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

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<sup>−/−</sup> mutants are rescued in Shh<sup>−/−</sup>;Gli3<sup>−/−</sup> compound mutants. Here we investigate if the loss of Pax6 can also ameliorate defects seen in Shh<sup>−/−</sup> mutants. In support of this notion, we observe that the fusion of the cerebral vesicles seen in Shh<sup>−/−</sup> mutants is partially corrected in E12.5 Shh<sup>−/−</sup>;Pax6<sup>−/−</sup> compound mutants. Investigation of pan-ventral markers such as Dlx2 also shows that, unlike Shh<sup>−/−</sup>, a broad domain of expression of this gene is observed in Shh<sup>−/−</sup>;Pax6<sup>−/−</sup> 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<sup>−/−</sup>;Pax6<sup>−/−</sup> 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 Sbb<sup>−/−</sup>;Gli3<sup>−/−</sup> 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 Sbb mutants and dorsalized in Gli3 mutants, DV patterning is largely restored in compound Sbb;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 Sbb<sup>−/−</sup>;Pax6<sup>−/−</sup> mutants. Surprisingly, we find that complete removal of Pax6 gene function from a Sbb<sup>−/−</sup> background partially rescues multiple aspects of the Sbb null phenotype. Most notably, the expression of the pan-ventral gene Dlx2 is partially rescued in these mutants. Interestingly, another defect in the Sbb 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 Sbb 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 Sbb 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 Sbb<sup>−/−</sup>;Pax6<sup>−/−</sup>
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; Littingtung 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 10 μm for in situ hybridization analysis. Section in situ hybridizations were performed as previously used non-radioactive digoxigenin-labeled probes. The following cDNA probes were employed: Nkx2.1, Lbx6, 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. 1A-C). Other defects, most obviously the syntactic limb abnormality, remain as severe as seen in Shh mutants alone (compare Fig. 1B,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. 2C,LI).

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 ventral-most aspect of the telencephalon, and the genes Nkx2.1 and Lbx6 provide excellent developmental markers for ventricular zone and subventricular zone MGE progenitors at E12.5 (Grigoriou and others 1998; Susel and others 1999). Based on the loss of expression of Nkx2.1 and Lbx6 in Shh\(^{-/-}\):Pax6\(^{+/+}\) compound mutants (Fig. 2A-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. 4B,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. 2G-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. 2M) (Garel and others 1997; Nery and others 2002; Stenman and others 2003), ER81 is expressed in the dorsal portion of the LGE (Fig. 2J), 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. 2L,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.
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

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). E881 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).
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. 3A–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. 3E,F). In contrast, *Wnt3a*, a secreted signaling molecule expressed within the cortical hem, is not rescued in *Shh*−/−;*Pax6*−/− mutants.

**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. 3G,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 showed that similar aspects of telencephalic patterning can be restored in Shh\(^{-/-}\) mutants by removal of one or both copies of Gli3, suggesting 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. 4A-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 both morphological and gene expression. We find that based on both morphological 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...
The Pallial-Subpallial Boundary Is Rescued in Shh<sup>+/−</sup>;Gli3<sup>+/−</sup> Mutants

The most complete rescue observed in Shh<sup>+/−</sup>;Gli3<sup>+/−</sup> 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<sup>+/−</sup> and Pax6<sup>+/−</sup> single mutants, this boundary could also be restored in Gsh2<sup>+/−</sup>;Pax6<sup>+/−</sup> 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<sup>+/−</sup>;Pax6<sup>+/−</sup> or Shh<sup>+/−</sup>;Pax6<sup>+/−</sup> 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<sup>−/−</sup>;Pax6<sup>−/−</sup> compound mutants (G. Fishell, unpublished data). Indeed, our preliminary analysis suggests that the Gli3<sup>−/−</sup>;Pax6<sup>−/−</sup> compound mutant strongly resembles the Ems<sup>+/−</sup>;Pax6<sup>−/−</sup> double mutant (Muzio and others 2002; G. Fishell, unpublished data).

The Dorsal Midline Is Partially Rescued in Shh<sup>+/−</sup>;Pax6<sup>−/−</sup> 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<sup>+/−</sup>;Pax6<sup>−/−</sup> compound mutants argues that expression of Pax6 in the dorsal midline of Shh<sup>−/−</sup> mutants 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<sup>−/−</sup> and Shh<sup>−/−</sup>;Gli3<sup>−/−</sup> compound mutants, patterning in this region is rescued in Shh<sup>−/−</sup>;Gli3<sup>−/−</sup> compound mutants (Rallu and others 2002, G. Fishell, unpublished data). This suggests that either too much (as seen in Shh<sup>+/−</sup> mutants) or too little Gli3 repressor function (as seen in Shh<sup>−/−</sup>;Gli3<sup>−/−</sup> compound mutants) results in the loss of the dorsal midline. One possible interpretation of the partial rescue observed in Shh<sup>−/−</sup>;Pax6<sup>−/−</sup> 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<sup>−/−</sup> and Shh<sup>−/−</sup>;Pax6<sup>−/−</sup> 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<sup>−/−</sup>;Pax6<sup>−/−</sup> 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

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References