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

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Accepted 19 May; published on WWW 5 July 1999

**SUMMARY**

The telencephalon is organized into distinct longitudinal domains: the cerebral cortex and the basal ganglia. The basal ganglia primarily consists of a dorsal region (striatum) and a ventral region (pallidum). Within the telencephalon, the anlage of the pallidum expresses the *Nkx2.1* homeobox gene. A mouse deficient in *Nkx2.1* function does not form pallidal structures, lacks basal forebrain TrkA-positive neurons (probable cholinergic neurons) and has reduced numbers of cortical cells expressing GABA, DLX2 and calbindin that migrate from the pallidum through the striatum and into the cortex. We present evidence that these phenotypes result from a ventral-to-dorsal transformation of the pallidal primordium into a striatal-like anlage.

Key words: *Nkx2.1*, Telencephalon, Regionalization, mouse

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**INTRODUCTION**

The telencephalic basal ganglia are major components of the extrapyramidal motor system, whose two principal subdivisions are the striatum and the pallidum (Gerfen, 1992). The analysis of regulatory gene expression in the telencephalon suggests that the striatum and pallidum are separate longitudinal domains that are under distinct genetic control (Puelles and Rubenstein, 1993; Rubenstein et al., 1994). Whereas the *Dlx* homeobox genes are expressed in the primordia of both the striatum and the pallidum (Bulfone et al., 1993; Liu et al., 1997), other homeobox genes are only expressed in precursor cells of the pallidum. These include *Nkx2.1* (Guazzi et al., 1990; Price et al., 1992; Shimamura et al., 1995).

*Nkx2.1* is a member of the vertebrate Nkx family (Price et al., 1992; Qiu et al., 1998; Pera and Kessel, 1998). *Nkx2.1* is also known as *TTFI* and *T/ebp* because of its ability to regulate the expression of thyroid-specific gene products (Guazzi et al., 1990; Mizuno et al., 1991; Kimura et al., 1996). The initial expression of *Nkx* genes in the mouse central nervous system (CNS) is in the medial neural plate which overlies the SHH-secreting axial mesendoderm (Shimamura et al., 1995; Shimamura and Rubenstein, 1997). This observation is consistent with the fact that SHH can induce *Nkx2.1*, *Nkx2.2* and *Nkx6.1* (Barth and Wilson, 1995; Ericson et al., 1995; Shimamura and Rubenstein, 1997; Pera and Kessel, 1997; Dale et al., 1997; Qiu et al., 1998; Kohutz et al., 1998). Within the medial neural plate and ventral neural tube, expression of the *Nkx* genes have distinct anteroposterior (A/P) and dorsoventral (D/V) boundaries (Qiu et al., 1998). For instance, while *Nkx2.2* and *Nkx2.9* are expressed along the entire CNS in a narrow column of cells adjacent to the floor plate (Shimamura et al., 1995; Pabst et al., 1998), *Nkx2.1* expression is restricted to the forebrain (Price et al., 1992; Shimamura et al., 1995; Qiu et al., 1998).

The vertebrate *Nkx* genes are related to the invertebrate *NK* genes. The *Nkx2* subfamily has homology with *Drosophila NK2* or *ventral nervous system defective* (*vnd*). Like the *Nkx2* genes, *NK2/vnd* is expressed in neuroblast precursors along the ventral part of the embryonic CNS (Kim and Nirenberg, 1989; Jiminez et al., 1995). Recent studies have demonstrated that *NK2/vnd* is required to specify ventral CNS cell fates, and in its absence, the ventral CNS appears to be respecified into more dorsal identity (McDonald et al., 1998; Weiss et al., 1998; Chu et al., 1998). Like the *NK2/vnd* mutants, mice lacking *Nkx2.2* also appear to have a ventral-to-dorsal respecification of the *Nkx2.2*-deficient neurons in the spinal cord (Briscoe et al., 1999).

In this study, we analyzed whether *Nkx2.1* specifies ventral regional fate within the telencephalon. *Nkx2.1* is one of the earliest known genes to be expressed in the forebrain; its transcripts can be observed in the hypothalamic primordium at the one somite stage (Shimamura et al., 1995; Ericson et al., 1995; Shimamura et al., 1995; Briscoe et al., 1999).
The sequences of the Nkx2.1 mutant allele and the other for detection resulting from the intact S' Nkx2.1 genomic DNA that is still present in the homozygous mutant animals. Additional cDNA probes include: Dist2 and Dist5 (Liu et al., 1997); other probes were kindly provided by A. McMahon (Shh), P. Gruss (Pax6), V. Pachnis (Lhx6, Lhx7/L3), H. Ingraham (SCIP/Oct6/Tst1), A. Wanaka (Lhx7/L3), C. Gerfen (D2R), T. Jessell (ER81), and R. Axel (Golf). Whole-mount in situ hybridization was performed as described by Shimamura et al. (1994).

Immunohistochemistry
Immunohistochemistry was performed as described by Anderson et al. (1997a,b). The following antibodies were used: mouse monoclonal anti-MAP2 (Sigma, 1:2000), mouse monoclonal anti-βIII tubulin (TuJ1) (Promega, 1:2000), rabbit polyclonal anti-calbindin (Swiss antibodies, 1:5,000), anti-calretinin (Chemicon, 1:1000), anti-tyrosine hydroxylase (Pel-Freez, 1:1000), anti-Gad67 (Chemicon, 1:2000), anti-GABA (Sigma, 1:5000), anti-TrkA (kindly provided by L. Reichardt, 1:10,000), anti-DLX2 (1:200; Porteus et al., 1994).

Organotypic cultures
Organotypic cultures were performed exactly as described by Anderson et al. (1997a,b).

RESULTS

Nkx2.1 expression in the basal telencephalon
Nkx2.1 expression is first detectable in the basal telencephalon of the mouse at approximately the 11 somite stage (Shimamura et al., 1995). As the basal telencephalon develops, Nkx2.1 is maintained in regions that form morphologically distinct structures such as the medial ganglionic eminence (MGE), as well as parts of the septum, anterior entopeduncular area and preoptic area (POA) (Fig. 1a-d; L. Puelles, L. S. and J. R., unpublished data). For this study, we have focused on the role of Nkx2.1 in the development of the MGE, a proliferative zone that gives rise to pallidal components of the basal ganglia (Rubenstein et al., 1998; Salvador Martinez, personal communication).

At E10.5 in the mouse, the MGE is a neuroepithelial protrusion out of the wall of the anterobasal telencephalon into the lateral ventricle (LV). As shown in Fig. 1a and b, Nkx2.1 RNA is expressed uniformly throughout the MGE neuroepithelium. Between E11.5 and E12.5, a second morphologically distinct basal ganglia anlage, the lateral ganglionic eminence (LGE), emerges between the MGE and the cortex (Figs 1, 3, 7). At these stages the LGE lacks Nkx2.1 expression (Fig. 1c). By E12.5, the MGE is composed of three molecularly distinct cell layers. The ventricular zone (VZ), which is adjacent to the telencephalic ventricle, is composed undifferentiated, rapidly dividing cells. VZ cells contribute to the subventricular zone (SVZ), a secondary proliferating population of cells (Halliday and Cepko, 1992; Bhide, 1996). Postmitotic cells migrate from the proliferative zones to
generate the mantle zone. Nkx2.1 is expressed in all three layers of the MGE (Figs 1c,d, 7). At E14.5 and later stages, Nkx2.1 expression is prominent in the developing globus pallidus (GP) (Fig. 1). As development continues, Nkx2.1 expression can be detected in several other ventral telencephalic structures including the bed nucleus of the stria terminalis (BNST), parts of the septum, the ventral pallidum and parts of the amygdala (Fig. 1d; L. Puelles, E. Kuwana, A. Bulfone, K. Shimamura, J. Keleher, S. Smiga, E. Puelles and J. L. R. R., unpublished data; L. Puelles, L. S. and J. L. R. R., unpublished data).

Nkx2.1 mutants are defective in basal ganglia development

To assess the extent of basal telencephalic defects in the Nkx2.1 mutant, we compared Nissl-stained sections from E18.5 Nkx2.1 mutant embryos (Fig. 1f,h,j) to wild-type control littermates (Fig. 1e,g,i). E18.5 was the only age that was previously shown to have morphological defects in the telencephalon (Kimura et al., 1996). At E18.5, we found that the mantle zones of the MGE and LGE are not cytoarchitecturally distinct and that pallidal structures, such as the globus pallidus, are either missing or unrecognizable (n=4). Likewise, the axon tract that traverses this region (anterior commissure, AC) does not cross the midline. Conversely, the striatum, an LGE derivative, appears to be enlarged and extends into the brain region normally occupied by the pallidal structures. These results were confirmed by molecular analyses (see below, Fig. 4i–p).

Because several components of the basal ganglia are affected in the Nkx2.1 mutant, we studied whether there were corresponding molecular and histological defects within the nascent basal telencephalon. We began our analysis at E10.5, when a morphologically distinct MGE is forming. The Nkx2.1 mutant allele was created in such a way that we are able to use mutant Nkx2.1 transcripts as a marker of the MGE (see Materials and Methods). In the Nkx2.1 mutants, a structure that resembles a small MGE and expresses high levels of Nkx2.1 RNA is evident at E10.5 and E11.5 (Fig. 2a,d,g,j). We refer to this structure as the MGE*.

Telencephalic Sonic hedgehog expression is severely reduced in Nkx2.1 mutant embryos

Sonic hedgehog (SHH) secretion from the axial mesendoderm is required for patterning of the anteromedial neural plate, including the hypothalamus and basal telencephalon (Chiang et al., 1996). In addition, SHH can induce markers of the basal telencephalon, such as Nkx2.1 (Ericson et al., 1995; Shimamura and Rubenstein, 1997; Dale et al., 1997; Pera and Kessel, 1997) and Dlx2 (Khotz et al., 1998). Shh begins to be expressed in the VZ of the ventral-most regions of the basal telencephalon (preoptic and anterior entopeduncular areas; POA, AEP) between the 10-12 somite stage, at approximately the same time as Nkx2.1 begins to be expressed in the basal telencephalon.
Subsequently, Shh expression spreads into the SVZ and mantle of the MGE (Fig. 2).

The ventral border of Pax6 marks the beginning of the MGE. (f) In the mutant, LGE levels of Pax6 expression are found more ventrally (arrowhead in f), and overlap with Nkx2.1 expression in the MGE* (arrowhead in d). (g-l) Serial coronal sections from wild-type (g-i) and Nkx2.1 mutant (j-l) E11.5 embryos. (g,j) At E11.5, the MGE in the mutant is less pronounced, but Nkx2.1 expression remains strong. The arrowhead indicates the ventral border of Pax6 expression which overlaps the Nkx2.1-positive region. Pax6 does not extend into the POA. Pax6 expression in the LGE mantle also extends ventrally (*). (m-p) Serial parasagittal sections of wild-type (m-n) and mutant (o-p) E11.5 embryos. (o) Nkx2.1 transcript can be detected in the MGE* and POA*. (p) Shh RNA is not detectable in the basal telencephalon, but can still be clearly detected in the tegmental (Teg) region of the midbrain. (q-r) Coronal sections from the same series of wild-type and mutant sections shown in g-l. Maturing neurons are present in the MGE* based upon the TuJ1 immunoreactivity (r). Scale bars, (a,r) 100 μm; (m) 400 μm.

(Shimamura et al., 1995).
Dorsalization of the ventral telencephalon in the Nkx2.1 mutants

To determine whether the defects in Nkx2.1 and Shh expression affect patterning of the ventral telencephalon, we studied the expression of Pax6, a homeobox gene that is repressed by SHH and which represses Nkx gene expression (Chiang et al., 1996; Ericson et al., 1997). In the telencephalon of wild-type embryos, Pax6 expression is strong in the cortex, weak in the LGE and not detectable in the Nkx2.1-expressing areas (MGE and POA) (Fig. 2c,i; also see Stoykova et al., 1996). In the Nkx2.1 mutants, at E10.5, prior to the formation of a LGE, low levels of Pax6 transcripts are detected in the basal telencephalon tissue that expresses the mutant Nkx2.1 gene (Fig. 2f). The ventral expansion of Pax6 expression becomes more obvious by E11.5 and E12.5, when it clearly overlaps with Nkx2.1 expression in the VZ of the MGE*, although it does not expand into the mutant preoptic area (POA*) (Figs 2i,l, 3k-n). This suggests that the mutant MGE* has dorsal characteristics that are not present in the wild-type MGE. In addition to the dorsalization of the VZ, there is a ventral expansion of Pax6 expression in the subpial mantle of the basal telencephalon (Fig. 3n).

Nkx2.1 mutants lack morphological and molecular maturation of the MGE

By E12.5, the dorsal/ventral molecular abnormalities are reflected by clear histological defects. At this stage in wild-type mice, the MGE and LGE are distinct structures (Fig. 3a). However, in E12.5 Nkx2.1 mutant embryos, the sulcus that is normally found between the MGE and LGE (see arrowhead in Fig. 3a) is almost completely absent, and there appears to be a single ganglionic eminence (Fig. 3b). Despite the morphological homogeneity, the medial (ventral) part of the mutant ganglionic eminence still expresses Nkx2.1 (Fig. 3d), demonstrating that the MGE* still exists.

To further characterize the MGE*, we looked at expression of the LIM-homeobox genes Lhx6 and Lhx7 (L3), whose onset of expression in the telencephalon is restricted to the Nkx2.1 territory (Wanaka et al., 1997; Grigoriou et al., 1998). Telencephalic expression of both genes is not detectable in the Nkx2.1 mutant at E12.5 (Fig. 3f and h). The lack of Shh, Lhx6 and Lhx7 in the SVZ and mantle suggests that the MGE* does not follow the normal developmental program of the MGE. Furthermore, the morphological appearance of the MGE*, the presence of Pax6 in the VZ of MGE*, combined with the enlarged striatum that is evident at E18.5, suggest the hypothesis that the MGE* may have become respecified to produce striatal tissue. To test this model, we analyzed the expression of genes that are expressed in the LGE and developing striatum.

Evidence that the MGE* is respecified to form an LGE which differentiates into the striatum

The Dlx genes encode homeobox transcription factors that are essential for differentiation of the LGE and MGE.
Four Dlx genes are expressed in progressively more advanced stages of differentiation in the MGE and LGE (Liu et al., 1997; Anderson et al., 1997b). There is also evidence that SHH may be required for their expression (Kohut et al., 1998). We assayed for the expression of Dlx2 and Dlx5.

(Anderson et al., 1997a,b; K. Yun, T. Stuehmer and J. L. R. R., unpublished results). Four Dlx genes are expressed in progressively more advanced stages of differentiation in the MGE and LGE.
**Forebrain respecification in Nkx2.1 mutants**

*Fig. 5. MGE cells migrate into the LGE and cortex. (a) At E14.5, Nkx2.1 is expressed in the MGE and in a small population of cells emanating from the MGE into the striatum. (b) By P6, there is a large population of Nkx2.1-expressing cells scattered throughout the striatum. (c) Dil was placed in the MGE SVZ in 250 μm thick coronal brain slices from E12.5 embryos. After 36 hours in culture, labeled cells are present in the striatum and a few have migrated into the cortex (arrowhead). (d-e) Lhx6 RNA expression in coronal hemisections of E14.5 wild-type (d) and mutant (e) brains. By E14.5 in wild-type mice, Lhx6 expression extends through the striatum and into the cortex. In the Nkx2.1 mutant, Lhx6 transcripts are not detectable. Scale bars, 500 μm.*

**Dlx5, and found that their expression appears normal in both the LGE and MGE* (Fig. 3i,j, and data not shown). Thus, unlike the MGE-specific markers, Dlx expression is not affected by the loss of Nkx2.1 function. There are at least two explanations for this result: either Dlx expression in the MGE* represents an Nkx2.1-independent population of MGE cells, or the MGE* has taken on LGE-like properties.

To distinguish between these possibilities, we analyzed at E14.5 the expression of two LGE-restricted markers: SCIP/Tst1/Oct6 encodes a POU-homeodomain transcription factor that is expressed in the SVZ of the LGE (Fig. 4b; Alvarez-Bolado et al., 1995; Anderson et al., 1997a) and **GOLF encodes a guanine nucleotide binding protein that is expressed in the mantle of the LGE (Fig. 4c; Belluscio, 1998). Neither gene is expressed at high levels in the MGE or its derivatives at E14.5 (Fig. 4d). However, in the Nkx2.1 mutant, SCIP expression has expanded into the SVZ of the MGE* and now overlaps with the Nkx2.1-expressing region (Fig. 4f,h). In addition, GOLF expression is ventrally expanded into the region normally occupied by the MGE mantle (Fig. 4g). Although there are only a small number of Nkx2.1-positive cells present in the MGE* mantle, these cells appear to be in the region containing the ventral-most GOLF-expressing cells (arrowhead in Fig. 4b). Therefore, this evidence suggests that in Nkx2.1 mutants, the MGE* becomes respecified to produce striatal-like tissue, instead of the globus pallidus.

**Histological analysis of the basal telencephalon at E18.5** also revealed expansion of striatal tissues and loss of pallidial structures in the Nkx2.1 mutants. For instance, expression of dopamine receptor 2 (D2R), which marks striatal projection neurons is greatly expanded (Fig. 4k,o), whereas expression of the ER81 ETS transcription factor, which labels neurons in the globus pallidus, is lost (Fig. 4l). Striatal histology and connectivity in the Nkx2.1 mutants is indistinguishable from that in normal littermates based on Nissl staining (Fig. 4j,n), expression of D2R and its innervation by tyrosine hydroxylase (TH) fibers (Fig. 4i,m).

Not all of the Nkx2.1-expressing region is equally dorsalized. As noted above, Pax6 expression is not detectable in the VZ of the POA*. Likewise, expression of SCIP and Golf also do not expand into this region. Consistent with these molecular results, striatal tissue does not expand into this ventral-most region of the telencephalon.

**Nkx2.1 cells migrate from the MGE into the LGE**

During our analysis of Nkx2.1 expression in the wild-type basal telencephalon, we noticed the appearance of scattered Nkx2.1-expressing cells in the striatum by E14.5 (Fig. 5a). As development proceeded, the expression of Nkx2.1 was maintained in a subset of striatal cells (Fig. 5b). To determine whether the Nkx2.1-positive striatal cells arose in situ or whether they migrated from the MGE, we tracked the migration of cells out of the MGE in slice cultures (Anderson, et al., 1997). Crystals of Dil were placed into the VZ and SVZ of the MGE in brain slices from E12.5 mice. After 24 to 36 hours in culture, many labeled cells were present throughout the striatum, suggesting that a population of striatal cells arrive via a tangential migration from the MGE (n=13; Fig. 5c).

The dynamic expression pattern of the Lim homeobox gene, Lhx6, supports this hypothesis. At E12.5, Lhx6 expression is restricted to cells of the MGE (SVZ and mantle zone) and its derivatives at E14.5 (Fig. 4d). However, in the Nkx2.1 mutant, Lhx6 RNA expression in coronal hemisections of E14.5 wild-type (d) and mutant (e) brains. By E14.5 in wild-type mice, Lhx6 expression extends through the striatum and into the cortex. In the Nkx2.1 mutant, Lhx6 transcripts are not detectable. Scale bars, 500 μm.

**Cortical interneurons are reduced in Nkx2.1 mutant mice**

Recent studies have identified a migration of cells from the basal ganglia to the cerebral cortex (De Carlos et al, 1996; Anderson et al., 1997b; Tamamaki et al., 1997). There is evidence that these migrating cells are GABAergic and may be the source of many of the neocortical interneurons (Anderson et al., 1997b). Furthermore, neonatal mice lacking Dlx1 and Dlx2 expression, which have a block in differentiation of the
LGE, have a four-fold reduction in neocortical GABAergic interneurons and have no detectable GABAergic interneurons in their olfactory bulbs (Anderson et al., 1997b, Bulfone et al., 1998). Our previous studies did not establish whether the migrating cells were coming from the LGE or the MGE. Our finding that MGE cells can migrate to the cortex, and that Lhx6 expression spreads from the MGE to the cortex prompted us to assess the cortical phenotype in the Nkx2.1 mutants.

Overall, the cortex appears histologically normal in E18.5 Nkx2.1 mutants (Fig. 6a,b). However, immunohistochemical analysis of calbindin, GAD67, and DLX2 (Fig. 6) shows that both the neocortex (NCx) and paleocortex (PCx) have reductions in the numbers of interneurons. Calbindin is almost completely missing (>99%; n=3) from the cortical plate (CP), intermediate zone and subventricular zone of the NCx and deep layers of the PCx (Fig. 6b and d). In the subpial region of the marginal zone, there are normal numbers of calbindin-expressing cells, which may correspond to the calretinin-positive Cajal-Retzius cells that are postulated to be derived from a proliferative zone proximal to the olfactory bulb (Meyer et al., 1998). On the other hand, in deeper regions of the marginal zone there is a large reduction of calbindin-positive cells (Fig. 6c,d). These results suggest that the majority of calbindin-expressing neocortical interneurons are derived from the MGE. Since many of the calbindin neurons are also GABAergic, we tested the Nkx2.1 mutants for GAD67 immunoreactivity, and found an approximately 40% reduction of GAD67-positive cells (n=3; Fig. 6j). We also see a reduction (approx. 50%) in DLX2-expressing cells in the cortical plate, intermediate zone and subventricular zone of the NCx, and there is almost a 75% reduction of DLX2-expressing cells in the marginal zone (Fig. 6f and 6l; n=3). While there are large
reductions in DLX2-positive cells in the marginal zone, calretinin expression in this region is normal, suggesting that Cajal-Retzius neurons are not derived from the MGE (Fig. 6g,h).

We next studied interneuron development in the Nkx2.1 mutant olfactory bulb because Dlx1 and Dlx2 mutants lack detectable GABAergic cells in this structure (Bulfone et al., 1998). Surprisingly, in the Nkx2.1 mutant, interneuron development in the olfactory bulb appears normal, based on the expression of GABA, calbindin and DLX2 (Fig. 6o,p and data not shown). This would suggest that the MGE/Nkx2.1 region does not contribute GABAergic or calbindin-positive interneurons to all regions of the telencephalon.

**Cholinergic neurons are missing from the striatum of Nkx2.1 mutant mice**

The cells migrating from MGE to LGE may be the cortical interneurons that are passing through the LGE, or they may represent a distinct population of cells that end their migration in the striatum. Since Shh and Nkx genes have been implicated in cholinergic neuron development in the spinal cord (Chiang et al., 1996; Ericson et al., 1996; M. Sander, S. Paydar, M. German and J. L. R. R., unpublished data), and cholinergic neurons constitute some striatal, septal and pallidal projection neurons, we tested whether basal telencephalic cholinergic neurons were affected by the Nkx2.1 mutation. To test for the presence of cholinergic neurons, we assayed for the TrkA neurotrophin receptor, a recognized marker for cholinergic cells (Sobreviela, et al., 1994). As shown in Fig. 6n, TrkA is not detectable in the Nkx2.1 mutant striatum at E18.5. In fact, no TrkA cells could be identified in the entire basal telencephalon (data not shown).

**DISCUSSION**

**Loss of Nkx2.1 function causes a ventral-to-dorsal molecular transformation within the anlage of the basal ganglia: evidence that the primordium of the globus pallidus (the MGE) forms striatal tissue**

In this study, we demonstrate that Nkx2.1 is expressed in the proliferative and postmitotic cells of the MGE from its inception, and continues to be expressed in maturing MGE derivatives (Figs 1a-d, 5u,b). Furthermore, Nkx2.1 is required
for development of pallidal-related structures of the ventral telencephalon; in its absence, none of the derivatives from Nkx2.1-positive telencephalic primordia are identifiable (Figs 1, 4). Our analysis suggests that loss of Nkx2.1 function results in a molecular repatterning and respecification of the MGE into an LGE-like tissue (Fig. 7). The evidence supporting this hypothesis is based on the following results. First, at E10.5 a MGE-like structure (MGE*), which expresses Nkx2.1 (Fig. 2d,j,o), is morphologically present (best seen in Fig. 2q,p). Second, the MGE* lacks additional molecular characteristics of the MGE (Shh, Lhx6, Lhx7 are absent; Fig. 3) and subsequently acquires molecular characteristics of the LGE (Pax6 in the VZ; SCIP in the SVZ; GOLF in the mantle) (Figs 3n and 4f-h). By late in gestation, we observe an enlargement of LGE derivatives (e.g. the striatum) and a lack of MGE derivatives (e.g. the globus pallidus) (Figs 1, 4). It is important to note that we have not proved at single cell resolution that the cells expressing Pax6, SCIP and GOLF also express the Nkx2.1 truncated transcript. This level of analysis is needed to strengthen the argument supporting a fate respecification of MGE* cells. However, aside from this caveat, we suggest that the evidence is strong enough to hypothesize that Nkx2.1 has a primary role in regional specification of the basal telencephalon; in its absence, the MGE takes on an LGE fate. We also suggest that the co-expression of the Nkx2.1 and Dlx genes co-define pallidal identity, whereas expression of the Dlx genes alone defines striatal identity.

Nkx2.1 is related to a Drosophila gene named ventral nervous system defective (vnd). Vnd encodes the NK2 homeodomain protein which is expressed in the ventral part of central nervous system (Kim and Nirenberg, 1989; Jiminez et al., 1995). The CNS in fly embryos lacking vnd have a ventral-to-dorsal transformation (McDonald et al., 1998; Weiss et al., 1998; Chu et al., 1998), analogous to the phenotype in the Nkx2.1 mutants. There are several Nkx genes expressed in the ventral CNS of vertebrates, including Nkx2.2 and Nkx6.1 (Price et al., 1992). Mutations of Nkx2.2 and Nkx6.1 also have ventral-to-dorsal transformations (Briscoe et al., 1999; M. Sander, S. Paydar, M. German and J. L. R. R., unpublished data). In both of these cases, Shh expression was unaffected, suggesting that Nkx2.2 and Nkx6.1, like vnd, have primary roles in ventral specification. We cannot be sure that Nkx2.1 alone is necessary for fate specification of the MGE, because Shh expression, which is essential for ventral specification (Chiang et al., 1996), is also reduced in the Nkx2.1 knockout mice. However, the described functions of vnd, Nkx2.2 and Nkx6.1 support the hypothesis that Nkx2.1 has a primary role in regional specification, like its homologues. Furthermore, in all regions of the CNS, the Nkx genes are expressed before Shh (Shimamura et al., 1995; P. Crossley and J. L. R. R., unpublished observations), supporting the model that Nkx2.1 is upstream of Shh in the basal telencephalon. Finally, in Gli2 mutant mice, Shh is not expressed in the floor plate, yet Nkx2.2 is expressed and motor neurons form (Matis et al., 1998). Thus, while Shh expression in the axial mesendoderm is essential for ventral specification of the CNS (Chiang et al., 1995), Shh expression in neural tissue may not have a major role in regionalization.

The fact that a morphologically distinct MGE* forms in the mutants (Fig. 2p,q) suggests that its early growth is not dependent on Nkx2.1. This implies that there are distinct genetic pathways for MGE regional specification and proliferation. We are unaware of other Nkx genes that are expressed in the proper pattern to be candidates for these genes.

Previous studies have demonstrated that SHH can induce Nkx2.1 expression (Ericson et al., 1995; Shimamura and Rubenstein, 1997; Dale et al., 1997; Pera and Kessel, 1997; Qi et al., 1998; Kohtz et al., 1998). Here we find that the Nkx2.1 gene transcription is induced and maintained, despite the massive reduction in Shh expression in the forebrain. We suggest that SHH produced in the anterior mesendoderm is sufficient to induce Nkx2.1 at neural plate stages, and that high levels of SHH expression in the forebrain is not required to maintain Nkx2.1 expression. In addition, neuroepithelial expression of Shh appears to require Nkx2.1. It will be important to test whether neuroepithelial expression of Shh in other CNS regions also depends upon expression of other Nkx genes.

The trace levels of Shh in the region of the mutant preoptic area (POA*) (Fig. 2e), may explain why Pax6 expression does not extend into this region (Figs 2f,l, 3n). This may also explain why the ectopic striatal tissue doesn’t enter this territory (compare expression of Nkx2.1 with SCIP and GOLF in the POA; Fig. 4). We do not know the identity of the tissue that develops in the mutant POA*.

SHH can induce DLX expression, and anti-SHH immunoglobulins reduce DLX expression in forebrain explants (Kohtz et al., 1998). However, despite the lack of Shh expression in the MGE*, there are roughly normal levels of Dlx2 and Dlx5 transcripts in Nkx2.1 mutants (Fig. 3i,j and data not shown). This could be due to the extremely low level of Shh expression in the POA* (Fig. 2e), or due to long lasting effects of SHH produced in the anterior mesendoderm at neural plate stages (Shimamura et al., 1997) (note: Dlx genes turn on around E9.5, about 24 hours after the medial neural plate begins to be exposed to SHH). In either case, it suggests that normal levels of Shh expression in the forebrain are not required for induction or maintenance of Dlx expression, and perhaps suggests that other secreted substances have a more direct role for inducing Dlx expression in this region.

Despite the loss of Shh and Nkx2.1 expression, and the apparent transformation of ventral telencephalon into a more dorsal fate, Nkx2.1 mutants form a LGE/striatum, have a normal subcortical/cortical boundary (interface of Dlx5 and high Pax6 expression; Fig. 3i,j,m,n) and form the major regions of the cerebral cortex (see Fig. 6). Thus, disruption of ventral patterning does not significantly affect dorsal and anteroposterior patterning. For instance, Fgf8 and Otx1, two genes implicated in anteroposterior patterning of the forebrain (Shimamura et al., 1997; Acampora et al., 1997), continue to be expressed normally (data not shown).

Nkx2.1 mutants have reduced basal forebrain cholinergic neurons and cortical interneurons: evidence for migrations from the ventral telencephalon into the striatum and cerebral cortex

In the absence of Nkx2.1, the MGE* does not produce normal ventral telencephalic cell types. Evidence is accumulating that dorsoventral patterning of the vertebrate CNS is regulated by common mechanisms along the entire anteroposterior axis (Hynes et al., 1995; Lumsden and Graham, 1995; Ericson et
REFERENCES


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