Tohoku J. Exp. Med., 2001, **193**, 163–174

Review

The Role of Pax6 in Brain Patterning

NORIKO OSUMI

Department of Developmental Neurobiology, Tohoku University Graduate School of Medicine, Sendai 980–8575

OSUMI, N. *The Role of Pax6 in Brain Patterning.* Tohoku J. Exp. Med., 2001, **193**(3), 163–174 —— *Pax6* gene encodes a transcription factor that plays a pivotal role in various aspects of brain development. Here I review the molecular and cellular mechanisms of how the early brain is patterned, and introduce recent studies on the role of Pax6 in brain patterning, neuronal specification, neuronal migration and axonal extension. ———— brain development; patterning; Pax6; small eye; cadherin

Pax6 is a member of the vertebrate Pax gene family, which is structurally related to the *Drosophila* pair-rule gene, *paired*. There are nine members in *Pax* gene family, which are categorized into four subclasses (Table 1; Dahl et al. 1997). *Pax6* was initially considered as a "master control gene" of the eye development (see review by Gehring and Ikeo 1999). Spontaneous mutations in the mouse and rat *Pax6* gene result in an eyeless phenotype in the homozygous condition (Hill et al. 1991; Matsuo et al. 1993; Osumi et al. 1997), and those in the human Pax6 gene are found in patients with a variety of eye disorders, including aniridia and Peter's anomaly (for review see Prosser and van Heyningen 1998). *Pax6* is highly conserved not only in vertebrates but also in invertebrates; *eyeless*, the counterpart of *Pax6* gene in the fly, also leads to lack of the eye in the loss-of-function condition, and ectopic eye in the gain-of-function condition (Halder et al. 1995). *Pax6* is also involved in the development of other organs (Dahl et al. 1997). In the pancreas, Pax6 is expressed in alpha cells that secrete the hormone, glucagon, and regulates specification of these cells (Dohrmann et al. 2000). Pax6 also plays an important role in the development of the pituitary gland (Dosen and Rosenfeld 1999). In this review I focus only on the role of Pax6 in early brain development, although this gene is expressed in various cells in the adult brain, e.g., the ependymal cells of the ventricles, granule cells in the olfactory bulb and cerebellum, and neurons in the amygdala and hippocampus (Stoykova et al. 1994; Engelkamp et al. 1999; and unpublished observation in my laboratory).

Received and accepted for publication March 2, 2001.

Address for reprints: Noriko Osumi, Department of Developmental Neurobiology, Tohoku University Graduate School of Medicine, 2–1 Seiryomachi, Aoba-ku, Sendai 980–8575, Japan.

This review is based on Millenium Lecture at 73th Annual Meeting for the Japanese Biochemical Society on October 13, 2000 in Yokohama.

TABLE 1. *Nine members of Pax gene family categorized into four subclasses*

Pax genes	Basic structure			Localization		Rouse mutant		Human syndrome
	PD	OP	HD	Mouse	Human	Natural	Targeted	
Pax 1	N– –C			2	20p11	*Undulated*		Spina bifida (?)
Pax 9				12	14q12–q13			
Pax 2				19	10q25	*Pax2^{1Neu}*	Yes	Renal coloboma
Pax 5	N– –C			2	2q12–q14			
Pax 8				4	9p13		Yes	
Pax 3	N– –C			1	2q35	*Splotch*		Waadenburg syndrome I, III
Pax 7				4	1p36.2		Yes	
Pax 4				6	7q32		Yes	
Pax 6	N C			2	11p13	*Small eye*	Yes	Aniridia Peter's anomaly

Structures of protein, chromosomal loci in human and mouse, mutant strains, and responsible syndromes in human are shown. PD, paired domain; OP, octapeptide; HD, homeodomain.

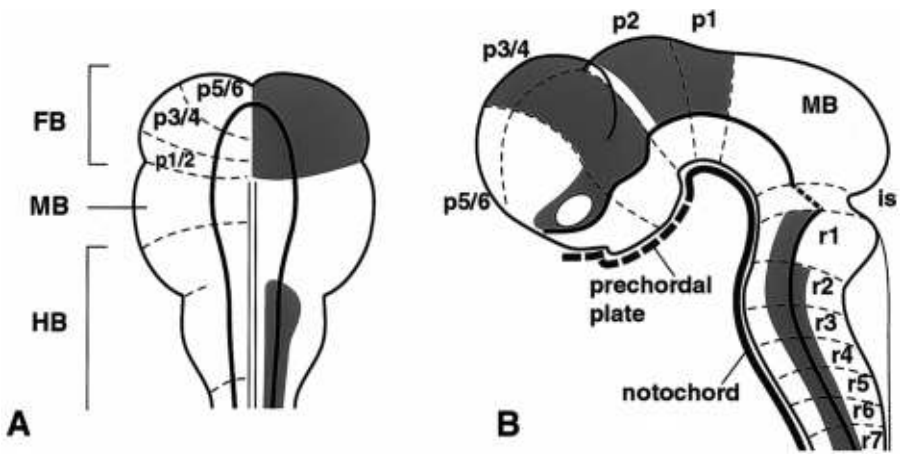

Fig. 1. Pax6 expression at the neural plate stage (A) and neuromere stage (B). FB, forebrain; MB, midbrain; HB, hindbrain; is, isthmus (=midbrain/hindbrain boundary); p1–6, prosomere 1–6; r1–7, rhombomere 1–7.

Brain patterning and its significance in brain development

In this section I briefly introduce the term "brain patterning" and describe a few but very important signal molecules in initial brain patterning. For more information, see reviews by Lumsden and Krumlauf (1996), Rubenstein and Beachy (1998), and Rubenstein et al. (1998).

The brain starts to develop from a simple sheet-like structure called the neural plate (4–6 somite stage; Fig. 1A). Neurulation converts the neural plate into a neural tube, which is subdivided into a series of vesicles that are the primordia of major brain regions: the forebrain, midbrain, and hindbrain. As development proceeds, transverse constrictions further subdivide the brain into neural segments, or neuromeres, thereby anteroposterior (A/P) patterning is established (about 30-somite stage; Fig. 1B). Neuromeres are present in the hindbrain, where they are called rhombomeres, and also probably in the forebrain where they are known as prosomeres. Furthermore, dorsoventral (D/V)

patterning generates longitudinally aligned columns of cells that are parallel to the long axis of the neural tube. In later development, the brain patterning is important for specification of neuronal subtypes. Various types of neurons are generated according to their positions along A/P and D/V axes in the brain. Brain patterning also functions as scaffolds or guidance for neuronal cell migration and axonal extension. Some neurons migrate along the boundary between the neuromeres, others migrate within one neuromere to reach its boundary, where they stop. Many of the initial networks are formed just precisely along the neuromere boundaries. There is accumulating molecular and cellular evidence that implicates brain patterning itself and its significance in later development.

Neuromere formation is controlled by the A/P patterning system, the molecular mechanisms of which are yet to be known. In contrast, the D/V patterning becomes clearer at the molecular level. Inductive signals that are secreted from the dorsal ectoderm belong to the TGFβ superfamily, including BMPs and dorsalin. These dorsalizing signals induce dorsal characters to the neuroepithelial cells in the developing neural tube. Conversely, a signal released from the ventral midline structures, the notochord and floor plate, is sonic hedgehog (SHH), which gives ventral characters to the neuroepithelial cells in the neural tube. Other local cues are also important in patterning the brain. For example, the midbrain-hindbrain boundary is the signaling center for both the midbrain and hindbrain to be patterned anteroposteriorly (Nakamura 2000).

Expression patterns of Pax6 in early brain development

In the developing brain, many transcriptional factors including Pax6 are expressed in region-specific manners (see Rubenstein et al. 1998). Expression of Pax6 begins at the neural plate stage (4–6 somite stage; embryonic day [E] 8 in mice) in the undifferentiated neuroepithelium of the forebrain, hindbrain and spinal cord (Fig. 1A; see also Inoue et al. 2000). At the neuromere stage (about 30-somite stage; E10 in mice), Pax6 expression patterns become more complex (Fig. 1B; see also Stoykova and Gruss 1994; Osumi et al. 1997; Ericson et al. 1997; Inoue et al. 2000): Pax6 is expressed in the dorsal part of the telencephalon and diencephalon as well as in the lateral-ventral part of the rhombencephalon and spinal cord. During these stages, most of Pax6-positive cells are observed in the ventricular zone, where undifferentiated neuronal progenitors of the central nervous system (CNS) exist, while mature neurons in the future thalamus and amygdala also express Pax6 (Stoykova et al. 1994). At E14 Pax6 is also expressed in the rhombic lip that later gives rise to the primordium of the cerebellum (Engelkamp et al. 1999).

Expression of *Pax6* in several organs is controlled by distinct tissue-specific cis-regulatory elements (Kammandel et al. 1999; Ashery-Padan et al. 2000). It was recently reported that *Pax6* expression in the neural tube is influenced by factor(s) released from the adjacent tissue, such as the paraxial mesoderm and newly formed somites (Pituello et al. 1999). FGFs released from the newly formed somites downregulate *Pax6* expression in the spinal cord (Bertrand et al. 2000). However, the factor(s) that initiates the expression of *Pax6* in the developing neural plate/tube remains to be identified. In the anterior CNS, Pax6 is expressed at first in the entire forebrain territory, but is gradually downregulated in the most anterior (later in the ventral) portion (see Fig. 3 in Inoue et al. 2000). This may also be due to the inhibitory effect of FGF that is expressed in the anterior margin of the forebrain (Rubenstein et al. 1998).

Role of Pax6 in forebrain patterning

The posterior expression boundary of Pax6 is at first rather vague, but eventually the bor-

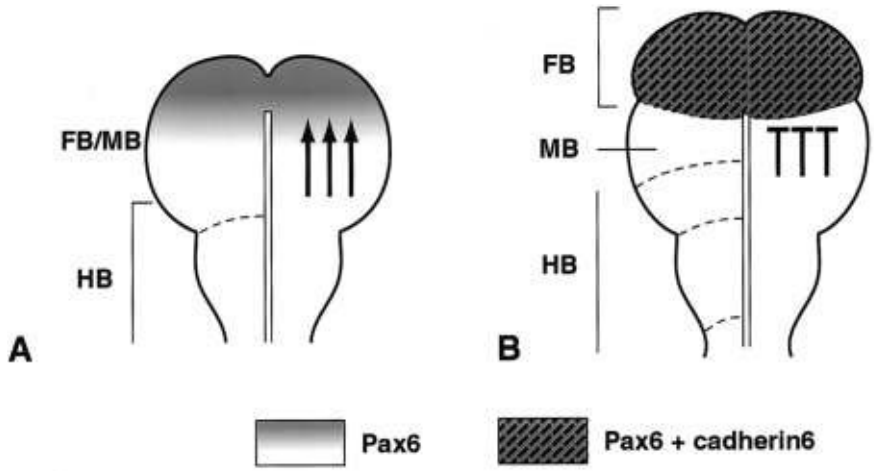

Fig. 2. Pax6 and patterning of the forebrain. (A) Pax6 starts to be expressed in the forebrain territory around 3–4 somite stage, but its posterior expression border is vague. During this stage, expression of cadherin6 is not observed yet, and neuroepithelial cells in the anterior neural plate move rostrally (arrows). (B) At 5-somite stage and thereafter, the posterior expression border of Pax6 becomes sharp, and expression of cadherin6 starts in the forebrain. Rostral movement of neuroepithelial cells is restricted at the forebrain/midbrain boundary (T-shaped bars).

der becomes sharp facing the Pax6-negative midbrain (Fig. 2A; also see Fig. 3 in Inoue et al. 2000). Mutual inhibition between Pax6 and Pax2 is observed at the boundary between the forebrain and midbrain (Matsunaga et al. 2000). Such inhibition between transcriptional factors seems to be a general feature in forming a boundary at adjacent brain regions; anteriorly expressed Otx2 and posteriorly expressed Gbx2 are involved in the formation of the midbrain/hindbrain boundary (see review by Nakamura 2000). Cell tracing analyses revealed that the forebrain/midbrain boundary indeed restricts intermixing of neuroepithelial cells (Inoue et al. 2000). Forebrain/midbrain boundary formation defect is observed in *Pax6* mutant mouse (Mastick et al. 1997; and unpublished observation in *Pax6* mutant rat in my laboratory) and in Pax2/5 double knockout mouse (Schwarz et al. 1999).

Pax6 is a transcriptional factor that functions in the nucleus. What kind of cell surface molecule is regulated by Pax6 and directly controls cell behavior in formation of the forebrain territory? Members of the cadherin superfamily, i.e., cell adhesion molecules, provide a selective adhesiveness via homophilic binding. One of the members, cadherin 6, is expressed in the forebrain in a coordinated manner with Pax6 (Inoue et al. 2000). It is therefore likely that cadherin 6 facilitates segregation of forebrain cells and midbrain cells, thereby ensuring the formation of the forebrain/midbrain boundary (Fig. 2B).

At neuromere stages, the forebrain subdivides into several regions. Pax6 expression is downregulated in the ventral part of the telencephalon and diencephalons (Fig. 1B). In later development of the diencephalons, the expression persists in the anterior thalamus (conventionally termed "ventral" thalamus) and the pretectum, but is downregulated in the body of the posterior (dorsal) thalamus (Grindley et al. 1997; Warren and Price 1997). Analyses of *Pax6* mutant mice suggest that normal expression of Pax6 is required for the regulation of diencephalic precursor proliferation (Warren and Price 1997). *Pax6* mutant embryos also showed early alterations in the expression of regulatory genes in the region destined to

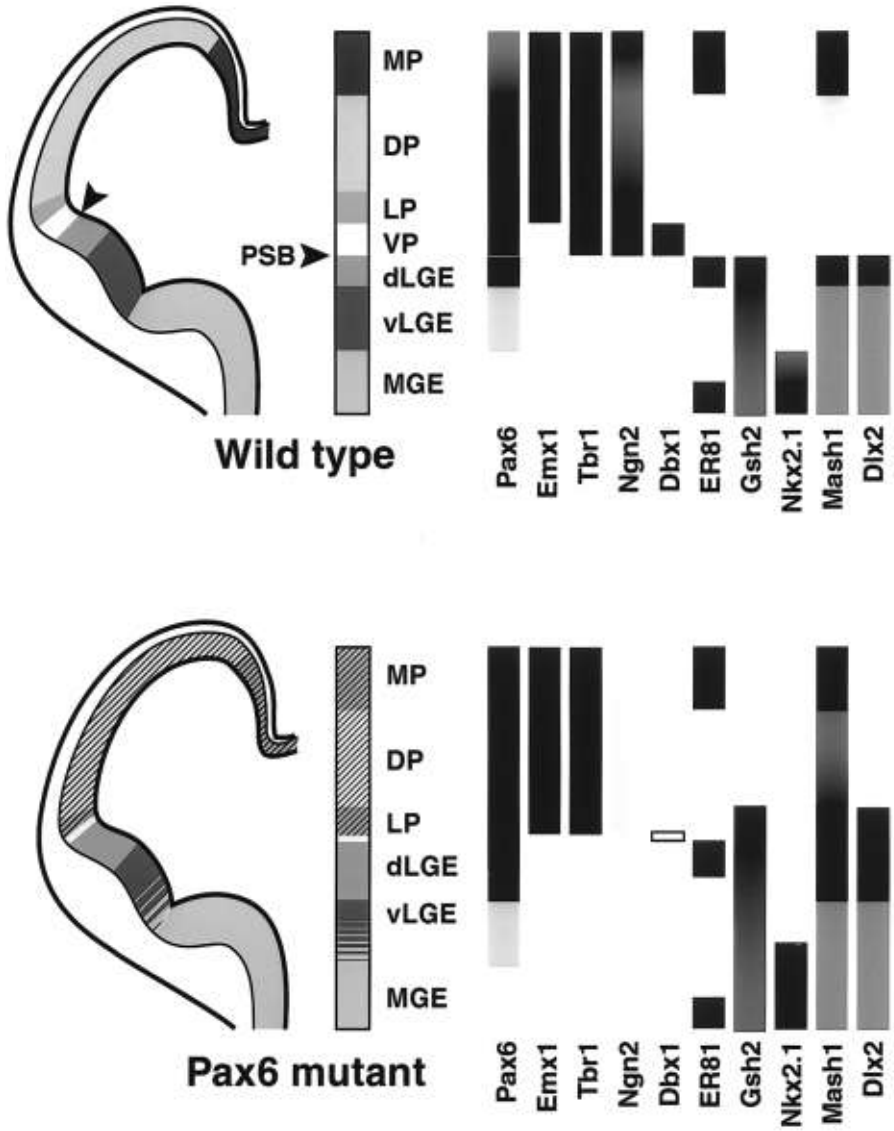

Fig. 3. Pax6 and D/V patterning of the telencephalon. Expression of genes involved in D/V patterning of the telencephalon in the wild type (upper) and *Pax6* mutant (lower) are shown based on results of Stoykova et al. (2000), Toresson et al. (2000), Yun et al. (2001), and on unpublished results in my laboratory. Pax6 is specifically expressed in the pallium and in the dorsal most of the LGE (dLGE). In the *Pax6* mutant telencephalon, LGE-specific genes, e.g., Dlx1/2, and Gsh2, are ectopically expressed within the ventral pallium (VP), and MGE-specific genes, e.g., Nkx2.1, are expressed in the LGE. Moreover, expression of the pallial genes, such as Neurogenin2 (Ngn2), is reduced, and that of the VP-specific genes, such as Dbx1, is missing in the *Pax6* mutant cortex. Thus, D/V patterning is impaired in the *Pax6* mutant telencephalon, which eventually results in the loss of the VP. MP, medial pallium; DP, dorsal pallium; LP, lateral pallium; PSB, pallial/subpallial boundary.

become the dorsal thalamus, suggesting that the primary role of Pax6 is to generate correct D/V patterns in the diencephalon (Warren and Price 1997; Grindley et al. 1997). Moreover, there is an apparent enlargement of the zona limitans (the boundary region between prosomeres p2 and p3), a blurring of the p1/p2 boundary, and a size reduction of the zona

incerta in p3. Thus, Pax6 is essential for the normal development and regionalization of the diencephalon.

In later stages, the developing telencephalon is subdivided into ventral (subpallial) and dorsal (pallial) territories. In mammals, the pallium primarily gives rise to the cerebral cortex, while the subpallium, which consists of the medial (MGE) and lateral (LGE) ganglionic eminences (Fig. 2A), later forms the striatal and pallidal components of the basal ganglia. Pax6 is specifically expressed in the pallium and in the dorsal most of the LGE (Fig. 3). In *Pax6* mutant mice, LGE-specific transcriptional factors, e.g., Dlx1/2, and Gsh2, are ectopically expressed in the ventral pallium (Fig. 3; Stoykova et al. 1997, 2000; Toresson et al. 2000; Yun et al. 2001; K. Sato and N. Osumi, unpublished data); thereby showing a dorsal-to-ventral transformation phenotype. Complementarily, in Gsh2 gene knockout mouse embryos, ectopic expression of allial markers is observed in the dorsal LGE, indicating dorsalization of this region of the subpallium (Corbin et al. 2000; Toresson et al. 2000; Yun et al. 2001). Mice lacking both Pax6 and Gsh2 show milder phenotypes than single mutants (Toresson et al. 2000; Yun et al. 2001), confirming that reciprocal regulatory interactions between these genes mediate D/V patterning on either side of the pallial/subpallial boundary. Molecular analyses revealed that *Pax6* mutant mice lack the ventral pallium, which is formed at the pallial/subpallial boundary (Fig. 3; Yun et al. 2001). Consistently, we observed loss of expression of a specific antigen at this boundary in *Pax6* mutant mouse embryos (Hirata and Osumi, unpublished data), although the role of the antigen in D/V patterning is not known yet.

The pallial/subpallial boundary also restricts the movement of neuroepithelial cells within the telencephalon (Inoue et al. 2001). Differential expression of the members of the cadherin superfamily is observed in the telencephalon; Rcadherin is expressed in the pallium and cadherin6 is in the subpallium (Inoue et al. 1997). Cadherins are thought to play a role in selective sorting of dorsal and ventral telencephalic cells as demonstrated in in vitro cell aggregation assay (Stoykova et al. 1997), and more directly by inducing ectopic expression of various cadherins at the pallial/subpallial boundary (Inoue et al. 2001). Thus, formation and/or maintenance of the pallial/subpallial boundary are regulated by exclusive expression of the cadherins. In analogy to the forebrain/midbrain boundary formation mentioned above, Rcadherin is downregulated in the *Pax6* mutant pallium (Stoykova et al. 1997). Thus, Pax6 is involved in brain patterning through the regulation of expression of cell adhesion molecules.

Cortical expression of Pax6 is higher anteriorly and ventrally than dorsally and posteriorly. In addition to the disruptions of ventral pallial development described above, loss of Pax6 function also leads to the expansion of posterior cortical domains at the expense of anterior domains (Bishop et al. 2000). Another homeobox gene, *Emx2*, is expressed at higher levels in posterior than in anterior areas of the dorsal telencephalon (Pellegrini et al. 1996; Yoshida et al. 1997). Loss of Emx2 function leads to expansion of anterior/lateral cortical domains, while posterior/medial domains are reduced or lost (Bishop et al. 2000; Mallamaci et al. 2000). Therefore, opposing activities of the homeodomain proteins Pax6 and Emx2 may generate graded positional identity within the dorsal cortex.

Role of Pax6 in specification of neurons

One outcome of early patterning of the brain is the specification of domains that produce different types of neurons. For example, the cortex produces glutamatergic neurons, the LGE produces GABAergic neurons, and the MGE produces both GABAergic and cholinergic neurons. Each domain produces neurons that migrate radially and maintain positional information essential for the generation of topo-

graphic connectivity maps. In addition, the LGE and MGE produce interneurons that migrate in two tangenitial pathways to populate adjacent territories with GABAergic and cholinergic cells (see review by Parnavelas 2000). Recently, Chapouton et al. (1999) demonstrated in *Pax6* mutant mice increased number of neuronal cells that migrate from subpallial to pallial regions and eventually increased GABAergic cells are found in the pallium. However, how Pax6 specifies neurons within the telencephalon is enigmatic at present. Ectopic expression of subpallial markers (Dlx, Mash1 etc.) in the *Pax6* mutant cortex strongly suggests mis-specification of cortical neurons *per se* (Stoykova et al. 1997, 2000; Toresson et al. 2000; Yun et al. 2001).

More information on the role of Pax6 in specification of neurons is available in hindbrain and spinal cord development. As mentioned in the previous section, SHH secreted from the notochord and the floor plate establishes the positional values within the ventral neural tube. The position that ventral progenitor cells occupy within a graded SHH activity directs their differentiation into specific neuronal subtypes (Fig. 4; Ericson et al. 1997). It should be noted that the specification of neurons is already determined in the precursors that exist in the ventricular zone of the neural tube. Several homeodomain proteins, Nkx2.2, Nkx6.1, Pax6, Irx3, Dbx2, and Dbx1, whose expression is regulated by SHH signaling, are expressed in the ventricular zone (Briscoe et al. 2000; for review see Stone and Rosenthal 2000). Consequently, generation of certain ventral neuronal subtypes is perturbed in mutants of these genes. In *Pax6* mutant mice and rats, expression of other homeodomain proteins is altered; distinct domains of the homeodomain proteins become obscure and shift dorsally or ventrally (Ericson et al. 1997; Pierani et al. 1999; and Takahashi and Osumi, unpublished data). *Pax6* mutants also show loss of somatic motor (SM) neurons in the hindbrain and En1-positive V1 interneurons

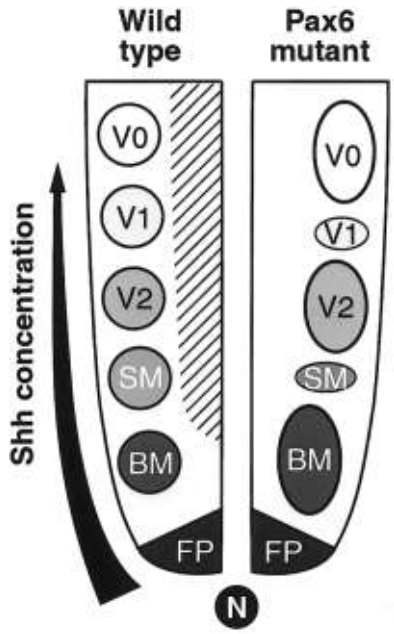

Fig. 4. Pax6 and specification of ventral neurons in the hindbrain. In the wild type embryo (left), various populations of ventral neurons marked with specific genes differentiate according to the graded SHH signal along the D/V axis. Expression of Pax6 is shown as a hatched area. In the *Pax6* mutant hindbrain (right), SM neurons and V1 interneurons are mostly missing, while populations of BM neurons and V0 interneurons are increased. V0–2, populations of ventral interneurons; SM, somatic motor neurons; BM, branchiomotor neurons; FP, floor plate; N, notochord.

both in the hindbrain and spinal cord, and conversely, more branchiomotor (BM) neurons are formed at the expense of SM neurons and more V0 interneurons (Evx1/2 positive) at the expense of V1 interneurons (Fig. 4; Burrill et al. 1997; Ericson et al. 1997; Osumi et al. 1997). Detailed analyses are in progress to elucidate the regulatory cascades downstream of Pax6 in mis-specification of these ventral progenitor cells.

Role of Pax6 in guiding neuronal migration and axonal extension

Pax6 also regulates neuronal migration in both cell-nonautonomous and cell-autonomous

manners. Layers of the cerebral cortex are formed by radial migration of neurons born in the pallidum and tangential migration of neurons born in the LGE and MGE (Parmavelas 2000). *Pax6* mutants exhibit defects in cortical layer formation; the ventricular and subventricular zones become thicker and the cortical plate becomes thinner (Schmahl et al. 1993; Stoykova et al. 1996; Caric et al. 1997; Warren et al. 1999; Fukuda et al. 2000). Transplantation of *Pax6* mutant cortical cells into the neonatal cortex of wild-type rats revealed that the impaired radial migration is caused cell-nonautonomously (Caric et al. 1997). This observation is also supported by the fact that Pax6 controls the differentiation of radial glial cells that serve as scaffolds for migration of cortical neurons (Goetz et al. 1998).

There are other tangenitial migrations of telencephalic cells from dorsal to ventral regions; neurons born relatively early (E9–10 in mice) in the pallium migrate ventrally and stop at the pallial/subpallial boundary (Tomioka et al. 2000). A subpopulation of these cells that express specific antigen recognized by a monoclonal antibody, lot1, guide the extension of the lateral olfactory tract (Sato et al. 1999). In *Pax6* mutant mice, pallial cells migrate ventrally, but many of these cells do not stop but cross the pallial/subpallial boundary (Nomura and Osumi, unpublished data). It will be interesting to know the molecule under the control of Pax6 that directs these cells to stop at the boundary.

In addition to these cell-nonautonomous effects on migration of neurons, Pax6 also controls neuronal migration in a cell-autonomous

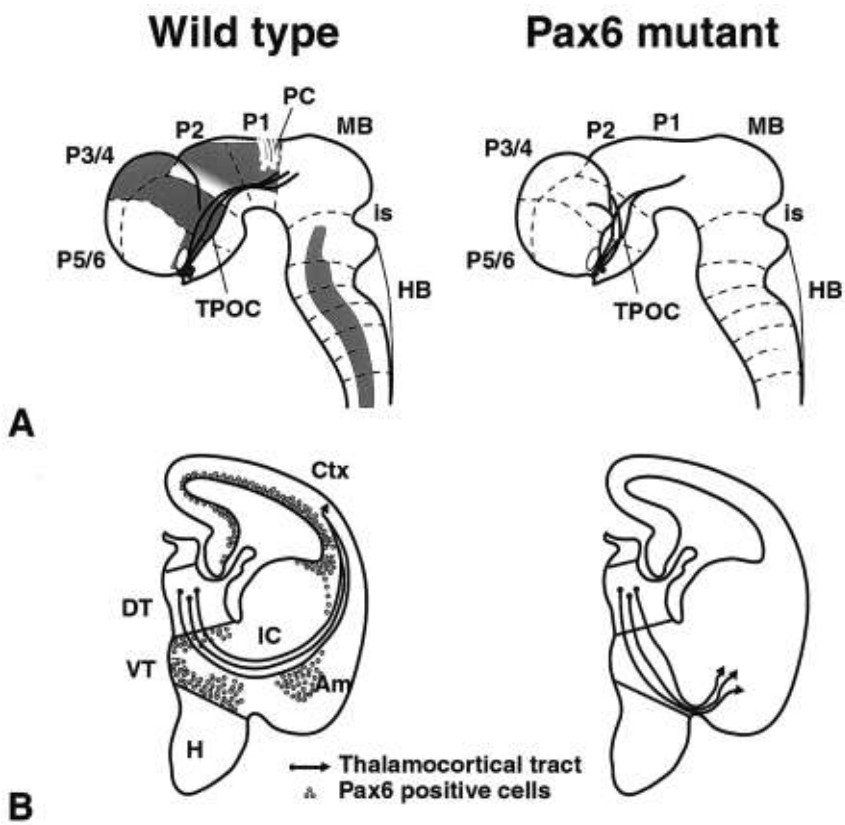

Fig. 5. Pax6 and formation of axonal tracts. Normal development of (A) the tracts of postoptic commissure (TPOC) and posterior commissure (PC), and (B) the thalamocortical pathway in the wild type (left) and *Pax6* mutant forebrain (right). p1–6, prosomere 1–6; MB, midbrain; is, isthmus (=midbrain/hindbrain boundary); HB, hindbrain; Ctx, cortex; DT, dorsal thalamus; VT, ventral thalamus; H, hypothalamus; IC, internal capsule; Am, amygdala.

manner. Granule cells in the postnatal cerebellum strongly express *Pax6* during differentiation and migration to form the inner granule cell layer (IGL). Formation of the IGL is impaired in the *Pax6* mutant mice and rats (Engelkamp et al. 1999; M. Kengaku in preparation). In culture, *Pax6* mutant granule cells show abnormal neurite formation and neuronal migration, and these defects are not rescued by co-culture with normal granule cells but recovered by transfecting Pax6 gene into mutant cells (M. Kengaku, in preparation). These data suggest that Pax6 controls the morphology and migration of cerebellum granule cells in a cell-autonomous manner.

As pointed in the early part of this review, borders of brain territories often coincide with pathways of axonal extension, and Pax6 offers typical examples (Fig. 5). Longitudinal tracts such as the tract of postoptic commissure (TPOC) and the nigrostriatal pathway are formed at the ventral border of the Pax6 domain in the forebrain. Formation of these tracts is impaired in *Pax6* mutants (Mastick et al. 1997; Warren and Price 1997; Vitalis et al. 2000; and unpublished results of Takahashi and Osumi). Identification of molecules that guide these tracts will be the focus of future studies.

Developmental defects of thalamocortical and mammilothalamic pathways are also observed in *Pax6* mutant mice and rats (Kawano et al. 1999; Pratt et al. 2000; Valverde et al. 2000). Pratt et al. (2000) tested whether the mechanism underlying the defect of thalamocortical pathway includes abnormalities of the dorsal thalamus itself by co-culture of dorsal thalamic explants from the wild type or *Pax6* mutant embryos with wild-type tissues from other regions of the forebrain. Whereas the wild type thalamic explants produced strong innervation of wild-type ventral telencephalic explants in a pattern that mimicked the thalamocortical tract in vivo, *Pax6* mutant explants failed to do so, indicating a defect in the ability of mutant dorsal thalamic cells to respond to signals normally present in the ventral telencephalon. Thus, the defect of thalamocortical pathway is considered to be caused cell-autonomously.

Concluding remarks

Since Pax6 is expressed in various regions of the developing brain and functions in many developmental aspects in highly context-dependent manners, identification of distinct partners and target genes of Pax6 would be a key to our understanding of the molecular mechanisms of brain patterning. In the adult brain, Pax6 is expressed in the ependymal layer of the lateral, third, and forth ventricles where neuronal stem cells are thought to exist (Valverde et al. 2000; Osumi, unpublished observation). It is thus interesting to know whether Pax6 is involved in the maintenance of neuronal stem cells.

Acknowledgments

I thank Drs. Y. Wakamatsu and T. Nomura for valuable comments on the manuscript, and Dr. T. Hirata, Dr. M. Kengaku, Mr. M. Takahashi, and Mr. K. Sato for providing unpublished information. Sincere gratitude is also extended to Mr. T. Nagase and N. Takashima for maintaining *Pax6* mutant rat colonies in National Institute of Neuroscience and Tohoku University Graduate School of Medicine, respectively. Finally I sincerely appreciate continuous support and encouragement from members of Shindan Laboratory in National Institute of Neuroscience and Department of Developmental Neurobiology in Tohoku University.

References

Ashery-Padan, R., Marquardt, T., Zhou, X. & Gruss, P. (2000) Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. *Genes Dev.*, **14,** 2701–2711.

Bertrand, N., Medevielle, F. & Pituello, F. (2000) FGF signalling controls the timing of pax6 activation in the neural tube. *Development*, **127,** 4837–4843.

Bishop, K.M., Goudreau, G. & O'Leary, D.D. (2000) Regulation of area identity in the mammalian

neocortex by Emx2 and Pax6. *Science*, **288,** 344–349.

Briscoe, J., Pierani, A., Jessell, T.M. & Ericson, J. (2000) A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell*, **101,** 435–445.

Burrill, J.D., Moran, L., Goulding, M.D. & Saueressig, H. (1997) PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development. *Development*, **124,** 4493–4503.

Caric, D., Gooday, D., Hill, R.E., McConnell, S.K. & Price, D. J. (1997) Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. *Development*, **124,** 5087–5096.

Chapouton, P., Gartner, A. & Gotz, M. (1999) The role of Pax6 in restricting cell migration between developing cortex and basal ganglia. *Development*, **126,** 5569–5579.

Corbin, J.G., Gaiano, N., Machold, R.P., Langston, A. & Fishell, G. (2000) The Gsh2 homedomain gene controls multiple aspects of telencephalic development. *Develpoment*, **127**, 5007–5020.

Dahl, E., Koseki, H. & Balling, R. (1997) Pax genes and organogenesis. *Bioessays*, **19,** 755–765.

Dohrmann, C., Gruss, P. & Lemaire, L. (2000) Pax genes and the differentiation of hormone-producing endocrine cells in the pancreas. *Mech. Dev.*, **92,** 47–54.

Dosen, J.S., & Rosenfeld, M.G. (1999) Signaling mechanisms in pituitary morphogenesis and cell fate determination. *Curr. Opin. Cell. Biol*., **11,** 669–677.

Engelkamp, D., Rashbass, P., Seawright, A. & van Heyningen, V. (1999) Role of Pax6 in development of the cerebellar system. *Development*, **126,** 3585–3596.

Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T.M. & Briscoe, J. (1997) Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell*, **90,** 169–180.

Fukuda, T., Kawano, H., Osumi, N., Eto, K. & Kawamura, K. (2000) Histogenesis of the cerebral cortex in rat fetuses with a mutation in the Pax-6 gene. *Dev. Brain Res.*, **120**, 65–75.

Gehring, W.J. & Ikeo, K. (1999) Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet*, **15,** 371–377.

Goetz, M. Stoykova, A. & Gruss, P. (1998) Pax6 controls radial glia differentiation in the cerebral cortex. *Neuron*, **21,** 1031–1044.

Grindley, J.C., Hargett, L.K., Hill, R.E., Ross, A. & Hogan, B.L. (1997) Disruption of PAX6 function in mice homozygous for the Pax6Sey-1Neu mutation produces abnormalities in the early development and regionalization of the diencephalon. *Mech. Dev.,* **64,** 111–126.

Halder, G., Callaerts, P. & Gehring, W.J. (1995) Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. *Science*, **267,** 1788–1792.

Hill, R.E., Favor, J., Hogan, B.L., Ton, C.C., Saunders, G.F., Hanson, I.M., Prosser, J., Jordan, T., Hastie, N.D. & van Heyningen, V. (1991) Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature*, **354**, 522–525.

Inoue, T., Chisaka, O., Matsunami, H. & Takeichi, M. (1997) Cadherin-6 expression transiently delineates specific rhombomeres, other neural tube subdivisions, and neural crest subpopulations in mouse embryo. *Dev. Biol.*, **183,** 183–194.

Inoue, T., Nakamura, S. & Osumi, N. (2000) Fate mapping of the mouse prosencephalic neural plate. *Dev. Biol.*, **219,** 373–383.

Inoue, T., Tanaka, T., Takeichi, M., Chisaka, O., Nakamura, S. & Osumi, N. (2001) Role of cadherins in maintaining the compartment boundary between the cortex and striatum during development. *Development*, **128,** 561–569.

Kammandel, B., Chowdhury, K., Stoykova, A., Aparicio, S., Brenner, S. & Gruss, P. (1999) Distinct cis-essential modules direct the time-space pattern of the Pax6 gene activity. *Dev. Biol.*, **205,** 79–97.

Kawano, H., Fukuda, T., Kubo, K., Horie, M., Uyemura, K., Takeuchi, K., Osumi, N., Eto, K. & Kawamura, K. (1999) Pax-6 is required for thalamocortical pathway formation in fetal rats. *J. Comp. Neurol.*, **408,** 147–160.

Lumsden, A. & Krumlauf, R. (1996) Patterning the vertebrate neuraxis. *Science*, **274,** 1109–1115.

Mallamaci, A., Muzio, L., Chan, C.H., Parnavelas, J. & Boncinelli, E. (2000) Area identity shifts in the early cerebral cortex of Emx2–/– mutant mice. *Nat. Neurosci*., **3**, 679–686.

Mastick, G.S., Davis, N.M., Andrew, G.L. & Easter,

S.S., Jr. (1997) Pax-6 functions in boundary formation and axon guidance in the embryonic mouse forebrain. *Development*, **124,** 1985–1997.

Matsunaga, E., Araki, I. & Nakamura, H. (2000) Pax6 defines the di-mesencephalic boundary by repressing En1 and Pax2. *Development*, **127,** 2357–2365.

Matsuo, T., Osumi-Yamashita, N., Noji, S., Ohuchi, H., Koyama, E., Myokai, F., Matsuo, N., Taniguchi, S., Doi, H., Iseki, S., et al. (1993) A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. *Nat. Genet.*, **3,** 299–304.

Nakamura, H. (2000) Regionalization of the optic tectum: combinations of gene expression that define the tectum. *Trends Neurosci.*, **24,** 32–39.

Osumi, N., Hirota, A., Ohuchi, H., Nakafuku, M., Iimura, T., Kuratani, S., Fujiwara, M., Noji, S. & Eto, K. (1997) Pax-6 is involved in the specification of hindbrain motor neuron subtype. *Development*, **124,** 2961–2972.

Parnavelas, J.G. (2000) The origin and migration of cortical neurones: new vistas. *Trends Neurosci*., **23,** 126–131.

Pellegrini, M., Mansouri, A., Simeone, A., Boncinelli, E. & Gruss, P. (1996) Dentate gyrus formation requires Emx2. *Development*, **122,** 3893–3898.

Pierani, A., Brenner-Morton, S., Chiang, C. & Jessell, T.M. (1999) A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. *Cell*, **97,** 903–915.

Pituello, F., Medevielle, F., Foulquier, F. & Duprat, A.M. (1999) Activation of Pax6 depends on somitogenesis in the chick embryo cervical spinal cord. *Development*, **126,** 587–596.

Pratt, T., Vitalis, T., Warren, N., Edgar, J.M., Mason, J.O. & Price, D.J. (2000) A role for pax6 in the normal development of dorsal thalamus and its cortical connections. *Development*., **127,** 5167–5178.

Prosser, J. & van Heyningen, V. (1998) PAX6 mutations reviewed. *Hum. Mutat*., **11,** 93–108.

Rubenstein, J.L.R. & Beachy, P.A. (1998) Patterning of the embryonic forebrain. *Curr. Opin. Neurobiol.*, **8,** 18–26.

Rubenstein, J.L.R., Shimamura, K., Martinez, S. & Puelles, L. (1998) Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.*, **21,** 445–477.

Sato, Y., Hirata, T., Ogawa, M. & Fijisawa, H. (1998) Requirement for early-generated neurons recognized by monoclonal antibody lotl in the formation of lateral olfactory tract. *J. Neurosci*., **18**, 7800–7810.

Schmahl, W., Knoedlseder, M., Favor, J. & Davidson, D. (1993) Defects of neuronal migration and the pathogenesis of cortical malformations are associated with Small eye (Sey) in the mouse, a point mutation at the Pax-6-locus. *Acta Neuropathol* (*Berl*)., **86,** 126–135.

Schwarz, M., Alvarez-Bolado, G., Dressler, G., Urbanek, P., Busslinger, M. & Gruss, P. (1999) Pax2/5 and Pax6 subdivide the early neural tube into three domains. *Mech. Dev.*, **82,** 29–39.

Stone, D. & Rosenthal, A. (2000) Achieving neuronal patterning by repression. *Nat. Neurosci*., **3,** 967–969.

Stoykova, A. & Gruss, P. (1994) Roles of Pax-genes in developing and adult brain as suggested by expression patterns. *J. Neurosci.*, **14,** 1395–1412.

Stoykova, A., Fritsch, R., Walther, C. & Gruss, P. (1996) Forebrain patterning defects in Small eye mutant mice. *Development*, **122,** 3453–3465.

Stoykova, A., Gotz, M., Gruss, P. & Price, J. (1997) Pax6-dependent regulation of adhesive patterning, R-cadherin expression and boundary formation in developing forebrain. *Development*, **124,** 3765–3777.

Stoykova, A., Treichel, D., Hallonet, M. & Gruss, P. (2000) Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. *J. Neurosci*., **20,** 8042–8050.

Tomioka, N., Osumi, N., Sato, Y., Inoue, T., Nakamura, S., Fujisawa, H. & Hirata, T. (2000) Neocortical origin and tangential migration of guidepost neurons in the lateral olfactory tract. *J Neurosci*., **20,** 5802–5812.

Toresson, H., Potter, S.S. & Campbell, K. (2000) Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for pax6 and gsh2. *Development*., **127,** 4361–4371.

Valverde, F., Garcia, C., Lopez-Mascaraque, L. & de Carlos, J.A. (2000) Development of the mammilothalamic tract in normal and Pax-6 mutant mice. *J. Comp. Neurol*., **419,** 485–504.

Vitalis, T., Cases, O., Engelkamp, D., Verney, C. & Price, D.J. (2000) Defect of tyrosine

hydroxylase-immunoreactive neurons in the brains of mice lacking the transcription factor Pax6. *J. Neurosci*., **20,** 6501–6516.

Warren, N. & Price, D.J. (1997) Roles of Pax-6 in murine diencephalic development. *Development*, **124,** 1573–1582.

Warren, N., Caric, D., Pratt, T., Clausen, J.A., Asavaritikrai, P., Mason, J.O., Hill, R.E. & Price, D.J. (1999) The transcription factor, Pax6, is required for cell proliferation and differentiation in the developing cerebral cortex. *Cereb. Cortex*., **9,** 627–635.

Yoshida, M., Suda, Y., Matsuo, I., Miyamoto, N., Takeda, N., Kuratani, S. & Aizawa, S. (1997) Emx1 and Emx2 functions in development of dorsal telencephalon. *Development*, **124,** 101–111.

Yun, K., Potter, S. & Rubenstein, J.L. (2001) Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development*, **128,** 193–205.