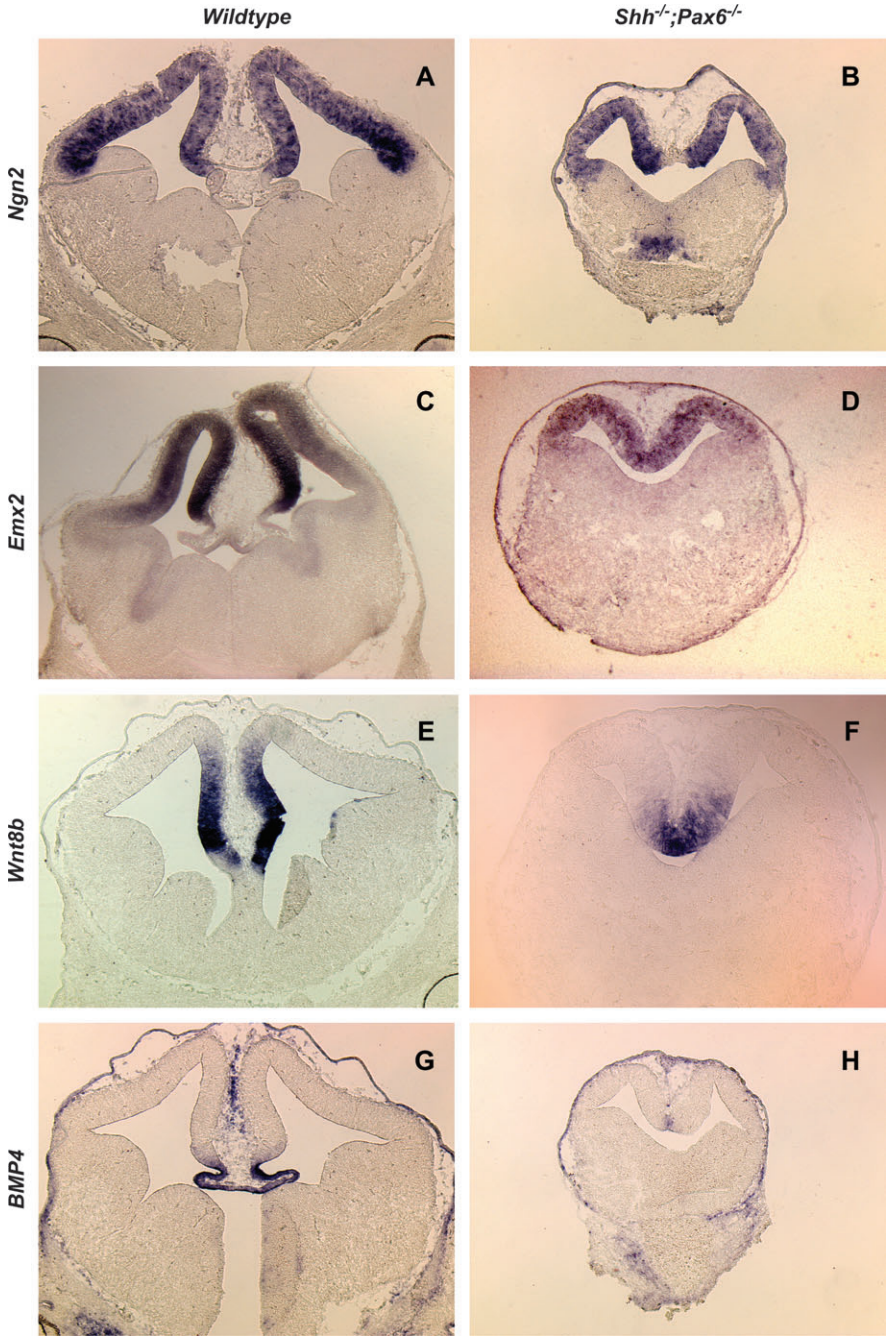

Figure 3. Partial rescue of the corticostriatal border and dorsal cortical midline. (*A–H*) Coronal sections of E12.5 wild-type (*A*, *C*, *E*, *G*) and *Shh*$^{-/-}$;*Pax6*$^{-/-}$ (*B*, *D*, *F*, *H*) tissue stained by in situ hybridization for dorsally expressed transcription factors. The sharp corticostriatal expression boundaries of *Ngn2* and *Emx2* are restored in the *Shh*$^{-/-}$;*Pax6*$^{-/-}$ double mutants (*A–D*). *Wnt8b*, a marker of the dorsal cortical midline is rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ double mutants (*E*, *F*).

mutants (data not shown). *BMP4* a gene that is expressed in the dorsal medial-most region, destined to become the choroid plexus epithelium, is present at low levels in the midline of *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants (Fig. 3*G*,*H*). Together these observations suggest that removal of *Pax6* from mice already lacking *Shh* partially rescues dorsal midline telencephalic structures.

Gli3 Expression Is Not Affected in either Shh or Pax6 Single or Compound Mutants

The observation that some of the defects observed in *Shh*$^{-/-}$ mutants can be restored by the compound removal of *Pax6* raises the question of how this is accomplished mechanistically. Our previous work showing that similar aspects of telencephalic patterning can be restored in *Shh*$^{-/-}$ mutants by removal of one or both copies of *Gli3* suggests a functional connection between *Pax6* and *Gli3*. The simplest potential mechanism is that the level of *Gli3* expression is under transcriptional control of *Pax6*. To explore this possibility, we examined the level of *Gli3* transcript expression in both *Pax6*$^{-/-}$ and *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants (Fig. 4*A–C*). *Gli3* is normally expressed highly throughout the ventricular zone of the cortex and LGE and at weaker levels in the MGE. In neither case did we observe a reduction in the expression of *Gli3*, suggesting that simple transcriptional regulation of *Gli3* does not account for the rescue observed in *Shh*$^{-/-}$;*Pax6* mutants.

Discussion

Here we have examined the genetic interaction between *Pax6* and *Shh* with regard to early DV patterning. We find that based on both morphology and gene expression, several defects observed in the *Shh*$^{-/-}$ single mutant are rescued in the *Shh*$^{-/-}$;*Pax6*$^{-/-}$ compound mutant. Specifically, we observe that a *Dlx2*-positive ventral domain is present in these compound mutants. Moreover, based on the absence of *Nkx2.1*, *Lhx6*, and *Ebf1* in these compound mutants and the expanded expression of *ER81*, we believe that the domain within the ventral telencephalon that is rescued is the dLGE and dCGE. We also observe that the pallial–subpallial boundary and the dorsal midline, both of which are lost in *Shh*$^{-/-}$ mutants, are rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. Given that a similar, albeit more pronounced rescue of DV pattern occurs in *Shh*$^{-/-}$;*Gli3*$^{-/-}$ mutants, combined with the observation that *Gli3* expression is unaffected in either *Pax6* or *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants, our results suggest that *Gli3* may require *Pax6* to function for certain aspects of DV patterning.

Loss of Pax6 Results in Partial Morphological Rescue of the Defects Observed in Shh$^{-/-}$ *Mutants*

We observed that compound *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants show some improvement in the telencephalic morphology compared with *Shh*$^{-/-}$ null mice. Specifically, both the DV patterning of the telencephalon and the organization of the dorsal midline are partially rescued. In contrast, the overall size of the telencephalon is only slightly larger in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants as compared with *Shh* nulls. The improvement in patterning without restoration of telencephalic growth demonstrates that patterning and growth are not inextricably linked. This is particularly intriguing given the evidence supporting a role for *Pax6* in cortical neural progenitors (Gotz and others 1998; Heins and others 2002). Indeed, it is possible that rather than antagonizing one another, *Pax6* and *Shh* signaling contribute in a common pathway to regulate the proliferation of cortical neuroblasts. Certainly, further work will be needed to understand the dual roles of *Shh* and *Pax6* in the proliferation/maintenance of cortical progenitors.

Patterning in the Ventral Telencephalon of Shh$^{-/-}$;*Pax6*$^{-/-}$ *Mutants*

At a morphological level, it is apparent that some rescue of ventral telencephalon occurs in compound *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. *Pax6* is expressed at low levels in the dLGE and dCGE, with both *Pax6* transcript and Pax6 protein levels diminishing rapidly in more ventral regions (Stoykova and others 1996, 2000). Consistent with this pattern of *Pax6* gene expression, and with the phenotype of *Shh*$^{-/-}$ null mutants, the MGE does not appear to be rescued in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ double mutants. Moreover, *Ebf1*, a marker of nascent striatal neurons thought to arise from both the vLGE (Garel and others 1999; Stenman and others 2003) and vCGE (Nery and others 2002), is also absent. By contrast, *ER81*, a gene expressed in the dLGE and dCGE, is expanded and expressed throughout the domain where we observe *Dlx2* expression. Although we interpret this to suggest that the dLGE and dCGE are rescued in these mutants, this conclusion is confounded by the broad expression of *ER81* within the MGE. We suggest however that the lack of both *Nkx2.1* and *Lhx6* argues that the expression observed does not reflect rescue of neurons arising from the MGE. If true, this suggests that only the dorsal-most aspect of the ventral telencephalon is restored in mice lacking both *Shh* and *Pax6* gene function. It is interesting that although the recovery seen in these compound mutants is striking, these mice show

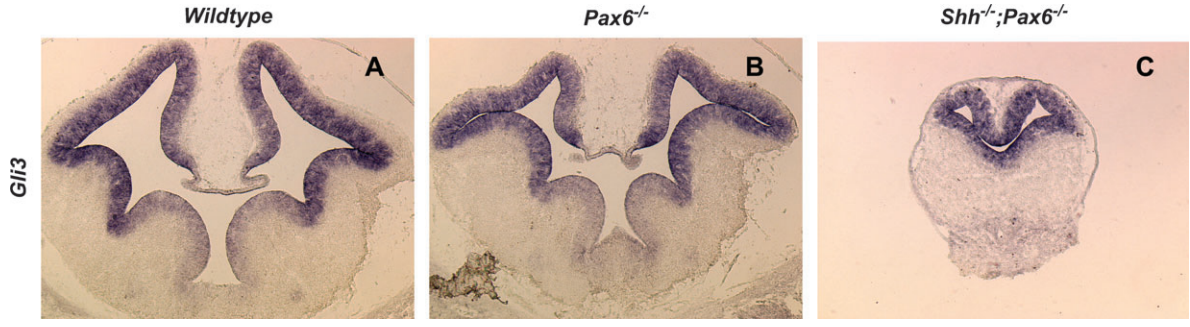

Figure 4. *Gli3* is not under the transcriptional control of Pax6. Coronal sections of E12.5 wild-type (*A*), *Pax6*$^{-/-}$ (*B*), and *Shh*$^{-/-}$;*Pax6*$^{-/-}$ (*C*) tissue stained by in situ hybridization for *Gli3* transcript. The wild-type expression pattern of *Gli3* (*A*), with high levels in the cortex and LGE and low levels in the MGE, is maintained in the *Pax6*$^{-/-}$ (*B*). The expression of *Gli3* in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants does not appear different from wild-type, with the exception of the MGE expression domain, which is absent from double mutants.

a less robust rescue than we have previously observed in mice that are null for *Shh* but lack only a single copy of the *Gli3* gene (i.e., *Shh*$^{-/-}$;*Gli3*$^{+/-}$, Rallu and others 2002). Moreover, in this previous study, we observed that complete recovery of DV patterning is observed in *Shh*$^{-/-}$;*Gli3*$^{-/-}$ mutants. Our prior results argue that the recovery we see in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants must relate to a decreased ability of the Gli3 repressor to suppress ventral patterning in the absence of *Shh* gene function. We suggest this indicates that *Pax6* is required for Gli3 repressor to be fully functional.

The Pallial–Subpallial Boundary Is Rescued in Shh$^{-/-}$;*Pax6*$^{-/-}$ *Mutants*

The most complete rescue observed in *Shh*$^{-/-}$;*Gli3*$^{-/-}$ mutants is found at the pallial–subpallial boundary. Because neither the MGE nor the vLGE/vCGE is restored in these mutants, interactions between the pallium and dLGE/dCGE are sufficient to establish a normal pallial–subpallial boundary. Previous work by a number of groups demonstrated that although this boundary was disrupted in *Gsh2*$^{-/-}$ and *Pax6*$^{-/-}$ single mutants, this boundary could also be restored in *Gsh2*$^{-/-}$;*Pax6*$^{-/-}$ compound mutants (Toresson and others 2000; Yun and others 2001). These observations are consistent with our own findings, suggesting the *Gsh2* is a target of *Shh* gene function (Corbin and others 2000). Taken together, these results suggest that *Shh* acts to initiate expression of *Gsh2*, which in turn is required to establish dLGE/dCGE fate. For this to occur, it appears that Shh must antagonize the Gli3R in this region. We suggest that the loss of *Pax6* in either *Gsh2*$^{-/-}$;*Pax6*$^{-/-}$ or *Shh*$^{-/-}$;*Pax6*$^{-/-}$ compound mutants argues that a critical role of *Shh* signaling for the establishment of the pallial–subpallial boundary is the *Shh*-mediated antagonism of *Pax6*. Consistent with the idea presented here that Shh is required to antagonize both Pax6 and the Gli3 repressor is our observation that the telencephalon is strongly ventralized in *Gli3*$^{-/-}$;*Pax6*$^{-/-}$ compound mutants (G. Fishell, unpublished data). Indeed, our preliminary analysis suggests that the *Gli3*$^{-/-}$;*Pax6*$^{-/-}$ compound mutant strongly resembles the *Emx2*$^{-/-}$;*Pax6*$^{-/-}$ double mutant (Muzio and others 2002; G. Fishell, unpublished data).

The Dorsal Midline Is Partially Rescued in Shh$^{-/-}$;*Pax6*$^{-/-}$ *Mutants*

One of the least understood aspects of Shh signaling in telencephalic patterning is its effect on dorsal midline patterning. Rigorous analysis of the developmental expression of *Shh* argues that there is never substantial early embryonic expression of *Shh* in dorsal regions (G. Fishell, unpublished data). This suggests that the action of *Shh* in patterning the dorsal midline might occur very early during development when the DV axis of the telencephalon is sufficiently small that ventrally derived Shh can affect dorsal midline patterning. Moreover, the partial restoration of the dorsal midline in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ compound mutants argues that expression of *Pax6* in the dorsal midline of *Shh*$^{-/-}$ mutants must partially account for the loss of the dorsal midline in this context. Again examination of the interactions between *Gli3* and *Shh* is interesting to consider in this context. Our previous work exploring the genetic interaction between *Gli3* and *Shh* revealed that although the dorsal midline is lost in both *Shh*$^{-/-}$ and *Shh*$^{-/-}$;*Gli3*$^{-/-}$ compound mutants, patterning in this region is rescued in *Shh*$^{-/-}$;*Gli3*$^{+/-}$ compound mutants (Rallu and others 2002, G. Fishell, unpublished data). This suggests that either too much (as seen in *Shh*$^{-/-}$ mutants) or too little Gli3 repressor function (as seen in *Shh*$^{-/-}$;*Gli3*$^{-/-}$ compound mutants) results in the loss of the dorsal midline. One possible interpretation of the partial rescue observed in *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mice is that the loss of *Pax6* sufficiently reduces the function of the Gli3 repressor to allow for the partial rescue of the dorsal midline.

Does Gli3 Requires Pax6 to Function within the Telencephalon?

Given our data suggesting that *Pax6* is required for the full function of the Gli3 repressor, the most obvious mechanism would be through Pax6-mediated transcriptional regulation of Gli3. The results in Figure 4 show that such a simple explanation for the genetic interaction between *Shh*, *Gli3*, and *Pax6* is unlikely, as *Gli3* expression appears to be normal in both *Pax6*$^{-/-}$ and *Shh*$^{-/-}$;*Pax6*$^{-/-}$ mutants. If our interpretation is correct, this suggests three possible ways in which Pax6 could affect the level of activity of the Gli3 repressor. First, Pax6 could influence the translation of the *Gli3* transcript. Second, Pax6 could be required for the efficient cleavage of the full length Gli3 protein into its repressor form. Finally, Pax6 could either directly or indirectly be required for the highest level of activity of the Gli3 repressor protein. The lack of an antibody that allows for the localization of the Gli3 protein, though immunocytochemistry (specifically the repressor fragment) makes these three possibilities impossible to discern at present. Nonetheless, the combined genetic evidence in both the present work and previous findings showing the genetic interaction between *Shh* and *Gli3* (as well as our unpublished findings concerning the phenotype observed in *Gli3*$^{-/-}$;*Pax6*$^{-/-}$ mutants) suggests a potential biochemical interaction between *Gli3* and *Pax6*. It will be important to study the biochemical functions of both these proteins to fully understand the importance of their interactions in telencephalic patterning.

Notes

We would like to thank Murielle Rallu for her thoughtful input and her preliminary work on this project and Josh Corbin for his in situ hybridization images. We would also like to thank Yuan Yuan Huang for her technical assistance. We would like to thank the following people for their gifts of cDNA probes: E. Lai, *Foxg1*; A. Simeone, *Emx2*; F. Guillemot, *Ngn2*; J. Rubenstein, *Dlx2*, *Lhx6*, and *Nkx2.1*; P. Charnay, *Ebf1*; S. Arber, *ER81*; A. Joyner, *Gli3*; A. McMahon, *Wnt8b*; and B. Hogan, *BMP4*. GF was supported by National Institutes of Health grants from the National Institute of Neurological Disorders and Strokes and the National Institute of Mental Health. *Conflict of Interest*: None declared.

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

References

Briscoe J, Chen Y, Jessell TM, Struhl G. 2001. A hedgehog-insensitive form of patched provides evidence for direct long-range morphogen activity of sonic hedgehog in the neural tube. Mol Cell 7:1279–1291.

Caric D, Gooday D, Hill RE, McConnell SK, Price DJ. 1997. Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. Development 124:5087–5096.

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. 1996. Cyclopia and defective axial patterning in mice lacking sonic hedgehog gene function. Nature 383:407–413.

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. 2005. Mice lacking Dlx1 show subtype-specific

loss of interneurons, reduced inhibition and epilepsy. Nat Neurosci 8:1059–1068.
Cooper MK, Porter JA, Young KE, Beachy PA. 1998. Teratogen-mediated inhibition of target tissue response to Shh signaling. Science 280:1603–1607.
Corbin JG, Gaiano N, Machold RP, Langston A, Fishell G. 2000. The Gsh2 homeodomain gene controls multiple aspects of telencephalic development. Development 127:5007–5020.
Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J. 1997. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90:169–180.
Garel S, Marin F, Grosschedl R, Charnay P. 1999. Ebf1 controls early cell differentiation in the embryonic striatum. Development 126:5285–5294.
Garel S, Marin F, Mattei MG, Vesque C, Vincent A, Charnay P. 1997. Family of Ebf/Olf-1-related genes potentially involved in neuronal differentiation and regional specification in the central nervous system. Dev Dyn 210:191–205.
Gotz M, Stoykova A, Gruss P. 1998. Pax6 controls radial glia differentiation in the cerebral cortex. Neuron 21:1031–1044.
Goulding MD, Lumsden A, Gruss P. 1993. Signals from the notochord and floor plate regulate the region-specific expression of two Pax genes in the developing spinal cord. Development 117:1001–1016.
Grigoriou M, Tucker AS, Sharpe PT, Pachnis V. 1998. Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development. Development 125:2063–2074.
Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M. 2002. Glial cells generate neurons: the role of the transcription factor Pax6. Nat Neurosci 5:308–315.
Hill RE, Favor J, Hogan BL, Ton CC, Saunders GF, Hanson IM, Prosser J, Jordan T, Hastie ND, van Heyningen V. 1991. Mouse small eye results from mutations in a paired-like homeobox-containing gene. Nature 354:522–525.
Hogan BL, Horsburgh G, Cohen J, Hetherington CM, Fisher G, Lyon MF. 1986. Small eyes (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse. J Embryol Exp Morphol 97:95–110.
Kroll TT, O'Leary DD. 2005. Ventralized dorsal telencephalic progenitors in Pax6 mutant mice generate GABA interneurons of a lateral ganglionic eminence fate. Proc Natl Acad Sci USA 102:7374–7379.
Litingtung Y, Chiang C. 2000. Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. Nat Neurosci 3:979–985.
Muzio L, DiBenedetto B, Stoykova A, Boncinelli E, Gruss P, Mallamaci A. 2002. Conversion of cerebral cortex into basal ganglia in Emx2(–/–) Pax6(Sey/Sey) double-mutant mice. Nat Neurosci 5:737–745.
Nery S, Fishell G, Corbin JG. 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat Neurosci 5:1279–1287.
Quinn JC, West JD, Hill RE. 1996. Multiple functions for Pax6 in mouse eye and nasal development. Genes Dev 10:435–446.
Rallu M, Machold R, Gaiano N, Corbin JG, McMahon AP, Fishell G. 2002. Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and hedgehog signaling. Development 129:4963–4974.
Roelink H, Augsburger A, Heemskerk J, Korzh V, Norlin S, Ruiz i Altaba A, Tanabe Y, Placzek M, Edlund T, Jessell TM, Dodd J. 1994. Floor plate and motor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. Cell 76:761–775.
Roelink H, Porter JA, Chiang C, Tanabe Y, Chang DT, Beachy PA, Jessell TM. 1995. Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. Cell 81:445–455.
Roessler E, Muenke M. 2003. How a hedgehog might see holoprosencephaly. Hum Mol Genet 12(Spec No 1):R15–R25.
Stenman J, Toresson H, Campbell K. 2003. Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. J Neurosci 23:167–174.
Stoykova A, Fritsch R, Walther C, Gruss P. 1996. Forebrain patterning defects in Small eye mutant mice. Development 122:3453–3465.
Stoykova A, Treichel D, Hallonet M, Gruss P. 2000. Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. J Neurosci 20:8042–8050.
Sur M, Rubenstein JL. 2005. Patterning and plasticity of the cerebral cortex. Science 310:805–810.
Sussel L, Marin O, Kimura S, Rubenstein JL. 1999. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. Development 126:3359–3370.
Theil T, Alvarez-Bolado G, Walter A, Ruther U. 1999. Gli3 is required for Emx gene expression during dorsal telencephalon development. Development 126:3561–3571.
Tole S, Ragsdale CW, Grove EA. 2000. Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J). Dev Biol 217:254–265.
Toresson H, Potter SS, Campbell K. 2000. Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. Development 127:4361–4371.
Wang B, Fallon JF, Beachy PA. 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. Cell 100:423–434.
Warren N, Caric D, Pratt T, Clausen JA, Asavaritikrai P, Mason JO, Hill RE, Price DJ. 1999. The transcription factor, Pax6, is required for cell proliferation and differentiation in the developing cerebral cortex. Cereb Cortex 9:627–635.
Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. 2004. Origins of cortical interneuron subtypes. J Neurosci 24: 2612–2622.
Yun K, Potter S, Rubenstein JL. 2001. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. Development 128:193–205.