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Pax and vertebrate development

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ABSTRACT ***Pax* genes encode transcription factors sharing a highly conserved sequence, the paired box. Their temporally and spatially restricted expression patterns during development indicate that *Pax* genes are involved in important steps of nervous system formation. Mutations in *Pax* genes have been correlated with three mouse mutants *(undulated, splotch, small eye)* and two human diseases (Waardenburg syndrome, aniridia). Recent data demonstrated that deregulation of *Pax* genes contributes to tumor formation.**

KEY WORDS: *Pax, Small eye, Splotch, Waardenburg, aniridia*

Introduction

Based on the homology to sequence motifs in the genome of *Drosophila melanogaster* different gene families have been identified in the mouse: the homeodomain (McGinnis *et al.,* 1984a,b; Kessel and Gruss, 1990), the paired domain (Bopp *et al.,* 1986; Deutsch *et al.,* 1988), the helix-loop-helix domain (Murre *et al.,* 1989), the zinc finger domain (Rosenberg *et al.,* 1986), the fork head domain (Jürgens and Weigel, 1988), the POU domain (Herr *et al.,* 1988; Rosenfeld 1991) and the leucine zipper (Landschultz *et al.,* 1988).

The family of *pa*ired bo*x (Pax)* genes share a highly conserved DNA sequence of 384 bp and was first described in *Drosophila* segmentation genes (Bopp *et al.,* 1986; Baumgartner *et al.,* 1987). Besides *Drosophila*, genes containing the paired box have been found in many vertebrate species such as zebrafish, frog, chick, mouse and human (Dressler *et al.,* 1988; Burri *et al.,* 1989; Krauss *et al.,* 1991b; Püschel *et al.,* 1992; Goulding *et al.,* 1993a) suggesting, as in the case of *Hox* genes, that fundamental events in body patterning are conserved between insects and vertebrates.

The murine *Pax* gene family consists of nine members *(Pax1* to *Pax9)* (Walther *et al.,* 1991; Wallin *et al.,* 1993). In contrast to *Hox* genes (Kessel and Gruss, 1990), *Pax* genes are not clustered and map on different chromosomal loci (Walther *et al.,* 1991; Stapleton *et al.,* 1993). Pax proteins contain additional conserved motifs, a conserved octapeptide (present in all except *Pax6* and *Pax4*) and/or a paired-type homeodomain (*Pax3, Pax4, Pax7* and *Pax6*). *Pax2, Pax5* and *Pax8* harbor a rudimentary homeodomain encoding only the first α-helix (Fig. 1). Pax proteins act as transcription factors. This is shown by their *in vitro* DNA-binding activity (Treisman *et al.,* 1989, 1991; Chalepakis *et al.,* 1991; Goulding *et al.,* 1991; Dressler and Douglass, 1992; Czerny *et al.,* 1993; Epstein *et al.,* 1994), their transcriptional activation (Chalepakis *et al.,* 1991) and their localization in the nucleus (Bopp *et al.,* 1989; Dressler and Douglass, 1992). Because of similarities in protein structure, genomic organization and expression during development, the murine *Pax* genes can be classified into subgroups, designated as paralogous genes (Fig. 1).

Expression of *Pax* genes in the developing CNS

The development of the nervous system starts with the interaction of the chordamesoderm and the overlying neuroectoderm resulting in the induction of the neural plate and subsequently in neural tube formation. Shortly after closure of the neural tube, it differentiates regionally into the spinal cord and three primary brain vesicles: the prosencephalon, the mesencephalon and the rhombencephalon. Later, the prosencephalon is further subdivided into diencephalon and telencephalon, while the rhombencephalon gives rise to the metencephalon and myelencephalon.

Pax genes and brain development

Along the anterior-posterior axis from the rhombencephalon to the tail, segment identity appears to be specified by the differential expression of *Hox* genes (Boncinelli *et al.,* 1991; Kessel, 1991; McGinnis and Krumlauf, 1992). Little is known about the molecular basis establishing regional properties more anterior in mes- and prosencephalon, especially in the region overlying the prechordal mesoderm. On the basis of expression patterns it is likely that members of the *Emx* (Simeone *et al.,* 1992b), *Dlx* (Porteus *et al.,* 1991), *Nkx* (Price, 1993), *Otx* (Simeone *et al.,* 1992a), *Wnt* (Nusse and Varmus, 1992) gene families are important in early prosencephalon patterning.

All *Pax* genes, except *Pax1* and *Pax9* (Deutsch *et al.,* 1988; Stapleton *et al.,* 1993; Wallin *et al.,* 1993), show spatially and temporally restricted expression during development of the central nervous system. The first group of early expressed *Pax* genes, namely *Pax3*, *Pax6* and *Pax7*, starts to be active around

Abbreviations used in this paper: p.c., post coitum.

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day 8.0-8.5 p.c. With the exception of *Pax6*, which excludes the mesencephalic roof from its expression domains, these genes are present in the entire developing neural tube (Fig. 2A) (Goulding *et al.*, 1991; Jostes *et al.*, 1991; Walther and Gruss, 1991). Different from *Pax6* which is expressed in forebrain in later stages and even after birth (Stoykova and Gruss, 1994), in midgestation embryos, *Pax3* and *Pax7* are retracted from the telencephalon to a rostral limit in the diencephalon (Fig. 2B,C).

A second group of late expressed *Pax* genes *(Pax2*, *Pax5*, and *Pax8)* is confined to the epichordal part of the CNS (spinal cord and hindbrain) and starts to be expressed at day 10.5 p.c. in postmitotic cells of the intermediate zone. The midbrain-hindbrain boundary is the rostral limit of *Pax2*, *Pax5* and *Pax8* expression, whereas *Pax5* is also detected in the posterior mesencephalic tegmentum (Fig. 2B) (Nornes *et al.*, 1990; Plachov *et al.*, 1990; Asano and Gruss, 1992).

The analysis of *Pax* gene activity in the developing brain reveals that the spatial expression is delineated by anatomical landmarks. First, *Pax2, Pax5* and *Pax8* are detected around the midbrain-hindbrain boundary (Fig. 2B) (Nornes *et al.*, 1990; Plachov *et al.*, 1990; Asano and Gruss, 1992). In zebrafish, injection of antibodies raised against the pax[zf-b] protein (most similar to *Pax2*) show default development of the isthmus (Krauss *et al.*, 1992), suggesting that members of the *Pax* family might play a critical role establishing the midbrain-hindbrain boundary.

Two recently reported models described the neuromeric organization of the forebrain in four (D1-D4, Figdor and Stern, 1993) or six (P1-P6 prosomeres, Puelles and Rubenstein, 1993) neuromeres, which are consistent with the restricted expression domains of a number of genes (*Emx*, Simeone *et al.*, 1992b; *Dlx*, Porteus *et al.*, 1991, *Nkx*, Price, 1993; *Otx*, Simeone *et al.*, 1992a; *Wnt*, Nusse and Varmus, 1992). In the developing diencephalon *Pax6* shows segment like expression confined to the ventral thalamus (D1, or the alar plate of P3) while *Pax3*, *Pax6* and *Pax7* transcripts are detected in the epithalamus (region of D2 or P3) and pretectum (D3-D4 or P1). Additional experiments are necessary to relate their expression domains to the proposed two neuromeric subdivisions (D3 and D4, Figdor and Stern, 1993) of the pretectum.

Pax gene expression was also detected in the adult brain (Fig. 2C) with a similar transcript distribution along the AP-axis compared to midgestation embryos (Stoykova and Gruss, 1994). In many cases, a correlation exists between the expression of particular *Pax* genes in adult brain structures and the embryonic region of their origin suggesting that *Pax* genes are not only involved in brain regionalization but also in the maintenance of the regional identity.

Pax genes in the developing spinal cord

Pax genes harboring a complete paired-type homeodomain (*Pax6*, and the paralogous *Pax3* and *Pax7*) (Goulding *et al.*, 1991; Jostes *et al.*, 1991; Walther and Gruss, 1991) are the first *Pax* genes expressed in the spinal cord. They are detected around day 8.5 p.c. in specific regions of the ventricular zone. While *Pax6* is present in the basal and intermediate plate, *Pax3* and *Pax7* are expressed in the alar plate. The expression domain of *Pax6* is partially overlapping with *Pax3* and *Pax7* (Fig. 3A). This region specific pattern of expression suggests that these transcription factors can play an important role during the establishment of the dorso-ventral polarity in the spinal cord.

Regionalization of the early spinal cord appears to be controlled by signals originating from ventral midline cell groups, the axial mesodermal cells of the notochord and the floor plate cells (Placzek *et al.*, 1991). A signal coming from the notochord is responsible for floor plate induction and subsequently the notochord and the floor plate will induce the differentiation of motor neurons (Placzek *et al.*, 1991; Ruiz i Altaba and Jessell, 1993). In chick embryo, experiments of surgical ablation or ectopic grafting of notochord show that these early expressed *Pax* genes modify rapidly their region of expression in response to signals coming from the notochord and floor plate centers (Goulding *et al.*, 1993a). These data indicate that the region specific expression of *Pax* genes is governed by the induction cascade originating from the notochord. This observation is consistent with the idea that *Pax* genes might play an important role in the early establishment of the dorso-ventral polarity in the spinal cord.

The second subgroup of *Pax* genes without homeodomain (*Pax2*, *Pax5* and *Pax8*) starts to be expressed around day 10.5 p.c. in a subset of post-mitotic neuroblasts located as two longitudinal columns in the intermediate grey on both sides of the sulcus limitans (Fig. 3B) (Nornes *et al.*, 1990; Plachov *et al.*, 1990; Asano and Gruss, 1992). The expression analysis of *Pax2* compared to the zebrafish homologue *pax [zf-b]* identified these cells as interneurons of the intermediate grey (Krauss *et al.*, 1991a; Mikkola *et al.*, 1992) supporting the idea that *Pax* genes may be involved in the cell fate determination to specific neuronal phenotypes.

Pax, mouse mutants and human diseases

It has been shown that three *Pax* genes are altered in classical mouse mutants (Hastie, 1991; Gruss and Walther, 1992) and two are associated with human diseases (Hill and van Heyningen, 1992; Strachan and Read, 1994). Recently, an additional transgene-induced mutation *(Krd)* including a *Pax2* deletion has been reported (Keller *et al.*, 1994).

Pax1 and undulated (un)

The paralogous *Pax1* and *Pax9* are the only *Pax* genes that are not expressed in the central nervous system (Deutsch *et al.*, 1988; Wallin *et al.*, 1993, 1994). At day 8.25 p.c. *Pax1* expression can be first detected in the ventral part of the somites (the prospective sclerotome) and subsequently in the developing vertebral column, the facial skeleton, the shoulder girdle, the segmented sternum, the pharyngeal pouches and the thymus anlage (Deutsch *et al.*, 1988; Timmons *et al.*, 1994). With exception of the thymus anlage and the facial skeleton, the expression domains are confined to structures undergoing chondrogenesis suggesting that *Pax1* plays a crucial role in that process including sclerotome specification.

All *Pax1* expression domains are corresponding to malformations affected in *undulated* mutant mice (Balling *et al.*, 1988, 1992; Dietrich *et al.*, 1993; Wallin *et al.*, 1994). The *Pax1* gene has been mapped on mouse chromosome 2 close to the recessive mutation *undulated* (Fig. 1) (Balling *et al.*, 1988). The analysis of *undulated* mice revealed that there are three different *undulated* alleles harboring mutations within the *Pax1* gene.

The first one identified was *undulated (un)* showing a single base pair mutation in a highly conserved position of the paired box (Balling *et al.,* 1988). This causes an amino acid exchange from glycine to serine reducing the DNA-binding activity of the protein dramatically (Chalepakis *et al.,* 1991). The *undulated* phenotype is characterized by a kinky tail, vertebral, shoulder girdle and sternum malformations (Wright, 1947; Grüneberg, 1963).

The *undulated extensive* (un^{ex}) allele (Wallace, 1979, 1980) presents a similar but more severe phenotype than *undulated*. The deletion of the 3′ part of the *Pax1* gene including the last exon and the poly (A) signal leads to a decreased *Pax1* mRNA level (Dietrich and Gruss, 1995).

Finally, *Undulated short tail* (Un^{s}) (Blandova and Egorov, 1975) presents the most severe phenotype, caused by the deletion of the whole *Pax1* gene (Balling *et al.,* 1992). In contrast to the other recessive *Pax1* alleles, Un^{s} is a semi-dominant mutation resulting in late embryonal or neonatal death in homozygous mice and similar vertebral malformations as the *un* allele in heterozygous mice (Balling *et al.,* 1992; Wallin *et al.,* 1993).

The comparison of phenotypes of all three *undulated* alleles supports the idea that *Pax1* is an important regulator during vertebral column formation.

Pax2 and kidney and retinal defects (Krd)

Pax2 expression during embryonic development is confined to the neural tube, the kidney and to the eye. *Pax2* starts to be expressed in the developing kidney at day 9.0 p.c. in the pro- and mesonephric tubules, the Wolffian duct and subsequently in the ureter and the condensed metanephric mesenchyme. In the developing eye, expression has been observed around day 9.0 p.c. in the optic vesicle, the optic cup and the optic stalk, in midgestation embryos in the optic disk and optic nerve.

Recently, a transgene-induced mutation *(Krd, kidney and retinal effects)* on chromosome 19 has been described, showing a large deletion including the *Pax2* locus (Keller *et al.,* 1994). Although the deletion encompasses possibly around 400 genes, some characteristic features of *Krd* are likely due to the deletion of *Pax2*. *Krd* shows in heterozygous state kidney defects including aplastic, hypoplastic and cystic kidneys. These data are also supported by observations in *Pax2* transgenic mice revealing severe functional and histological defects in the developing kidney (Dressler *et al.,* 1993). Furthermore, *Krd* exhibits retinal defects including abnormal electroretinograms and a reduced number of cells in the inner and ganglion layers of the retina. Heterozygous mice show growth retardation whereas homozygote *Krd* mice are early embryonic lethal. Another characteristic of the *Krd* mutation is the semidominance, that has been also described in the *undulated*, *small eye* and *splotch* mutations (Mansouri *et al.,* 1994).

Pax3 and splotch (Sp)

The *Pax3* expression pattern (Goulding *et al.,* 1991) and the correlation with defects in the mouse mutant *splotch (Sp)* (Auerbach, 1954) provides evidence that the *Pax3* gene is an important regulatory factor during neural tube development and neural crest migration (Moase and Trasler, 1990). Initially, *Pax3* has been mapped close to the *splotch* locus on chromosome 1 (Fig. 1) (Evans *et al.,* 1988; Goulding *et al.,* 1993b). Six different

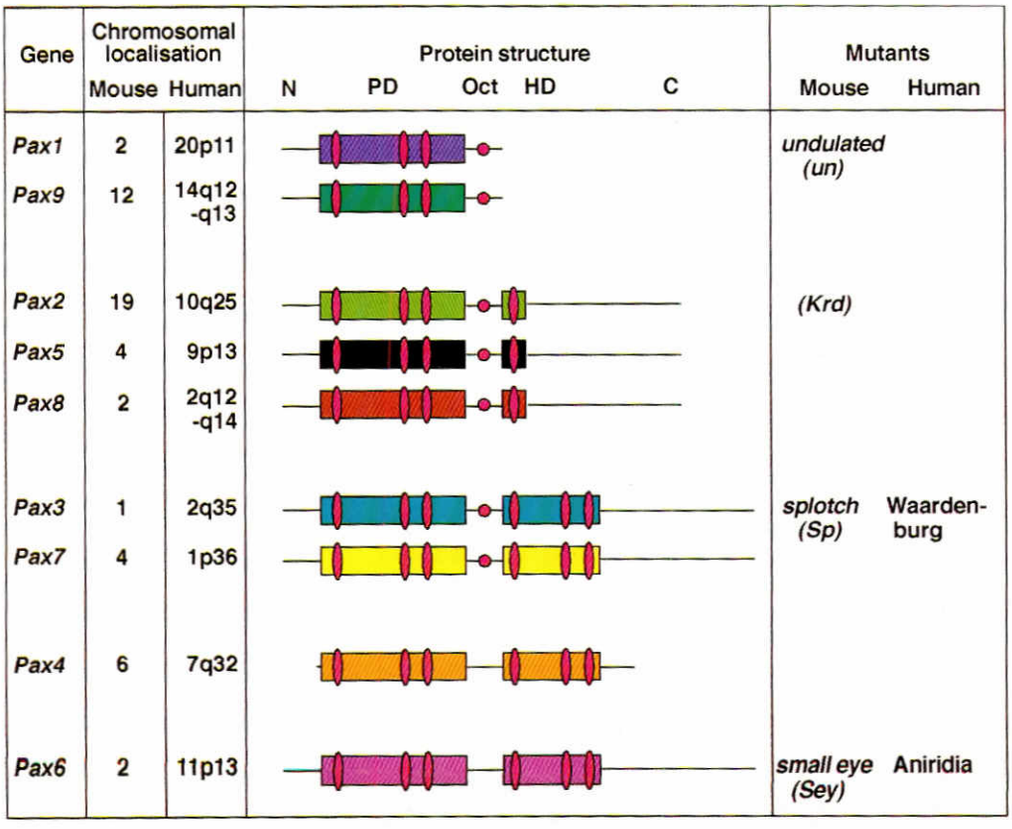

Fig. 1. Protein structure of members of the murine and human *Pax* gene family. *Chromosomal localisation of Pax genes and their corresponding mouse mutants and human diseases are indicated. The homeodomain and the paired domain are shown in coloured boxes, the octapeptide (circle) and the putative a-helices (ovals) in red. The paralogous genes are grouped together. C, carboxy terminus; HD, homeodomain; Krd, kidney and retinal defects; N, amino terminus; Oct, octapeptide; PD, paired domain.*

semi-dominant *splotch* alleles have been described in mouse: two spontaneously mutations, *splotch (Sp)* (Russel 1947, Auerbach 1954) and *splotch delayed (Sp^d)* (Dickie, 1964; Vogan *et al.*, 1993), and four induced, *splotch retarded (Sp^r)* , $Sp1^H$, Sp^{2H} and Sp^{4H} (Beechey and Searle, 1986). The molecular alterations have been identified in all *splotch* alleles corresponding to deletions and point mutations in the *Pax3* gene (Epstein *et al.*, 1991a,b, 1993; Goulding *et al.*, 1993b). DNA-binding studies of different *splotch* alleles revealed that these alterations affect the DNA-binding property of *Pax3* (Chalepakis *et al.*, 1994).

In a heterozygous state, all *splotch* alleles exhibit a phenotype showing characteristic pigmentation abnormalities (white spotting of the belly, limbs and tail) originating from neural crest deficiencies (Auerbach, 1954). In addition, heterozygous *splotch retarded (Sp^r)* mice are characterized by retarded growth (Evans *et al.*, 1988).

Homozygous *splotch* mice are embryonic lethal around day 13-14 p.c. The most prominent phenotype is observed during development of the neural tube. All *splotch* alleles cause severe abnormalities during the proper closure of the neural tube exhibiting spina bifida, cranial exencephaly, meningocele and overgrowth of nervous tissue (Auerbach, 1954). Consistent with the *Pax3* expression pattern in the dorsal part of the neural tube, an important role during the proper closure of the neural tube can be suggested.

Neural crest defects result in various severe deficiencies affecting melanocytes, Schwann cells, truncus arteriosus, thyroid, parathyroid, thymus, dorsal root ganglia and the cardiovascular system (Auerbach, 1954; Franz, 1989; Moase and Trasler, 1989, Grim *et al.*, 1992). Therefore, the *splotch* mutants provide a good model for neural crest defects perfectly matching with the *Pax3* expression (Auerbach, 1954; Goulding *et al.*, 1991). Thus, *Pax3* might be a crucial regulator in emigration of neural crest cells.

Pax3 is also expressed in the dermomyotome of the developing somite and in the limb buds. Recent analysis of the limb phenotype suggests that the lack of limb musculature is correlated with the absence of *Pax3* expressing cells that fail to migrate into the limb bud (Franz *et al.*, 1993; Bober *et al.*, 1994). Also the dermomyotome and myotome was shown to be disorganized in *splotch* mutants (Yang and Trasler, 1991). Both, defects in neural crest and myoblast precursors suggest a probable function of *Pax3* during cell migration and/or proliferation and specification.

PAX3 mutations and the Waardenburg syndrome

Mutations in the human *PAX3* gene have been described in the genome of patients with Waardenburg syndromes (WS type 1 and 3) (Fig. 1) (Waardenburg, 1951). Waardenburg syndrome is an autosomal dominant hereditary disease with a frequency of approximately 1 in 100,000 (Tassabehji *et al.*, 1993). The WS 1 has been mapped to a chromosomal region syntetic to the region of the murine *splotch* locus (Ishikiriyama *et al.*, 1989; Foy *et al.*, 1990). A variety of *PAX3* mutations have been identified in WS1 patients. Different mutations like splice site mutations (Tassabehji *et al.*, 1992, 1993), insertions and deletions (Morell *et al.*, 1992; Tassabehji *et al.*, 1993), missense mutations (Baldwin *et al.*, 1992; Hoth *et al.*, 1993; Tassabehji *et al.*, 1993) etc. have been assigned to the paired box of *PAX3*. Previously it was shown, that a point mutation in Waardenburg Brazil (Baldwin *et al.*, 1992) and a small deletion in WS.05 (Tassabehji *et al.*, 1992) completely abolishes the DNA-binding ability of *PAX3* (Chalepakis *et al.*, 1994). Phenotypic features of patients with WS 1 include a lateral displacement of the inner corner of the eye (dystropia canthorum), a prominent nasal root, deafness and pigmentation disturbances indicated by i.e. white forelock and eye lashes and heterochromia irides (Waardenburg, 1951; McKusick, 1992). Patients with WS 2 show a very similar phenotype as WS 1 except no dystropia canthorum (Tassabehji *et al.*, 1993). These phenotypes appear in a heterozygous state. Individuals homozygous for the Waardenburg syndrome most likely do not survive. Furthermore, WS 3 patients can be distinguished from WS 1 by additional limb abnormalities (Hoth *et al.*, 1993). This observed limb abnormality can be correlated with the underdevelopment of the muscle anlagen and a reduced size of the axial musculature in *splotch* mice (Bober *et al.*, 1994; Hoth *et al.*, 1993). Finally, also the observation of spina bifida and meningomyelocele in WS 1 patients are of particular interest (Arnvig, 1959; Lavergne, 1959; Pantke and Cohen, 1971; Carezani-Gavin *et al.*, 1992; Chatkupt *et al.*, 1993), reminiscent of splotch mice.

Pax6 and small eye (Sey)

Another developmental mutant could be associated with the *Pax6* gene (Walther and Gruss, 1991). *Small eye (Sey)* mutants are identified in mouse (Roberts, 1967; Hill *et al.*, 1991) and rat (Matsuo *et al.*, 1993). *Pax6* maps on chromosome 2 close to the *Sey* locus (Fig. 1) (Walther *et al.*, 1991). Early *Pax6* expression is confined to the optic sulcus, the optic stalk and the optic vesicle, subsequently in the retina and the surface ectoderm later developing into the lens (Walther and Gruss, 1991). Furthermore, Pax6 is expressed during nasal development.

Heterozygous *small eye* mutants have a characteristic eye malformation phenotype in tissues where *Pax6* is expressed during development. In particular, a reduced diameter of the eye, cataracts, vacuolated and dislocated lenses, iris hypoplasia etc. are observed (Hogan *et al.*, 1986, 1988). In comparison to wild-type litter mates, *small eye* mice are about 10% smaller (Roberts, 1967). In a homozygous state, *small eye* embryos fail to develop eyes, nose, the optic nerve and the olfactory bulbs, finally dying after birth because they cannot breath (Hogan *et al.*, 1986, 1988). Also the defects in the nose coincides with *Pax6* expression domains (Walther and Gruss, 1991). However, none of the analysed *Sey* alleles showed defects in the spinal cord. This is possibly due to compensating effects of other *Pax* family members, as has been proposed for other families of transcription factors (Joyner *et al.*, 1989; Chisaka and Capecchi, 1991; McMahon *et al.*, 1992) or possibly subtle alterations exist which have not been detected yet.

Four different semidominant *small eye* alleles have been detected and analyzed yet: *Sey* and Sey^{Neu} (Hill *et al.*, 1991) are phenotypic similar, whereas Sey^H and Sey^{Dey} exhibit a more drastic phenotype showing additionally Coloboma of the eyes (heterozygous) and prenatal death around implantation (homozygous) (Theiler *et al.*, 1978; Hogan *et al.*, 1986; 1988; Glaser *et al.*, 1990). The molecular analysis of these four alleles revealed that a total deletion of *Pax6* in Sey^H and Sey^{Dey} might be responsible for the stronger phenotype (Glaser *et al.*, 1990),

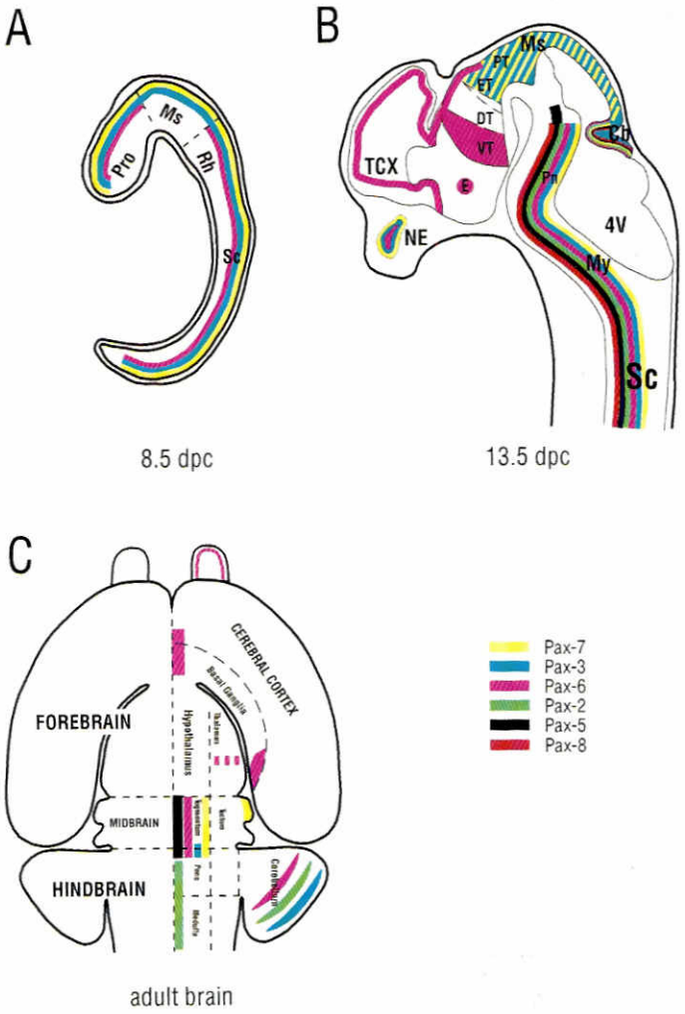

Fig. 2. Schematic representation of *Pax* gene expression in early and midgestation embryos and adult brain. (A) *Saggital section of day 8.5 p.c. embryo.* **(B)** *Saggital section of day 13.5 p.c. embryo.* **(C)** *Overview of Pax gene expression in the adult mouse brain. AP, alar plate; BP, basal plate; Cb, cerebellum; Di, diencephalon; dpc, day post coitum; DT, dorsal thalamus; E, eye; ET, epithalamus; FP, floor plate; IZ, intermediate zone; Ms, mesencephalon Mt, metencephalon; My, myelencephalon; NE, nasal epithelium; PN, pons; Pro, prosencephalon; PT, pretectum; Rh, rhombencephalon; RP, roof plate; Sc, spinal cord; Sl, sulcus limitans; TCX, telencephalic cortex; TL, telencephalon; VT, ventral thalamus; VZ, ventricular zone; 4V, 4th ventricle.*

while *Sey* and *Sey*^{Neu} are characterized by a point mutation and a 116 bp insertion respectively (Hill *et al.*, 1991).

The phenotypic features of all four *Sey* alleles correspond with the expression of *Pax6* during the development of the eye and nose. Recently, ablation experiments in chick revealed that *Pax6* expression in the optic vesicle is independent from the overlying head ectoderm (Li *et al.*, 1994), suggesting *Pax6* to be involved in the early establishment of competent regions in the head ectoderm.

PAX6 and human diseases

Mutations in the human *PAX6* gene have been identified in patients with aniridia syndrome (Glaser *et al.*, 1990; van der Meer de Jong *et al.*, 1990) and Peters´ anomaly (Hanson *et al.*, 1994) (Fig. 1). Aniridia is an autosomal dominant disorder with a frequency of around 1 in 64,000 to 1 in 96,000 characterized by defects affecting the iris, the lens, retina and optic nerve (Shaw *et al.*, 1960; Nelson *et al.*, 1984; Hittner, 1989). Aniridia maps on chromosome 11p13, which is the loci where *PAX6* has been located (Fig. 1) (Davis *et al.*, 1989; Ton *et al.*, 1991). Molecular analysis revealed that the *PAX6* gene was mutated in several aniridia patients, having point mutations or various deletions (Ton *et al.*, 1991; Glaser *et al.*, 1992; Jordan *et al.*, 1992). A compound heterozygous child has been reported with very drastic craniofacial abnormalities, absence of eyes, nose and adrenal gland (Glaser *et al.*, 1994). Recently, *PAX6* was also found to be mutated in patients with Peters´ anomaly characterized by defects of the anterior chamber of the eye and corneal malformations (Hanson *et al.*, 1994). Thus far, the malformations observed in aniridia and Peters´ anomaly show striking similarity to those described for *Sey*. Therefore, *Sey* provides a mouse model for both human diseases.

Pax genes and oncogenesis

Many transcription factors have been classified as proto-oncogenes controlling cellular processes. Recent data show that *Pax* genes are involved in oncogenesis. Overexpression of *Pax1, 2, 3, 6* and *8* in fibroblasts (NIH3T3) resulted in foci of transformed cells that are able to develop tumors when injected into nude mice (Maulbecker and Gruss, 1993). This oncogenic potential seems to depend on a functional paired domain since the point mutation in the Pax1^{un} protein inhibits the transforming activity of Pax1.

These *in vitro* studies are supported by *in vivo* observations in diverse human cancers. *PAX2* and *PAX8* are expressed in Wilms´ tumor, a kidney carcinoma, whereas both genes are normally expressed only during kidney development and not after terminal differentiation (Dressler and Douglass, 1992; Poleev *et al.*, 1992). Data showing that *Pax2* is required for conversion of mesenchyme to epithelial aggregates is also consistent with the characteristically mesenchymal origin of Wilms´ tumors (Dressler *et al.*, 1993). Moreover, it has been shown that the human *PAX3* in alveolar rhabdomyosarcomas is linked with cancer (Barr *et al.*, 1993). The rearrangement and translocation of *PAX3* to the carboxyterminus of a *fork head* gene *(FKHR)* results in the expression of a PAX-Forkhead fusion protein. Finally, rearrangements in highly malignant astrocytomas in the chromosomal region including the *PAX5* locus suggests the involvement of *PAX5* in the progression of astrocytomas. Studies showing altered *PAX5* expression seem to support this idea (Stuart *et al.*, 1994).

In summary, inappropriate *Pax* gene expression seems to be tightly linked to uncontrolled cell growth although the function of *Pax* genes in this process is not yet understood.

Perspectives and possible functions of *Pax* genes

Pax genes have turned out to be important transcriptional regulators in murine development. The functional analysis is still in its infancy but there is clearly available evidence that *Pax* genes can serve as a powerful tool to elucidate the molecular mecha-

nisms underlying embryonic development, in particular that of the nervous system.

First of all, the comparison of the detailed expression analysis and the loss-of-function mutations in mouse and man provided us with information about their tissue specific roles.

For two *Pax* mutants, *splotch* and *small eye*, human diseases have been described correlating with Waardenburg syndrome (Waardenburg, 1951) and aniridia (Glaser *et al.*, 1990; van der Meer de Jong *et al.*, 1990). These mouse models present a good system to analyse the molecular basis responsible for these human diseases.

So far, it is still not clear whether the phenotypic alterations of the mouse mutants can be correlated with a complete or partial loss of function. On one hand, the analysis of all mutant alleles suggests that *Pax* gene mutations are loss-of-function mutations since different disruptions (point mutations or extensive deletions) of the Pax proteins result in similar phenotypes. This would suggest that every functional subdomain (paired domain and homeodomain for DNA-binding, C- and N-terminus for transcriptional activation or inhibition) is essential for the gene function (Chalepakis *et al.*, 1991; Chalepakis and Gruss, unpublished data). On the other hand in the *undulated* alleles residual gene activity seems to argue against this hypothesis. Whereas the classical *undulated* allele, characterized by a point mutation, shows the least severe defect, the most severe phenotype is exhibited by the *undulated short tail* (Un^s) mutant, missing the whole *Pax1* gene (Blandova and Egorov, 1975; Balling *et al.*, 1988). Un^s can be considered as a null allele although the deletion encompasses more than the *Pax1* locus. Therefore, it can not be excluded that contiguous genes contribute to the Un^s phenotype.

Gain-of-function experiments expressing ectopically Pax protein in fibroblast cell lines (Maulbecker and Gruss, 1993) or the overexpression of *Pax2* in transgenic mice (Dressler *et al.*, 1993) indicate that also positive deregulation of Pax proteins causes abnormal cellular programs.

Another feature of *Pax* genes seems to be reflected by the semidominant nature of the mutant alleles in mouse and man. The phenotypic variations of e.g. the *splotch* mutant ranges from minor pigmentation defects in a heterozygous state up to severe neural crest, neural tube and limb muscle defects in a homozygous state (Auerbach, 1954; Bober *et al.*, 1994). This observation could be explained by a dosage effect, meaning that variable thresholds of Pax proteins are responsible for pathogenesis in different organs.

To further elucidate the *Pax* gene function, two different approaches might be useful. Firstly, there is need for more *Pax* gene mutants that could be generated as gain-of-function mutants in transgenic mice or as loss-of-function mutants by homologous recombination. In particular the loss-of-function approach could be performed by different methods taking into account that the *Pax* genes show temporal and spatial restricted expression. Therefore, the establishment of systems for temporal induced and tissue specific knock-outs are in progress.

Secondly, since *in vitro* studies revealed that Pax proteins act as DNA-binding transcription factors the question for downstream target genes arises. The identification of targets would certainly help to understand the biological functions of *Pax* genes in development as well as the molecular mechanisms underlying pathogenesis. Therefore, differential screening methods (subtracted cDNA-libraries (Wang and Brown, 1991) or differential display (Liang and Pardee, 1992) comparing wild-type mice with mutant mice or induced and non-induced cell culture systems would be of advantage. The approach to identify target genes by immunoprecipitation of chromosomal protein-DNA complexes might be another useful tool (Gould *et al.*, 1990).

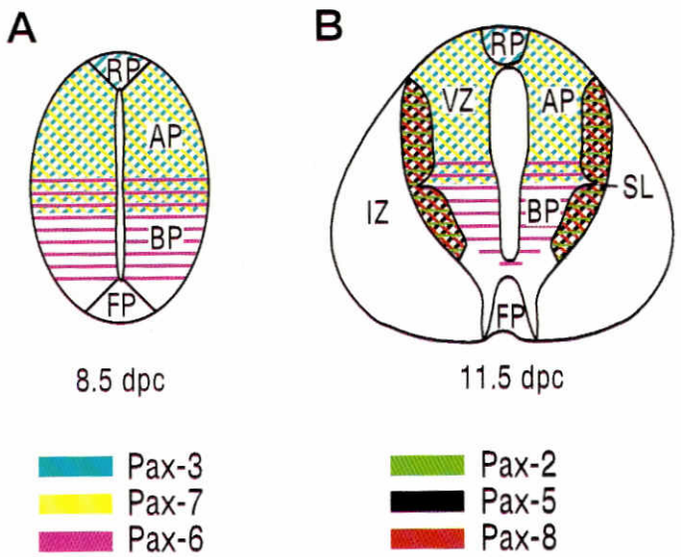

Fig. 3. Schematic representation of *Pax* gene expression in the developing spinal cord. *Restricted expression as observed in cross sections along the dorso-ventral axis.* **(A)** *Embryo day 8.5 p.c.* **(B)** *Embryo day 11.5 p.c. AP, alar plate; BP, basal plate; dpc, day post coitum; IZ; intermediate zone; FP, floor plate; SL, sulcus limitans; RP, roof plate; VZ; ventricular zone.*

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