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Review

Pax genes in development and maturation of the vertebrate visual system: Implications for optic nerve regeneration

M.R. Ziman[1], J. Rodger[1], P. Chen[1], J.M. Papadimitriou[2], S.A. Dunlop[1] and L.D. Beazley[1]
Departments of [1]Zoology and [2]Pathology, University of Western Australia, Nedlands, Western Australia, Australia

Summary. *Pax* genes play a pivotal role in development of the vertebrate visual system. *Pax*6 is the master control gene for eye development: ectopic expression of *Pax*6 in *Xenopus laevis* and *Drosphila melanogaster* leads to the formation of differentiated eyes on the legs or wings. *Pax*6 is involved in formation of ganglion cells of the retina, as well as cells of the lens, iris and cornea. In addition *Pax*6 may play a role in axon guidance in the visual system.

*Pax*2 regulates differentiation of the optic disk through which retinal ganglion cell axons exit the eye. Furthermore, *Pax*2 plays a critical role in development of the optic chiasm and in the guidance of axons along the contralateral or ipsilateral tracts of the optic nerve to visual targets in the brain.

During development *Pax*7 is expressed in neuronal cells of one of the major visual targets in the brain, the optic tectum/superior colliculus. Neurons expressing *Pax*7 migrate towards the pia and concentrate in the stratum griseum superficiale (SGFS), the target site for retinal axons. Together, expression of *Pax*2, 6 and 7 may guide axons during formation of functional retinotectal/collicular projections.

Highly regulated *Pax* gene expression is also observed in mature animals. Moreover, evidence suggests that *Pax* genes are important for regeneration of the visual system. We are currently investigating *Pax* gene expression in species that display a range of outcomes of optic nerve regeneration. We predict that such information will provide valuable insights for the induction of successful regeneration of the optic nerve and of other regions of the central nervous system in mammals including man.

Key words: Developmental genes, Eye development, Optic nerve regeneration, *Pax* genes, Neurogenesis

Offprint requests to: Mel R. Ziman, Department of Zoology, The University of Western Australia, Nedlands, Western Australia, 6907, Australia. Fax: 61-8-9346 2891. e-mai: mziman@cyllene.uwa.edu.au

Abbreviations: *PAX*: gene encoding the human nuclear protein, PAX; *Pax*: gene encoding the vertebrate nuclear protein, Pax.

Introduction: Scope of the review

We will discuss the development, maintenance and regeneration of the visual system in vertebrates, concentrating on the retina and a major primary visual centre in the brain - the optic tectum in lower vertebrates and its mammalian homologue, the superior colliculus.

The highly conserved *Pax* genes are known to play a role in development of several organs and tissues including the central nervous system. This discussion will centre on the role of three *Pax* genes, *Pax*2, 6 and 7 in development of the vertebrate visual system during cellular differentiation, axon pathfinding and the formation of retinotectal/collicular projections. *Pax* genes play a critical role in the formation of components of the eye and the differentiation of neural cells in the retina, as well as in the guidance of retinal axons to primary visual centres in the brain. In addition, *Pax* genes control differentiation of specific neurones of the tectum/superior colliculus.

The expression of *Pax* genes in adult tissues and their role in the maintenance of neural cell types will also be described. Finally, we will speculate on the possible role of *Pax* genes in regeneration of the optic nerve in adult vertebrates on the premise that genes shown to be crucial during development of the visual system are likely to be required during optic nerve regeneration. The highly conserved nature of *Pax* genes has allowed us to examine current information on their expression in species that display a range of regenerative responses following optic nerve crush, from full return of visual function in fish to an abortive response in mammals. We predict that such information will provide valuable insights for the induction of successful regeneration of the optic nerve, information that can be

applied to promote successful regeneration of other regions of the central nervous system in mammals including man.

Pax genes

The study of the genetic basis and molecular mechanisms underlying invertebrate and vertebrate development has resulted in the identification of different families of transcriptional regulators, one of which is the *Pax* (or Paired box containing) gene family (Reviewed in Noll, 1993). *Pax* genes are involved in the development of animals with strikingly different embryogenesis and mature architecture. Homologous members of the *Pax* gene family have been identified in organisms as diverse as sea urchin, nematode, fly, fish, mouse and human (Dressler, 1988; Burri et al., 1989; Chalepakis et al., 1993). We have identified, and are in the process of characterising, certain *Pax* genes from an Australian lizard, *Ctenophorus ornatus*.

Pax genes encode a family of highly conserved transcription factors. The characteristic feature of the *Pax* gene family is the presence of the paired box, a 384 bp DNA sequence that encodes a highly conserved 128-amino acid DNA binding domain situated close to the amino terminus of the protein. The paired box sequence was originally identified in the segmentation genes *paired*, *gooseberry* and *gooseberry neuro* of *Drosophila melanogaster* (Bopp et al., 1986); the name derives from the similarity of sequence with the pair-rule segmentation gene, *paired*. *Pax* genes can be grouped into different classes based on whether they also encode a full or partial homeodomain, a second highly conserved DNA binding domain present in *homeobox* (*hox*) genes. *Pax* genes may also contain a conserved octapeptide encoding region (Walther et al., 1991) (Fig. 1). The function of the octapeptide is as yet unknown but may be important in the regulation of DNA methylation of *Pax* genes (Ziman and Kay, 1998a).

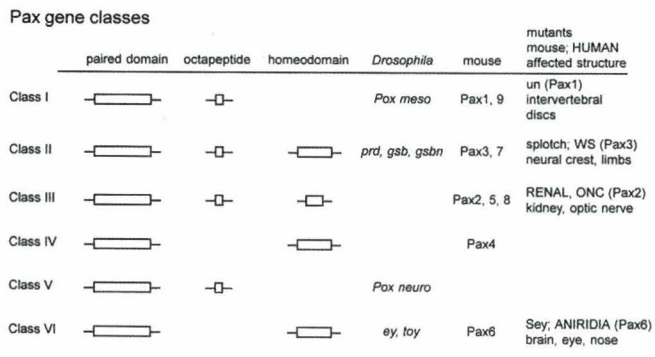


Fig. 1. A schematic representation of the six classes of *Pax* genes. All *Pax* gene products contain the highly conserved paired DNA binding domain near the N-terminus of the protein. *Pax* genes are classified by the presence of a conserved octapeptide encoding region and/or by the presence of a full or partial homeodomain encoding region. The homeodomain is a second highly conserved DNA binding domain in the protein product of some *Pax* genes. The *Pax* genes of *Drosophila melanogaster* and mouse are given. Mouse mutants and human syndromes and their associated defects are also given. Prd: paired; gsb: gooseberry; gsbn: gooseberry neuro; ey: eyeless; toy: twin of eyeless; un: undulated; sey: small eye; WS: Waardenburgís syndrome; ONC: optic nerve colobomas.

Recent data for species from different animal phyla suggest that *Pax* genes are key regulators of several aspects of embryogenesis such as embryonic patterning and organogenesis as well as development of the central nervous system, including the eye. Several mutations in the *Pax* genes affecting embryogenesis have been described in mouse and human (Noll, 1993; Stuart et al., 1994; Tremblay et al., 1995). In mouse, spontaneous mutations in *Pax*1, *Pax*3 and *Pax*6 are associated with *undulated* (Balling et al., 1988), *splotch* (Epstein et al., 1991) *and small eye* (Hill et al., 1991), respectively. Mutations in human *PAX* genes are also associated with several congenital disorders: for example, *PAX*2 mutations are linked with autosomal dominant renal anomalies and optic nerve colobomas (Sanyanusin et al., 1995), *PAX*3 mutations with Waardenburg's syndrome (Hoth et al., 1993) and *PAX*6 mutations with aniridia (Ton et al., 1991) (Fig. 1).

Pax genes in development of the central nervous system

The developing neural tube and spinal cord

Most *Pax* genes are initially expressed in the developing neural tube and brain. It has been suggested that they play a role in establishing the dorso-ventral axis at early stages of neural tube development (Pituello, 1997). At later stages, *Pax* genes play a role in specifying regional differentiation in the nervous system (Tanabe and Jessell, 1996).

In mouse, *Pax* genes that encode a paired and homeodomain (*Pax*3, *Pax*6 and *Pax*7) are expressed along the dorso-ventral axis of the developing spinal cord at embryonic day (E) 8-8.5 post coitum (E8-E8.5 p.c.), before the onset of cellular differentiation (Goulding et al., 1991; Jostes et al., 1991; Walther and Gruss, 1991). *Pax*6 and *Pax*7 are induced by signals from the notochord including the soluble factors Sonic Hedgehog (Shh) and bone morphogenetic proteins 4 and 7 (BMP4 and BMP7) (Goulding et al., 1992; Pituello, 1997). Recent studies have shown that the spatially restricted expression of *Pax*6 and *Pax*7 along the dorso-ventral axis primarily determines neuronal identity in the spinal cord, specifying a ventral or dorsal cell fate respectively (Goulding et al., 1992; Tanabe and Jessell, 1996) (Fig. 2).

The developing brain

At early stages of murine development, *Pax* genes play a role in specification of brain stem nuclei (Stoykova and Gruss, 1994). Evidence for the role of *Pax* genes in segmentation of the brain comes from clearly

delineated *Pax* expression domains in neuromeric structures: *Pax*2 and *Pax*5 appear to be involved in the formation of the midbrain-hindbrain boundary; *Pax*6 expression is confined to the ventral thalamus, whereas *Pax*3 and *Pax*7 are expressed in the entire diencephalon dorsal of the fasciculus retroflexus. At later stages of development, *Pax* genes specify differentiation of restricted subsets of brain cells in the telencephalon, diencephalon, mesencephalon, isthmus and granular layer of the cerebellum (Stoykova and Gruss, 1994).

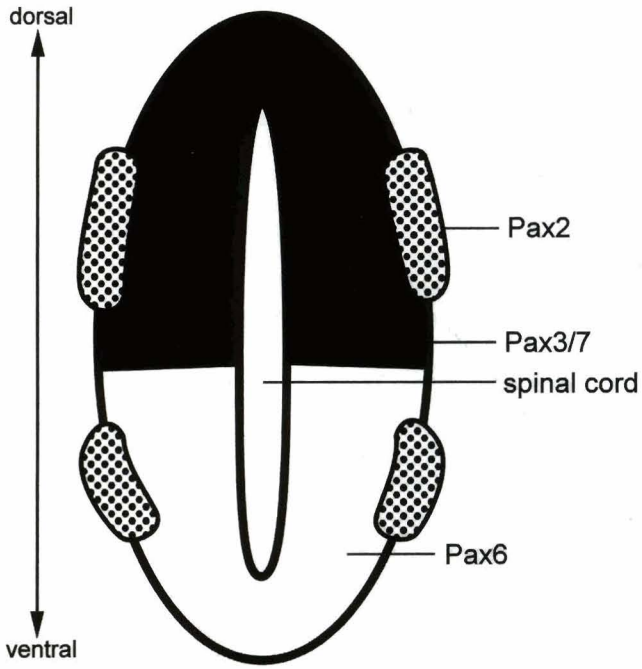
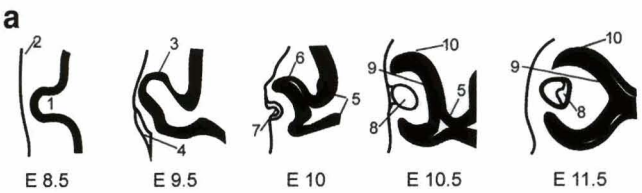


Fig. 2. A model for the role of *Pax* genes in neuronal specification in the spinal cord. The schematic representation shows a cross section of the developing spinal cord after neural tube closure. Distinct subpopulations of ventral progenitor neural cells are defined by the presence of *Pax*6 and the absence of *Pax*3 and *Pax*7. In contrast, dorsal progenitor cells are defined in the developing neural tube by the expression of *Pax*7.



Eye and visual pathway development

The vertebrate eye develops as two components, with the neural ectoderm and the head surface ectoderm each specifying differential development of specific regions (Jean et al., 1998). The neural ectoderm evaginates laterally at the base of the diencephalon and becomes the optic pit that will form the optic vesicle. Invagination of the optic vesicle results in a bilayered optic cup; the inner layer forms the neural retina, and the outer layer the retinal pigment epithelium. The optic cup is joined to the brain via the optic stalk that forms the optic nerve. The junction between the optic cup and the optic stalk narrows to form the optic disk through which retinal ganglion cell axons exit the eye (Fig. 3a). The head surface ectoderm forms the lens placode that thickens and invaginates to form the lens vesicle. The interaction between the lens placode and optic vesicle is important for continued eye development (Callaerts et al., 1997). Light passes through the lens, is detected by rod and cone photoreceptor cells in the retina and the resultant visual information is conveyed to the retinal ganglion cells. The axons of the retinal ganglion cells project along the optic nerve to the optic chiasm where they may either cross the midline and project along the contralateral tract or proceed via the ipsilateral tract to

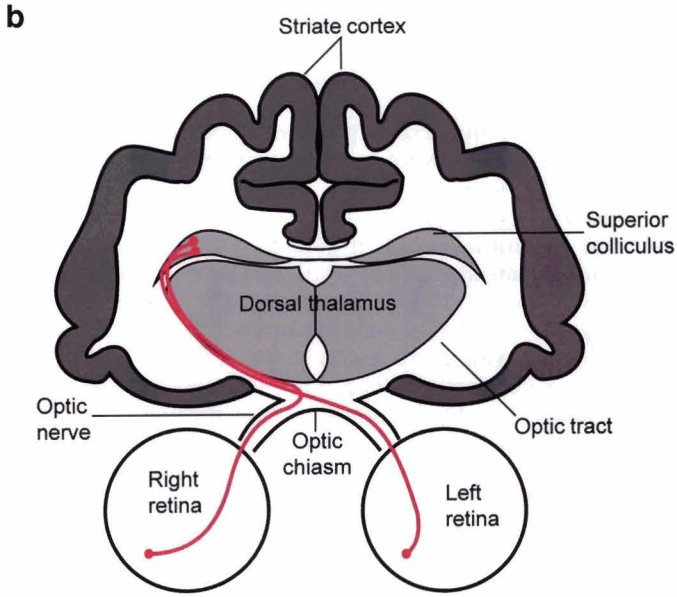


Fig. 3. A schematic representation of eye development and nature visual projections to the brain. **a)**. A representation of eye development in mouse embryo E8.5 to E11.5. The neural ectoderm (1) evaginates to form the optic vesicle (3). The optic vesicle invaginates to form the bilayered optic cup (6). At E10.5, the optic cup and optic stalk (5) differentiate into the optic nerve tract (5) and the retina, containing the inner neural layer (9) and the pigmented epithelial layer (10), respectively. The head surface ectoderm (2) forms the lens placode (4). The epithelium thickens (7) and invaginates to form the lens vesicle (8). *Pax*6 is expressed in the developing optic cup and lens, and surface epithelium (1-4) and in the retina (6, 9) during development and at maturation. *Pax*2 expression regulates differentiation of the optic disk and establishes the boundary between the optic cup and the optic stalk (5). **b)**. A representation of a sagittal view of a mouse brain indicating the projection of the optic axons from the retina to one of the primary visual centres of the brain, the superior colliculus. The temporal retina of the right eye and the nasal retina of the left eye project axons via the optic nerve and the optic tract to the right visual centre of the brain (Adapted from Bear et al., 996). Neural cells of the superior colliculus express *Pax*7 during development and in adulthood.

visual targets in the brain, a major one being the optic tectum/superior colliculus. In this way visual information is passed from the retina to visual centres within the brain (Fig. 3b).

In fish (Meyer, 1978) and frogs (Gaze et al., 1974) retinal neurogenesis and remodelling of retinotectal connections continues throughout life as new neurons are continually added at the retinal margin from surrounding neuroepithelial cells. The gene expression profile necessary for development may therefore be present throughout life and available to optic axons during regeneration of the optic nerve. Birds (Kahn, 1974), mammals (Kiernan, 1979) and lizards (Beazley et al., 1998) complete retinal neurogenesis and stabilise their retinotectal/collicular connections early in development (Beazley and Dunlop, 2000). We suggest that gene expression in the visual systems of adult birds and mammals may differ from that present during development and reflect only those components necessary for maintenance of the adult system.

The role of Pax6 in eye development

Evidence suggests that the structure and function of *Pax*6 are highly conserved throughout the animal kingdom and that *Pax*6 is the master control gene for eye development. Ectopic expression of *Pax*6 cDNA from *Drosophila* or mouse within the imaginal disks of *Drosophila* embryos results in the formation of eyes on the legs and wings (Halder et al., 1995). Moreover, ectopic expression of *Pax*6 in the frog *Xenopus laevis* leads to the formation of differentiated eyes that contain mature lens fiber cells, ganglion and Müller cells, photoreceptors and retinal pigment epithelial cells in a spatial arrangement similar to that of endogenous eyes (Chow et al., 1999).

*Pax*6 is considered vital for eye development because mutations result in defective eye formation in *Drosophila*, mouse (*Small eye*) and human (aniridia) (Callaerts et al., 1997). *Small eye* (*Sey*) alleles are homozygous lethal. *Sey* embryos at E9.5 days p.c. lack a lens placode and consequently the lens pit and lens vesicle are not formed. The optic vesicle is uniformly broad in contrast to the proximal constriction observed in wild-type embryos, and the optic stalk retains a lumen, unlike that in normal mouse (Grindley et al., 1995). The embryos continue to develop to term but die soon after birth, lacking eyes and nasal cavities and exhibiting severe brain defects.

During early stages of development in the mouse, *Pax*6 is expressed in a broad region of head surface ectoderm covering the prosencephalon and in an extensive part of head neural ectoderm, including the optic pit. It is expressed in almost all cells of the eye primordium including the optic cup, along with the developing lens and surface epithelium surrounding the lens (Kawakami et al., 1997) (Fig. 3a). At a later stage, when neural cells of the retina begin to differentiate, *Pax*6 expression is dramatically up-regulated in differentiating ganglion, amacrine and horizontal cells of the retina (Kawakami et al., 1997). The expression of *Pax*6 has been implicated in the control of neuronal differentiation in the retina. Specific retinal cell lineages may be defined by *Pax*6 together with other transcription factors and secreted ligands such as ChX10 (a homeodomain containing protein) and ciliary neurotrophic factor (CNTF) (Jean et al., 1998).

The expression of *Pax*6 in the head surface ectoderm and subsequently in the lens placode is correlated with the lens-forming competence of these cells. In normal mouse, the lens continues to express *Pax*6 as the lens fiber cells start to differentiate. It is of interest that heterozygous *Sey* mice (mutation in *Pax*6) exhibit a marked reduction in the size of the developing lens (Hogan et al., 1986, 1988).

Pax6 protein induces the expression of crystallins in the developing lens. The Pax6 protein has been shown to bind to the promoter sequences of crystallin genes in several species including the mouse (Cvekl et al., 1994; Gopal-Srivastava et al., 1996), guinea-pig (Richardson et al., 1995) and chicken (Cvekl et al., 1995). It is thought that Pax6 regulates crystallin gene expression in concert with other transcription factors activated by lens induction (Jean et al., 1998).

In addition, *Pax*6 has a crucial role in development of both the iris and cornea in a manner that is sensitive to gene dosage. Heterozygous *Sey* (*Pax*6 mutant) mice display abnormal development of the iris as well as optic nerve hypoplasia (aniridia syndrome) (Hogan et al., 1988). Aniridia also occurs in humans as a result of mutations at the *PAX*6 locus (Glaser et al., 1992). Normal corneal development requires the correct dosage of *Pax*6; opaque corneas are found in heterozygous *Sey* mice (Hogan et al., 1986) and humans with a mutation in one *PAX*6 gene (Peter's anomaly) (Hanson et al., 1994). The equivalent phenotypes in humans are associated with several heterozygous *PAX*6 null mutations that occur throughout the gene suggesting that eye formation is particularly sensitive to *PAX*6 gene dosage. An autoregulatory loop acting in the developing eye (Grindley et al., 1995) may explain why the eye is so sensitive to *PAX*6 dosage (Glaser et al., 1994).

The role of Pax2 in optic nerve formation

The expression of *Pax*2 regulates differentiation of the optic disk. Furthermore, the limits of the *Pax*2 expression domain establish the optic disk as the boundary between the optic cup and the optic stalk and prevent differentiating retinal cells from extending into the optic stalk (Jean et al., 1998) (Fig. 3). In both the zebrafish mutant of the *Pax*2 equivalent, *Noi* (no isthmus) (Macdonald et al., 1997) and the *Pax*2 null mutant mouse (Torres et al., 1996) pigment epithelial cells extend into the optic stalk and the optic disk fails to form. In humans optic nerve coloboma syndrome is the phenotypic condition linked to mutations in *PAX*2 (Sanyanusin et al., 1995).

It is likely that *Pax*2 is activated by Shh-mediated signals regulating development of the medial optic vesicle. The concept is supported by the finding that there is no embryonic expression in optic tissue of *Pax*2 in the Shh null mutant mouse (Chiang et al., 1996). Moreover, ectopic expression of Shh in the zebrafish leads to a lateral extension of the expression domain of *Noi*, the *Pax*2 equivalent, as well as a loss of *Pax*6 positive retinal cells (Macdonald et al., 1995). These findings strongly suggest that *Pax*2 mediates differentiation of the optic disk and formation of a boundary between the optic cup and optic stalk, restricting *Pax*6 expression to the temporal portion of the optic vesicle and optic cup.

Pax7 expression in the optic tectum/superior colliculus

In zebrafish, chick and mouse, *Pax*7 is involved at early stages of development in specification of brain prosomeric boundaries (Stoykova and Gruss, 1994). At later stages, *Pax*7 is restricted to cells in the optic tectum/superior colliculus and epiphysis and is expressed specifically in differentiating neuronal cells during development of the tectal/collicular cyto-architecture. *Pax*7-positive neurons migrate radially towards the pia and concentrate in the *stratum griseum superficiale* (SGFS) (Kawakami et al., 1997), the target site for retinal axons. These observations suggest that neurons destined to reside in the SGFS are specified at an early stage before overt differentiation. Studies in chick by Nomura et al., (1998), correlating *Pax* gene expression in the prosencephalon and the mesencephalon (from which the tectum/superior colliculus is derived), clearly show that the formation of the tectum consistently coincides with induction/maintenance of *Pax*7 and the suppression of *Pax*6. The result indicates that the switching-on or -off of these two *Pax* genes in a region-specific manner by region-specific factors is responsible for differentiation of brain vesicles into the tectum/superior colliculus. Similarly, floor plate factors suppress *Pax*7 and limit the tectum/superior colliculus to the dorsal mesencephalon (Nomura et al., 1998).

Pax genes in axon guidance

A particularly interesting suggestion is that *Pax* genes play a role in establishing axonal pathways at the borders of their expression domains. For example, the borders of expression of two zebrafish *Pax* genes, *Pax[zf-a]* and *Pax[zf-b]*, homologues of murine *Pax*2 and 6, coincide with primary axon tracts generated in the embryonic brain a few hours after the onset of expression of these genes (Krauss et al., 1991).

*Pax*6 may be involved in axonal pathfinding within the visual system. In *Pax*6 deficient mice the loss of the boundary between the procencephalon and the mesencephalon results in defective pathfinding of the tract of the postoptic commissure. Furthermore, in the *Pax*6 mouse mutant *Small eye* (*rSey2*), dorsal thalamo-cortical axons are remarkably disordered despite cortical efferent axons developing along normal trajectories. *Pax*6 expressing cell clusters along the thalamo-cortical pathway appear to be instrumental in guidance of axonal growth cones (Kawano et al., 1999).

*Pax*2 plays a critical role in optic chiasm development and in the guidance of axons along the optic tract. *Noi* mutant zebrafish (Macdonald et al., 1995) and null mutant *Pax*2 mice (Torres et al., 1996) develop an abnormal chiasm in which all (mouse) or most (zebrafish) optic axons are prevented from projecting across the midline into the contralateral optic tract. Therefore, it appears that *Pax* genes may play a pivotal role in the guidance of retinal axons to the visual centres of the brain where they form functional connections with their recipient target cells.

Pax genes as controllers of visual system development

The ability of Pax proteins to control many aspects of visual development is a result of their capacity to activate downstream target genes that perform specific functions in a time and position dependent manner. For example, the guidance of axons during their outgrowth from the retina and the spatially-related matching of these axons to their neuronal targets in the tectum/superior colliculus is effected by several factors some of which are downstream targets of *Pax* proteins (Jean et al., 1998).

*Pax*2 and *Pax*6 are upstream regulators of EN-1 and EN-2 (the mouse homologues of the Drosophila gene *engrailed*). EN in turn regulates Ephrin ligand/Eph receptor molecules that act as molecular guidance cues in the genetic cascade involved in controlling mid-hindbrain development, neural cell differentiation and topographic map formation in the developing visual system (Brennan et al., 1997; Mastick et al., 1997; Saueressig et al., 1999). In addition, Pax proteins bind to the promoter and regulate expression of the neural cell adhesion molecule (N-CAM) that mediates cell-cell interactions during neural cell development (Holst et al., 1997).

Given that *Pax*6 and *Pax*7 are expressed respectively in retinal cells and their targets within the tectum/superior colliculus, we propose that their complementary expression patterns are important for the establishment of functional connections between these two neuronal populations. We speculate that this circuitry is achieved via activation of complementary cell surface proteins, including Ephrin ligands, Eph receptors and neural cell adhesion molecules.

Pax genes in the mature visual system

Pax genes are expressed in adult tissues in patterns that correlate with their expression during specification of neural cells in the developing visual system (Stoykova and Gruss, 1994). In addition, *Pax* genes are expressed

in adult tissues of several vertebrate classes. Taken together, these findings suggest a crucial role for *Pax* genes in maintaining cellular differentiation patterns in the adult visual system.

*Pax*6 expression in the retina of adult goldfish, zebrafish, quail and mouse is observed in the ganglion cell layer, and the bipolar and amacrine cells of the inner nuclear layer (Krauss et al., 1991; Martin et al., 1992; Püschel et al., 1992; Hitchcock et al., 1996; Kawakami et al., 1997). Furthermore, in newts, *Pax*6 expression is observed in the ganglion cell and the inner nuclear layers (Kaneko et al., 1998). We have observed *Pax*6 expression in the retinal ganglion cell and inner nuclear layers of the retina in adult lizards (Ziman et al., 2000) (Fig. 6) reflecting the patterns described for other vertebrates.

In the adult mouse, *Pax*7 is expressed specifically in neurons of the superior colliculus, corresponding to embryonic expression patterns (Stoykova and Gruss, 1994; Rodger et al., 1999). Moreover, data from our laboratory using RT-PCR show that *Pax*7 expression in the brain is maintained into adulthood in zebrafish, lizard (Ziman et al., 2000) and mouse (Ziman et al., 1997).

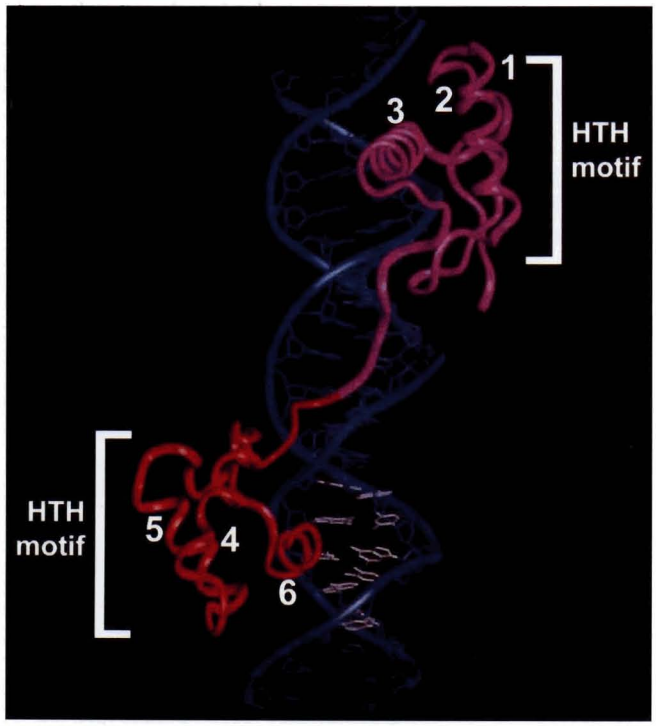


Fig. 4. A representation of the DNA binding domains of Pax proteins interacting with target DNA sequences. The figure represents the paired domain-DNA complex. Helices 1 and 2 are anti-parallel whereas helices 2 and 3 form a helix-turn-helix arrangement. Helix 3 makes contact in the major groove of the DNA. The paired domain contains two sets of helices. The homeodomain contains an additional set of α-helices. The homeodomain and paired domain may bind to target DNA sequences separately or in combination. The target DNA sequences differ for each binding domain and are different again from that bound by the paired domain and homeodomain together (Adapted from Callaerts et al., 1997).

Alternate transcripts of *Pax* genes

Pax proteins bind to specific DNA sequences in the promoters of downstream target genes by using their DNA binding domains (the paired domain and the homeodomain). The DNA binding domains contain helix-turn-helix (HTH) motifs, regions of α-helices separated by a ß-turn. Amino acids within the α-helices form the points of contact with target DNA. The paired domain and the homeodomain both contain HTH motifs that may be used in various combinations (paired domain alone; homeodomain alone or paired and homeodomain together) to influence binding site selection at specific target gene sequences (Fig. 4). This mechanism provides *Pax* proteins with the potential for complex, multiple DNA binding specificities (Underhill and Gros, 1997).

By altering the positions of the intron/exon splice sites within the coding region of the paired box, alternate transcripts of *Pax*6 and *Pax*7 are produced that have differing paired box sequences (Callaerts et al., 1997; Ziman et al., 1997; Kay and Ziman, 1999) (Fig. 5). Computer simulation studies (Ziman and Kay, 1998b) show that such alternate isoforms have modified protein structures within the highly conserved paired DNA binding domain. As a consequence, the isoforms have different DNA binding specificities and affinities as assessed by protein binding studies (Vogan and Gros, 1997) thus providing Pax proteins with an additional mechanism to increase their transcriptional versatility.

Expression of alternate transcripts is conserved in several vertebrate species, emphasising their probable functional relevance (Callaerts et al., 1997; Ziman et al., 2000). In zebrafish, alternate splicing at the 5' end of the paired box intron produces alternate transcripts of the *Pax*7 homologue whereas in mouse, *Pax*7 transcripts are produced by alternate splicing at the 3' end of the paired box intron (Kay and Ziman, 1999; Ziman et al., 2000).

*Pax*6 and *Pax*7 may use alternate transcripts to specify divergent differentiation pathways as indicated by their differential expression in a variety of tissues (Callaerts et al., 1997; Ziman and Kay, 1998b). Recent

Fig. 5. A schematic representation of the intron-exon structure of the paired box of *Pax*6 and *Pax*7 showing the alternate transcripts produced by altering the intron/exon splice sites within the paired domain encoding regions of *Pax*6 and *Pax*7. The paired box encoding region (grey shading) and the alternately spliced sequences (diagonal lines) are indicated. The alternate transcripts produce isoforms that differ within the highly conserved paired DNA binding domain of the proteins. The isoforms have different protein structures and therefore different DNA binding specificities (Callaerts et al., 1997; Ziman et al., 1998). Exons and introns of the paired box (not drawn to scale) are indicated.

experiments in our laboratory revealed that *Pax*7 transcripts are expressed in different proportions in brain as opposed to skeletal muscle in adult mouse (Ziman and Kay, 1998a,b). Further experiments using C2C12 cells, fibroblasts and P19 mouse embryonic cells confirmed that *Pax*7 transcripts are differentially expressed in various cell lineages and suggest a role for *Pax*7 in cellular differentiation (Ziman et al., 2000).

*Pax*6 expression in quail, mouse and human retina is regulated via two promoters, P_0 and P_1, with transcriptional start sites at exon 0 and exon 1 respectively. Furthermore, in quail, a retina-specific enhancer is located upstream of the P_0 promoter. Together these regulatory elements control the tissue-specific expression of *Pax*6 transcripts (Kammandel et al., 1999). The transcripts expressed from the two promoters show differing chronological patterns; whereas the transcripts of P_1 appear first and then slowly decrease, those of the P_0 promoter appear later, but increase and then remain constant until hatching, decreasing thereafter (Plaza et al., 1995a,b). Similar regulatory regions control the expression of *Pax6/PAX6* transcripts in mouse and human (Plaza et al., 1995a,b, 1999), with one transcript preferentially expressed in adult human brain (Okladnova et al., 1998).

Another important aspect of *Pax*6 is that it can regulate its own expression. The gene product of *Pax*6 can bind to sequences in its own gene promoter thereby transactivating its own expression. In this way, *Pax*6 expression is autoregulated in the surface ectoderm and retina of the developing eye (Grindley et al., 1995; Plaza et al., 1999). The autoregulation of *Pax*6 would enhance the sensitivity to gene dosage as observed in mice and humans heterozygous for mutations at the *Pax*6 locus (discussed earlier) (Glaser et al., 1994). Thus, autoregulation of *Pax*6 plays an important role in the developing eye. Moreover the level and pattern of *Pax* gene transcript expression would also be important for the maintenance of a functional visual system in adults.

Pax genes and regeneration of the visual system

On the basis that *Pax* genes are crucial for development of the visual system, they are likely to be required during regeneration. Recent work from several laboratories suggests that *Pax*6 is closely associated with regeneration of the adult visual system. In the mature newt, regeneration of the lens from iris pigment epithelial cells appears to be associated with continued *Pax*6 expression in the dorsal iris (Del Rio-Tsonis et al., 1995). By contrast, the axolotl, a salamander with good regenerative abilities of the limb and tail, is nevertheless unable to regenerate a lens. The lack of regenerative capacity was thought to be associated with a decrease in *Pax*6 expression in cells of the eye as the axolotl matures (Del Rio-Tsonis et al., 1995).

*Pax*6 expression has also been associated with regeneration of the retina. In newts, an entire retina can be regenerated from the retinal pigment epithelium after retinal ablation, a process accompanied by a strong up-regulation of *Pax*6 in neural cells of the regenerating retina (Kaneko et al., 1998). In mature goldfish, *Pax*6 expression is observed in some ganglion cells as well as in the neuronal progenitors and newly postmitotic neurons at the retinal margin (Hitchcock et al., 1996). After injury to the retina, antibodies to the *Pax*6 protein label mitotically active neuronal progenitors at the edges and adjacent to the ablated region. By contrast, rod precursors, (proliferating cells that normally give rise solely to rod photoreceptors) are not immunostained by *Pax*6 antibodies suggesting that these cells are not involved in the regenerative response. Therefore, in goldfish, *Pax*6 expression in neuronal progenitors may be associated with the ability of retinal neurons to continue to be generated throughout life.

Optic nerve regeneration

Central nerve regeneration is an important area of research aimed at providing return of function after trauma. Optic nerve regeneration is a widely used model since the visual system is easily accessible. In addition the nerve contains essentially only one class of axons, those arising from retinal ganglion cells projecting via the optic nerve to visual centres in the brain.

In adult birds and mammals including man, optic nerve regeneration is abortive: after lesioning the optic nerve to sever all optic axons, damaged axons form growth cones that fail even to penetrate the lesion site (Kiernan, 1979). Peripheral nerve regeneration, by contrast, is successful with axons re-growing to their target tissue and restoring function (Barron, 1983). In lower vertebrates such as fish and frog, optic axons successfully regenerate across the lesion site and re-innervate the visual centres within one to two months (reviewed Beazley and Dunlop, 2000). A retinotopic map is reformed and consolidated such that functional vision is restored. In fish, almost all retinal ganglion cells survive axotomy and complete regeneration (Meyer and Kageyama, 1999). For frog, the re-establishment of functional connections occurs despite the loss of approximately one third or more of retinal ganglion cells (Beazley, 1981; Humphrey and Beazley, 1985).

Work from our laboratory on reptiles, phylogenetically intermediate between the fish and frogs and the birds and mammals, has highlighted the sequence of events necessary for successful optic nerve regeneration. In the lizard, *Ctenophorus ornatus,* optic axons re-grow to the visual centres, incurring a loss of retinal ganglion cells that is similar to that observed in frog (Beazley et al., 1997). The regenerative process in lizard, however, is inaccurate with axons failing to re-form a retinotopic map and with a proportion of axons leaving the visual pathway altogether (Dunlop et al., 2000). At an intermediate phase of regeneration between 5-7 months, electrophysiological experiments have revealed a crude retinotopic map; however, the map fails to consolidate and breaks down with the consequence

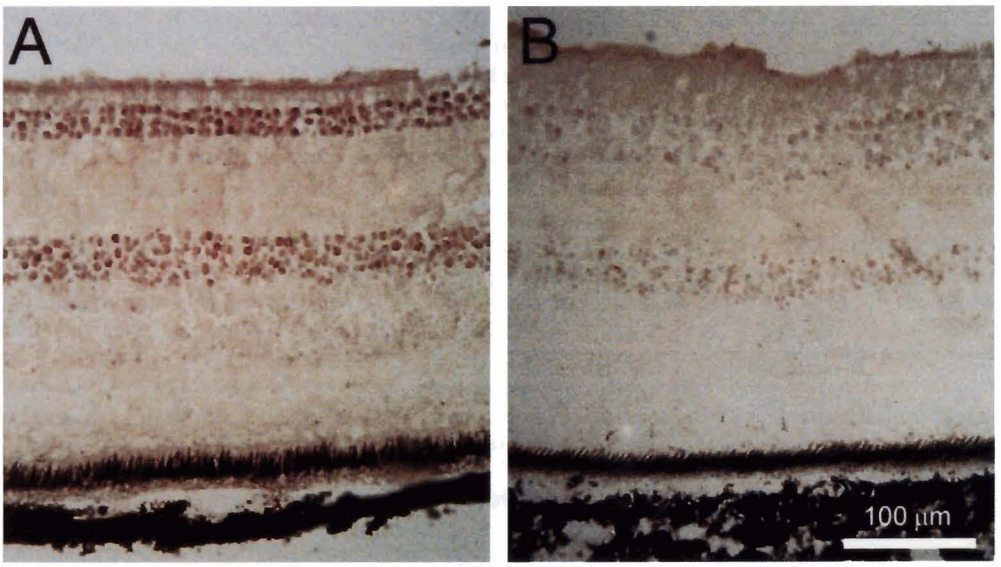


Fig. 6. *Pax*6 expression in the retina of normal adult lizard (a) and one with a regenerating optic nerve six months after axotomy **(b)**. Radial sections (6 µm) with ganglion cell layer uppermost taken from equivalent retinal regions for **(a)** and **(b)** were incubated at 4 °C overnight with a *Pax*6 monoclonal primary antibody (DSHB) followed by peroxidase labelled streptavidin (Biogenix). Sections were treated with the chromogen diaminobenzidine (Pierce) and visualised by light microscopy. Expression of *Pax*6 in the ganglion cell and the inner nuclear layers is apparent in the retina of the normal lizard, however the level of expression is reduced in the retina from the experimental lizard. Scale bar: 100 µm.

that animals remain blind via the operated eye (Stirling et al., 1999).

Pax gene expression and optic nerve regeneration

In our laboratory, recent experiments have focused on the role of *Pax* genes in regeneration of the optic nerve in adult vertebrates. Preliminary experiments in the lizard have shown that *Pax*6 is down regulated within the retina during optic nerve regeneration with expression being dramatically reduced in retinal ganglion cells and cells of the inner nuclear layer (Fig. 6). A possible interpretation is that the loss of *Pax*6 expression in retinal neural cells of the lizard may be instrumental in the failure of optic axons to restore function after axotomy (Stirling et al., 1999). It is possible then that during regeneration *Pax*6 is not associated with retinal ganglion cell axon growth per se but rather with establishment of functional retino-tectal connections.

Moreover, it is anticipated that loss of *Pax* gene expression during optic nerve regeneration would have a negative effect on downstream target genes associated with guidance of regenerating axons and cell adhesion. During optic nerve regeneration in goldfish, various cytoskeletal proteins and ephrins are transiently up-regulated to coincide with the phases of optic axon re-growth and map formation respectively. However, in the lizard, a persistent upregulation of such proteins was observed (Bartlett et al., 2000; Chen et al., 2000; Rodger et al., 2000), consistent with the view that a reduction in *Pax*6 expression results in a loss of regulation of axon guidance and cell-cell adhesion.

*Pax*2 may play an important role in axon guidance during optic nerve regeneration. As previously mentioned, *Pax*2 expression in cells of the optic chiasm is necessary for guidance of retinal ganglion cell axons into the ipsilateral or contralateral tracts during development (Macdonald et al., 1995; Torres et al., 1996). Results from our laboratory indicate that another group of reptiles, the gekkos, do not restore a functional retino-tectal projection after injury to the optic nerve. Preliminary findings suggest that a small proportion of axons cross the lesion site and reach the optic chiasm but fail to traverse the midline to enter the contralateral optic tract. Instead some axons enter the ipsilateral tract or the opposite optic nerve. We anticipate that *Pax*2 expression will be affected in cells of the optic chiasm of gekkos after the optic nerve is severed.

Work currently in progress in our laboratory aims to determine the role of *Pax*2, 6 and 7 in optic nerve regeneration by assessing expression of these genes in zebrafish, lizards and mice, species that display a range of outcomes of optic nerve regeneration.

Conclusion

Highly regulated *Pax* gene expression appears to control specification of cells in the visual system during development and at maturity. We suggest therefore that the expression of a transcript profile of *Pax* genes that mirrors development will be essential for successful optic nerve regeneration. A detailed analysis of differences between the sites, levels and times of *Pax*2, *Pax*6 and *Pax*7 transcript expression in several vertebrate species with a range of regenerative abilities may lead to identification of expression patterns that are a pre-requisite for successful optic nerve regeneration in humans.

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