Pax6 Modulates the Dorsoventral Patterning of the Mammalian Telencephalon

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The Pax6 gene encodes a transcription factor with a restricted expression in the ventricular zone of the pallium and subpallium. We tested whether the function of Pax6 is necessary for the correct patterning and morphogenesis of the vertebrate telencephalon. Homozygous embryos of the Pax6/Small eye mutant lack functional Pax6 protein because of a point mutation of the gene. In the mutant Small eye embryos we detected a ventralization of the molecular patterning of the telencephalon at two borders, the pallium/subpallium and the lateral/medial ganglionic eminence. The results indicate that Pax6 controls the lateral limit of the expression of Nkx2.1, Shh, and Lhx6 in the prechordal neural tube, the telencephalon. This finding is in agreement with previous studies and supports a model for a common genetic mechanism for modulation of the dorsoventral patterning of the prechordal and epichordal CNS. The pattern defects caused by the loss of Pax6 function result in multiple morphological abnormalities in the Small eye brain: dysgenesis of the piniform, insular, and lateral cortices, the claustrum–endopiriform nucleus, and a failure in the differentiation of a subpopulation of the cortical precursors. Together the results demonstrate that Pax6 has an essential role for the modulation of the dorsoventral patterning of the embryonic telencephalon, influencing thereby the forebrain morphogenesis.

Key words: Pax6; Small eye; dorsoventral patterning; telencephalon; borders; pallium/subpallium; MGE/LGE

The two main subdivisions of the embryonic telencephalon, pallium (cortex) and subpallium (basal ganglia), have a distinct molecular patterning and strikingly different developmental potentials. During development, the initial sheet of uniform pseudostratified neuroepithelium generates dorsally the six-layered cortex and ventrally the three eminences, the medial ganglionic eminence (MGE), lateral ganglionic eminence (LGE), and caudal ganglionic eminence (CGE), which later differentiate into the nuclei of the basal ganglia.

The Pax6 gene plays a crucial role in the development of the vertebrate CNS. The mouse Small eye (allele Sey) mutation is caused by a point mutation in the Pax6 gene, resulting in the production of a nonfunctional protein (Hill et al., 1991). The homozygous Small eye animals die at birth with multiple CNS defects in the eye, forebrain, cerebellum, and spinal cord (Schmah et al., 1993; Stoykova et al., 1996; Burrill et al., 1997; Caric et al., 1997; Ericson et al., 1997; Grindley et al., 1997; Mastick et al., 1997; Osumi et al., 1997; Engelkamp et al., 1999; Warren et al., 1999). We have previously found that Pax6 mediates the establishment of distinct adhesive properties between the dorsal and ventral compartments of the embryonic telencephalon (Stoykova et al., 1997) and that Pax6 controls the differentiation of the cortical radial glia cells (Götz et al., 1998). Here we explore the role of Pax6 in the control of the dorsoventral (DV) regionalization of the telencephalon and the consequences for the brain morphogenesis in loss of Pax6 function.

In the embryonic telencephalon, the expression of Pax6 is confined to the mitotically active ventricular neuroepithelium (Ne) of the pallium (Walther and Gruss, 1991). The pallium is classically subdivided into the medial pallium (MP), dorsal pallium (DP), and lateral pallium (LP), giving rise to the archicortex (hippocampus), neocortex, and paleocortex, respectively. In addition, Pax6 exhibits a particularly strong expression in a small lateralmost region of the ventricular zone of the LGE at the level of the pallial/subpallial border (Stoykova et al., 1996, 1997). This domain is intercalated between the neuroepithelium of the striatum and the lateral pallium (Fig. 1A) and was recently designated as “ventral pallium” (VP) (Puelles et al., 1999, 2000) or “intermediate zone” (Smith-Fernandez et al., 1998). The Pax6 mRNA level shows a lateral-to-medial gradient, being highest in the region of the VP (Walther and Gruss, 1991; Stoykova et al., 1997; Puelles et al., 1999). Pax6 is also expressed in the ventricular zone (VZ) of LGE, although at a very low level (Hallonet et al., 1998; Puelles et al., 1999). A number of transcription factors and regulatory molecules with a restricted expression in the embryonic telencephalon are respecting the pallial/subpallial and MGE/LGE border (for review, see Rabenstein and Shimamura, 1997; Rubenstein et al., 1998). We examined therefore whether the strikingly different Pax6 expression levels at these two boundaries might have a biological function for the regionalization of the telencephalon. We show in this work that a similar constellation of genes, including Pax6, Nkx2.1/2.2, and Shh, appears to modulate the DV patterning not only in the epichordal part of the neural tube (Ericson et al., 1997; Briscoe et al., 1999), but also in the prechordal part of the CNS, the telencephalon. Furthermore, we found that the disruption of the normal DV patterning in the Sey/Sey brain leads to a hypoplasia of the basolateral cortex, affecting the structures that derive from the region of the ventral pallium. Our results further suggest that Pax6 possibly controls the activity of the neural determination gene Ngn2 in a subpopulation of the cortical precursors.

MATERIALS AND METHODS

Animals. Embryos were derived from crosses of heterozygous Small eye mice, Sey allele (Roberts, 1967; Hogan et al., 1986) on a C57BL/6JxDBA/2J background. The point mutation in the Pax6 gene results in the generation of truncated non-functional protein (Hill et al., 1991), whereas the transcription is not affected, thus allowing us to study the activity of the gene in the affected brain regions. The day of the vaginal plug was considered as embryonic day 0.5 (E0.5). The brains of matched homozygous and wild-type littermates were used for the expression analysis.

Received April 6, 2000; revised Aug. 11, 2000; accepted Aug. 14, 2000.

This work was supported by the Max-Planck-Gesellschaft. We thank A. Simeone, M. Price, F. Guillemit, V. Pachnis, A. Bullone, Chica Schaller, A. McMahon, and V. Tarabykin for providing us with Emx1, Emx2, and Otx1, Dlx1, Dlx5, Tbr1, SorLA, Shh, and redin probes for the in situ analysis. We thank L. Puelles and M. Götz for fruitful discussions. The excellent technical assistance of S. Eckert is highly acknowledged. Thanks are due to S. Heinemann for correcting this manuscript.

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In situ hybridization and immunohistochemistry. Sectioning, in situ hybridization, and emulsion autoradiography were performed as previously described (Stoykova and Gruss, 1994). 35S-labeled sense and antisense RNA probes were synthesized in the presence of two radioactive nucleotides from linearized plasmid templates according to the supplier’s instructions (Promega, Madison, WI). Two independent in situ analyses for each stage were performed. For the colocalization of the Ngn2 and Pax6 antigens first a nonradioactive in situ hybridization with the Ngn2 in situ probe was performed on 12 μm cryostat sections from E13.5 wild-type brain as described by Gradwohl et al. (1996). For the antibody staining the sections were further proceeded for immunohistochemistry according to Götz et al. (1998) using the anti-mouse Pax6 antibody (Development Studies Hybridoma Bank, Iowa City, IA), 1:200 and “Alexa” 568 goat anti-mouse conjugate (MoBiTec), 1:500. The terminology is in accordance with the rat brain atlases of Paxinos et al. (1994), Altman and Bayer (1995) and Foster (1998).

RESULTS

Ventralization of the molecular patterning of the pallial neuroepithelium in the Pax6/Small eye mutant telencephalon

To examine whether Pax6 plays a role in the dorsoventral region-alization at the pallial/subpallial border, we studied the molecular patterning by in situ hybridization in sections of E12.5 wild-type (WT) and homozygous Small eye (Sey/Sey) brains using the following markers: Emx1 (Simeone et al., 1992) as a dorsal telencephalic marker, which is expressed in the whole pallium except for the VP (Puelles et al., 1999; 2000); Pax6 (Walther and Gruss, 1991), Ngn2 (Gradwohl et al., 1996), and Tbr1 (Bulfone et al., 1995; Puelles et al., 1999, 2000) as pallial markers that include the VP in their expression domains and Dlx1 (Bulfone et al., 1993), Fax1 (Hallo-net et al., 1998), Mash1 (Guillemot and Joyner, 1993), and Six3 (Oliver et al., 1995) as ventral telencephalic markers. The comparative analysis was performed at three rostrocaudal levels of sectioning, and the detected patterns are illustrated in Figure 1.

At E12.5, Emx1 is expressed in mitotic and postmitotic cells in the anlage of the medial, dorsal, and lateral pallium (Fig. 1B). In the mutant brain, Emx1 expression was retraced from the depth of the basolateral wall, except for some Emx1+ cells, located very superficially (Fig. 1B’). The expression of Tbr1 is restricted to early postmitotic cells in the pallium (Bulfone et al., 1995). In the basolateral telencephalon, the Tbr1 expression extends more medially than Emx1 so that the subventricular zone (SVZ), submantle, and mantle zone of the VP expresses the Tbr1, but not the Emx1 gene (Fig. 1F, arrowheads). Thus, the medialmost expression domain of Tbr1 in the basolateral telencephalic wall seems to consist of postmitotic cells that are generated predominantly from the neuroepithelium of the VP (Puelles et al., 2000). In Sey/Sey, the expression of Tbr1 was abolished in the SVZ, submantle, and mantle zone of the VP and appeared less affected in the postmitotic neurons of the preplate in the DP and LP (Fig. 1F’).
Pax6 is expressed in the VZ of the entire pallium, showing a particularly strong signal within the region of the VP (Figs. 1A, 2A). In Sey/Sey the mutant transcripts were much less abundant in the VP, and the pallial/subpallial border was not well delineated (Figs. 1A', 2A'). The Pax6 and Ngn2 expression domains overlap in the pallial VZ (Fig. 1E; see Fig. 5A,B). Interestingly, the expression of Ngn2 in Sey/Sey was completely abolished in the region of the VP, substantially reduced in the LP and DP, but appeared at a normal level in the MP (Fig. 1E').

In the WT brain, the transcripts of the subpallial markers Dlx1, Vax1, and Mash1 in the VZ–SVZ and Six3 in the mantle zone were clearly not detectable in the VP (Fig. 1C,D,G,H). In the Pax6-deficient brain, the markers for the ventral telencephalic VZ–SVZ were ectopically expressed within the Ne of the VP and LP (Fig. 1C',D',G'). Similarly, the expression of Six3 expanded into the mantle zone of the VP (Fig. 1H'). In addition, the limit of the Dlx1 and Vax1 expression extended more dorsally in the mutant septum (Fig. 1C',D', arrowheads), which appeared enlarged as compared with the septum of the wild-type brain.

To test whether the extremely low level of the expression of Pax6 in the VZ of the entire LGE may have a biological significance for the patterning of the basal telencephalon, we studied the expression of several markers for the MGE in sections of WT and Sey/Sey brains at stages E11.5–E14.5. From E10.5 onward, the proliferative sector of the VZ–SVZ of the entire LGE was much more widespread, strongly suggesting that the mutant LGE contains a higher number of Lhx6+ cells (Fig. 3A,E). In the WT brain, the transcripts of the subpallial markers Dlx1, Vax1, and Mash1 in the VZ–SVZ and Six3 in the mantle zone were clearly not detectable in the VP (Fig. 1C,D,G,H). In the Pax6-deficient brain, the markers for the ventral telencephalic VZ–SVZ were ectopically expressed within the Ne of the VP and LP (Fig. 1C',D',G'). Similarly, the expression of Six3 expanded into the mantle zone of the VP (Fig. 1H'). In addition, the limit of the Dlx1 and Vax1 expression extended more dorsally in the mutant septum (Fig. 1C',D', arrowheads), which appeared enlarged as compared with the septum of the wild-type brain.

**Ventralization of the subpallial patterning in Sey/Sey**

To test whether the extremely low level of the expression of Pax6 in the VZ of the entire LGE may have a biological significance for the patterning of the basal telencephalon, we studied the expression of several markers for the MGE in sections of WT and Sey/Sey brains at stages E11.5–E14.5. From E10.5 onward, the proliferative sector of the VZ–SVZ of the entire LGE was much more widespread, strongly suggesting that the mutant LGE contains a higher number of Lhx6+ cells (Fig. 3A,E). In the WT brain, the transcripts of the subpallial markers Dlx1, Vax1, and Mash1 in the VZ–SVZ and Six3 in the mantle zone were clearly not detectable in the VP (Fig. 1C,D,G,H). In the Pax6-deficient brain, the markers for the ventral telencephalic VZ–SVZ were ectopically expressed within the Ne of the VP and LP (Fig. 1C',D',G'). Similarly, the expression of Six3 expanded into the mantle zone of the VP (Fig. 1H'). In addition, the limit of the Dlx1 and Vax1 expression extended more dorsally in the mutant septum (Fig. 1C',D', arrowheads), which appeared enlarged as compared with the septum of the wild-type brain.

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Together these data indicate that in the Sey/Sey telencephalon, the domain of the VP and LP is ventralized so that the limit of the Emx1 expression is retracted to a more dorsal position within the pallium, the expression of Ngn2 and Tbr1 is abolished in the VZ and SVZ-mantle of the VP, respectively, and the subpallial markers Dlx1, Vax1, Mash, and Six3 are ectopically expressed into more dorsal pallial domains.

**Figure 2. Ventralization of the neuroepithelium of the LGE in Sey/Sey.** In situ hybridization on coronal (A–D') and cross (E,E') sections from the WT and Sey/Sey brains. Different markers for the MGE were tested at stages: E13.5 (A–C'), E12.0 (D,D') and E14.5 (E,E'). In A, note the strikingly different level of Pax6 expression in the VZ of the VP and LGE. The arrowheads in A and A' point to Pax6+ cells that appear to stream out from the Ne of the VP toward the basolateral telencephalon. In the mutant telencephalon, the lateral limit of the expression of Shh (B,B') and Nkx2.1 (C,C') extends from the MGE into the adjacent territory of the LGE. In D', the large and small arrows point to the lateral limit of the strong and the faint ectopic expression of Nkx2.1 within the VZ of the mutant LGE, respectively. In E and E', note the enlarged MGE (which includes at this stage the adjacent LGE domain with a ventralized identity) and the differentiating globus pallidus, labeled by the Nkx2.1 probe (open arrowhead). F, F'. Coronal sections from E13.5 WT (F) and Sey/Sey (F') brain at the level of the preoptic area stained with neutral red, illustrating the enlarged MGE in the Sey/Sey telencephalon.
Sey/Sey brains at different rostrocaudal levels. The expression of Lhx6 along the entire rostrocaudal axis was much more abundant in the mutant as compared with the wild-type LGE (Fig. 3B–D'). Furthermore, while present in the MZ of the Sey/Sey cortex, Lhx6+ cells were not detectable in the lower part of the mutant cortical plate (Fig. 3B–D'). In accordance with previous data showing that the Lhx6+ cells originate mainly from the Ne of the MGE (Grigriou et al., 1998) at a very rostral level the WT septum contained only a few Lhx6+ cells (Fig. 3B). In contrast, the SVZ–submantle of the septum in Sey/Sey was abundantly populated with Lhx6+ cells that seem to migrate directly into the mutant LGE (Fig. 3B'). Thus, in Pax6 loss of function the Ne of the rostral septum and MGE appears to produce a higher number of Lhx6+ cells that migrate into the territory LGE, but these cells fail to populate the lower part of the mutant cortical plate. Different possibilities may account for the observed wider expression of Lhx6 in the basolateral telencephalon in Sey/Sey: (1) enhancement of the rate of the Lhx6 mRNA synthesis implicating a transcriptional regulation between Pax6 and Lhx6; (2) increase of the number of the generated Lhx6+ cells and/or enhanced ventrodorsal cell migration between the MGE and LGE as a result of the ventralization of a part of the Ne of LGE, as noticed above; and (3) accumulation of Lhx6+ cells within the mutant LGE because of a malformation of the corticopetal axons (Kawano et al., 1999) that normally help the subpallial cells in their tangential migration toward the cortex. Further experimentation will be required to definitively distinguish between these possibilities.

Taken together, the results from the performed analysis of the patterning of the basal telencephalon indicate that in the lack of a functional Pax6 protein a more dorsal domain (LGE) of the basal telencephalon achieves characteristics of a more ventral domain (MGE).

Defects in the rostral basolateral telencephalon of the Sey/Sey brain

The origin of the telencephalic basolateral structures is still under debate. Morphological studies suggested that whereas the caudate nucleus has a neocortical origin, the endopiriform nucleus and piriform cortex originate from the Ne of the corticostriatal wedge (Bayer and Altman, 1991a) and/or from the Ne of the LGE (Valverde and Santacana, 1994; De Carlos et al., 1996).

At stage E12.5, cells expressing Pax6 or Tbr1 appear to extend out from the Ne of the VP toward the basolateral telencephalon (Fig. 1A,F; see Fig. 7). To study which basolateral structures were specifically patterned by either of the two pallial markers, we examined the patterning at stage E18.5. In agreement with Bufone et al. (1995), Tbr1 transcripts were detected in postmitotic cells of the neocortex and paleocortex, classical claustrum (Cl) (Fig. 4A,C,F), and the dense layer II of the piriform cortex (Fig. 4B, open arrowhead). Recent results indicate that the classical claustrum is a derivative of the “dorsolateral claustrum” whose precursors are possibly generated from the Ne of the Tbr1+/Emx1+ lateral pallium (Puelles et al., 1999, 2000). Rostrally, Pax6 was not expressed in the differentiating classical claustrum (Fig. 4D). However, Pax6 transcripts were detected in the presumptive domain of the olfactory tubercle (Tu) and in the ventral part (presumptive layer I) of the piriform cortex (Fig. 4E, filled arrowhead). In addition, Pax6 was expressed in a region, which has been designated by different authors as the endopiriform nucleus, anterior amygdalar area, ventral pallidum, and/or lateral striatal area (Fig. 4E,F, arrow). At early developmental stages this domain was referred to as the “ventromedial caudrum”, a derivative of the VP (Fig. 1F, arrowheads) (for discussion, see Puelles et al., 1999, 2000). Results from autoradiographic studies indicated that early-born cells from the Ne of the corticostriatal wedge (included in the territory of VP) are divided by the growing tip of the cortical plate at late developmental stages into a superficial part corresponding to layer I and a deep part, corresponding to cells located in the adult layer III of the piriform cortex (Valverde and Santacana, 1994). Thus, our results suggest that the early- and the late-born constituents of the piriform cortex (the primary olfactory cortex) are differentially patterned by Pax6 and Tbr1.

As illustrated in Figure 4, the piriform cortex, the claustrum (assumed to represent the deep layers of the insular cortex), the endopiriform nucleus, and the reservoirs cells (t) (Bayer and Altman 1991a,b) were not detectable in the rostral Sey/Sey telencephalon. Likewise, the lateral cortex including the prospecitive insular cortex was severely disorganized without a recognizable cortical plate at a very rostral level (Fig. 4C,H). Cells expressing defective Pax6 transcripts were detectable in the stream that extends from the Ne of the VP along the pallial/subpallial border (Fig. 1A'), implicating that Pax6 would not have a cell autonomous function for the generation of the early-born cells of the piriform cortex. We assume rather that the dysgenesis of the piriform, lateral cortex and claustrum in the Sey/Sey telencephalon is a consequence of the prominent ventralization of the molecular patterning of the VP in Sey/Sey as demonstrated in this work.
Figure 4. Differently patterned structures by Pax6 and Tbr1 are distorted in the Sey/Sey basolateral telencephalon. A, B, D, E. Adjacent coronal sections from the E18.5 WT brain were hybridized with probes for Tbr1 (A, B) and Pax6 (D, E). C is a bright-field picture of an adjacent section to the section (B) after hematoxylin–cosin (HE) staining. F is a close-up of C for the indicated field. A, B, Tbr1 expression is detected in the differentiating claustrum proper (Cl) and in the dense layer II (B, open arrowhead) of the piriform cortex. D, E, Pax6 is expressed in the olfactory tuberculum (Tu), in the ventral part (presumptive layer I) of the piriform cortex (E, arrowhead), and in the presumptive anlage of the anterior amygdala–endopiriform nucleus, a thin arrow in E and F. In E, note that the dense layer of the piriform cortex is Pax6-negative (open arrowhead). G–J are adjacent coronal sections from the E18.5 Sey/Sey brain, hybridized with Tbr1 (G) and Pax6 (I) probes or stained with HE (H, J). In C, F, H, and J note that in the mutant brain, the piriform cortex, the claustrum proper, the endopiriform nucleus–anterior amygdala and the reservoir (r) are not distinguishable. The dark-stained structures in H and J are cells from the pallial germinative neuroepithelium that form clumps (or a thick band at other levels) located all along the pathway of the lateral migratory stream (Fig. 5) in the Sey/Sey pallium.

Defects in the differentiation of the cortical plate in Sey/Sey

The intriguing finding that at E12.5 Pax6 and Ngn2 have overlapping expression domains and that the activity of Ngn2 was downregulated within the Sey/Sey pallium was confirmed also later in development (Fig. 5). Recent data (Hartfuss et al., 2000) indicated that a subpopulation of acutely dissociated cortical progenitors colocalize Ngn2 and Pax6, implicating that a direct regulation of the activity of the proneural gene Ngn2 might contribute to the complex cortical phenotype in Sey/Sey.

A prominent feature of the Sey/Sey pallium is the thin cortical plate (CP) and the enlarged germinative neuroepithelium (VZ–SVZ) that occupies the IZ domain (Fig. 6A, A*; for discussion, see also Warren et al., 1999). Highly accumulated cells appear adherent to each other all along the lateral migratory stream (Fig. 6B*), a pathway that normally carries postmitotic cells populating the basolateral cortex. At stage E14.5, the early differentiation markers Tbr1 and SorLA showed only a faint expression in the superficial zone of the forming CP, but were not detected in the mutant VZ–SVZ (data not shown). We tested the differentiation of the mutant cortex further at stage E18.5 using the available layer-specific markers: Emx1 and Tbr1 for all layers of the cortex (Bulfone et al., 1995), Otx1 for layer V–VI (Franz et al., 1994), mSorLA for layers V–II (Hermans-BorgMeyer et al., 1998; our unpublished observations) and reelin for the MZ (D’Arcangelo et al., 1995; Ogawa et al., 1995). In the abortive CP, the Emx1, mSorLa, and Otx1 showed a diffuse expression at a similar strength.
Given the key role of reelin in the laminar cortical development (Lambert de Rouvroit and Goffinet, 1998), it is of special interest to note that whereas the reelin expression in the Sey/Sey MZ was at a higher level as compared with the wild-type brain, the reelin transcripts were lacking from the IZ of the pallium in the mutant brain (Fig. 6D′, arrowhead).

**DISCUSSION**

Pax6 modulates the DV regionalization of the neuroepithelium along the entire anteroposterior axis of the developing CNS

Accumulating evidence indicates that the expression of Shh in the axial mesendoderm is essential for the ventral specification of the developing CNS, including the forebrain (Ericson et al., 1995; Chiang et al., 1996; Rubenstein and Shimamura, 1997). In the ventral neural tube the Shh signal secreted from the floor plate mediates a long-range repression of the Pax6 level, forming thereby four zones of distinct progenitors, a most ventral Pax6−/Nkx2.2+ domain and progressively more dorsally located domains with low, moderate, and high levels of Pax6 expression (Ericson et al., 1995, 1997). The progenitors of these domains generate distinct neuronal
from the expression analysis performed on coronal sections at a rostral level of the telencephalic Ne (Puelles et al., 1999, 2000) and the results obtained and MGE/LGE borders. The drawing is based on the proposed subdivision embryonic telencephalon is ventralized at the level of the pallial/subpallial in the absence of functional Pax6 protein, the molecular patterning of the ventrodorsal domains of the telencephalic neuroepithelium for the expression of Pax6 telencephalon of the Figure 7. Schematic representation of the DV pattern defects in the

Figure 7. Schematic representation of the DV pattern defects in the
telencephalon of the Pax6;Small eye mutant. The scheme illustrates that in the absence of functional Pax6 protein, the molecular patterning of the embryonic telencephalon is ventralized at the level of the pallial/subpallial and MGE/LGE borders. The drawing is based on the proposed subdivision of the telencephalic Ne (Puelles et al., 1999, 2000) and the results obtained from the expression analysis performed on coronal sections at a rostral level of the E12.5 wild-type (WT) and homozygous Small eye (Sey/Sey) brain. The pallial and subpallial markers have been color-coded as indicated. The upper points to the morphological corticostriatal sulcus. The filled arrow points to the pallial/subpallial border, from where a of Pax6+ stream of cells (red dots) and Tbr1+ (black dots) cells migrate toward the basolateral telencephalon as described in the text. Noteworthy, results from a very recent homolog study in chick and mouse suggest that the Pax6+ cells migrate within the striatal territory (Puelles et al., 2000). The open arrowhead points to the boundary between the MGE (pallidum) and the LGE (striatum).

populations of the motor neurons and the three columns of interneurons. Our analysis revealed a similar characteristic in three ventrodorsal domains of the telencephalic neuroepithelium for the expression of Pax6 and Nkx2.1 which is another member of the Nkx gene family with a restricted expression in the anlage of the MGE (Price et al., 1992; Shimamura et al., 1995; Sussel et al., 1999). It should be noted however that the “dorsoventral” terminology used to describe our observations is preliminary because the topological relationship of the telencephalic subdivisions is still an open question (for discussion, see Rubenstein et al., 1998).

We found that the most ventrally located domain, the VZ of the MGE, that generates the cells of the pallidum is a Pax6−, but Nkx2.1+/Dlx1,2+/Vax1+/Mash1+ region. In the MGE, Shh is initially expressed in the VZ (Sussel et al., 1999), and later it is expressed in the SVZ and mantle zone. The next domain is the VZ of the LGE, which produces the striatum. It expresses Pax6 at a very low level and is Nkx2.1−/Dlx1,2+/Vax1+/Mash1+. The domain of the VP is the third zone, located further dorsally. It contributes to the generation of the caudal–endopiriform nucleus, the piriform cortex, and a part of the amygdala (Bayer and Altman, 1991; for further discussion see Puelles et al., 2000). Here the expression of Pax6 (and Ngr2 as well) is very high, whereas the transcripts of Nkx2.1, Dlx1, Vax1, and Mash1 are absent.

In the caudal neural tube, the loss of Pax6 function leads to a dorsal expansion of ventral markers and to a change of the cell fate (Ericson et al., 1997). Likewise we found that the Pax6 mutation leads to an expansion of the expression of the MGE marker genes Shh, Nkx2.1, and Lhx6 into the territory of the more dorsally located LGE. This pattern defect appears to result in the alteration of the regional identity of the adjacent LGE area reflected in an enlargement of the MGE territory at midgestation and underdevelopment of the striatum later on—a puzzling morphological phenotype for the Pax6 mutant brain for a long time (Glaser et al., 1994). We found further that the ventralization of the Ne of the VP, where Pax6 is expressed at a very high level, causes defects in the generation of the piriform, rostral lateral (insular) cortex, and the caudal–endopiriform nucleus. These defects are reminiscent to observations in the Sey/Sey hindbrain and spinal cord where the columns of the dorsally and more ventrally located neurons, produced by domains with a very high and low level of Pax6 expression, are either missing or show an altered identity, respectively (Burrill et al., 1997; Ericson et al., 1997). Thus, in Pax6 loss of function in appears that domains that normally have a comparable level of Pax6 expression, show similar morphological disturbances in the epichordal and prechordal part of the CNS as a result of the ventralization of the molecular identity of adjacent regions. These results indicate that the level of Pax6 expression is an essential determinant of the DV regionalization of the Ne along the entire anteroposterior axis of the developing CNS.

In the spinal cord of Nkx2.2−/− mice the fate of the most ventral column of neurons is dorsalized into the fate of the somatic motoneurons, but without a change in the Pax6 expression—a fact implicating that Nkx2.2 has a decisive role for interpreting the ventralizing activity of the Shh protein produced by the notochord and floor plate (Briscoe et al., 1999). Although Shh, which is produced by the rostral mesendoderm, is an essential factor for establishing the ventral identity in the forebrain (Ericson et al., 1995; Shimamura and Rubenstein, 1997), the final specification of the DV domains in the telencephalon seems to include additional mechanisms. The expression of Nkx2.1 and Shh in the MGE and Pax6 in the pallium appears almost simultaneously at E10.5 (Hentges et al., 1999). In Pax6 loss of function we observed ectopic expression of both Shh and Nkx2.1 into more dorsal telencephalic domains. A recent analysis of Nkx2.1−/− mice revealed opposite pattern defects as compared with the Small eye telencephalon (Sussel et al., 1999). In these mice, the lateral domain of the MGE is dorsalized showing ectopic expression of Pax6, whereas the expression of Shh in the MGE is suppressed. The alteration of the patterning leads to a lack of the globus pallidus and an enlargement of the striatum. Thus, in the Sey/Sey and Nkx2.1−/− mutants, although the anlage of the MGE and LGE is specified presumably by the ventralizing activity of the mesendodermal Shh, these structures show a complementary DV pattern and morphological defects in the adjacent domains of the MGE or LGE. It is worthy to note that in the absence of the low level expression of Pax6 in the VZ of the LGE, the dorsal ectopic expression of Nkx2.1 includes the VZ of a part of the LGE. Together, these data suggest that either a direct regulation of the activity of these genes or protein–protein interactions between their products might contribute for the maintenance of the MGE/LGE border in the telencephalon.
Pax6 and the patterning of the cortex

The development of the cortex is severely affected in the Sey/Sey mutant: the CP is hypocellular without radial alignment of the cells, whereas the germinative neuroepithelium (VZ–SVZ) is enlarged and consists of accumulated precursors in large clumps that occupy the area of the IZ (Schmahli et al., 1993; Warren and Price, 1999). These cells show a high level of expression of the mutant Pax6 message (Stoykova et al., 1997; this study) and active incorporation of BrdU after pulse labeling at early (E10–E12.5) (Warren et al., 1999) and later (E12.5-E18.5) stages (Brunjes et al., 1998; Götz et al., 1998).

We show in this work a severe defect of the DV patterning in the Sey/Sey telencephalon. As a result of the early developmental ventralization of the NE at the pallial/subpallial border, the morphogenesis of the basolateral cortex appears to be strongly affected, as shown by the malformation of the claustrum, endopiriform nucleus, piriform, and lateral cortex.

From E14.5 onward, the pallium of the Sey/Sey mutant fails to properly differentiate. The accumulated cells in the mutant VZ–SVZ express the neuron-specific marker TuJ1 (Caric et al., 1997). Progenitors. Most intriguingly, the misexpression of Ngn2 is detected only in those Pax6 and Sanes, 1992). In accordance with recent results indicating that potential (Alvarez-Buylla et al., 1990; Lendahl et al., 1990; Gray 1999; Cai et al., 2000). Our previous results indicated that the expression of Pax6 is a characteristic trait of the cortical RC2+ radial glial cells with an essential role for their differentiation (Götz et al., 1998). The cortical radial glial cells might have a neurogenic potential (Alvarez-Buylla et al., 1990; Gray and Sanes, 1992). In accordance with recent results indicating that Ngn2 is detected only in those Pax6+/RC2+ radial glial cells, that contain neither the astrocyte-specific glutamate transporter (GLAST) nor the brain-lipid-binding protein (BLBP) (Götz, 2000; Hartfuss et al., 2000) we show here that at E13.5 the expression of Ngn2 and Pax6 normally colocalizes only in some cortical progenitors. Most intriguingly, the misexpression of Ngn2 in the cortical progenitor cells results in the production of neurons (Cai et al., 2000). Furthermore, isolated radial glial cells from Sey/Sey cortex generated in vitro only 44% of the neuronal clones produced by the WT radial glial cells (Malatesta and Götz, 2000). Thus, our results and the literature data support the possibility that the differentiation of not all cortical precursors in the Sey/Sey pallium is affected; indeed the mutant CP shows expression of all tested cortical markers. We favor rather the idea that only a portion, mainly the Ngn2+/Pax6+ progenitors of the ventrolateral pallium are hampered to differentiate in Pax6 loss of function.

Accumulating evidence indicates that some postmitotic cells born in the subpallium invade the pallium. Thus, a part of the cortical interneurons are produced in the subpallial Ne and populate through a tangential migration the CP as postmitotic Dlx-, GABA-, GAD67-, Lhx6-, calbindin-, calretinin-, or reelin-positive cells (for review, see Parnavelas, 2000). The absence of Dlx1/2 (Anderson et al., 1997a,b) and Mash1 (Casarosa et al., 1999) in the MGE/LGE leads to an almost complete loss of the GAD67+ cells in the CP or in the MZ, respectively, whereas the loss of Nkx2.1 in the MGE (Sussel et al., 1999) is associated with absence of calbindin+ cells. We show in this work that early in development the proliferative Ne of the VP and LP in Sey/Sey expresses ectopically Dlx1, Mash1, Vax1, and Six3, whereas the restricted expression of Nkx2.1 and Lhx6 to the MGE expands into the adjacent LGE territory. Therefore it is likely that the ventralized Ne in the basal telencephalon of Sey/Sey produces progenitors with altered identity, increasing thereby the portion of the subpallial cells that migrate into the cortex. This is in line with data showing that the lateral telencephalon of Sey/Sey contains twice as much postmitotic GABA+, calbindin+ and calretinin+ cells as compared with the wild-type littermates (Chapouton et al., 1999). Thus, the defects of the dorsoventral patterning of the telencephalic neuroepithelium in Pax6 loss of function are part of the complex cortical phenotype of the Small eye mutant.

REFERENCES


