Pax proteins and eye development

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Homologous members of the Pax gene family are required for eye development in *Drosophila* and vertebrates. Despite superficial similarities in the phenotypes of vertebrates with mutations in *pax-6* and *Drosophila eyeless* mutants, it remains uncertain whether the two proteins encoded by these genes have comparable functions. The genetic cascade triggered by *eyeless* leads to eye formation, whereas *pax-6* is not necessary for optic vesicle formation, but is required at other stages of eye development. A second vertebrate Pax gene, *pax-2*, is also required during eye development and appears to play a role during closure of the choroid fissure.

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Abbreviations

HH	Hedgehog
Sey	*Small eye*
SHH	Sonic Hedgehog
TWHH	Tiggywinkle Hedgehog

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Introduction

Paired-box (*pax*) genes encode a family of transcription factors involved in the regulation of many aspects of early development (reviewed in [1–5]). In vertebrates, at least two Pax genes, *pax-6* and *pax-2*, are required during eye development (Table 1). Mice that lack Pax-2 (M Torres, P Gruss, personal communication; [6,7]) and humans heterozygous for a mutation in the *pax-2* gene [8•] exhibit optic nerve coloboma, a condition in which closure of the choroid fissure is arrested or fails altogether, and which can lead to defects in the retinal pigmented epithelium and poor vision. In the absence of Pax-6, eyes fail to develop in both rats [9•] and mice [10–12], and, remarkably, *eyeless*, a *Drosophila* homologue of *pax-6*, is also essential for fly eye development [13••].

This review focuses on the roles that Pax-6 and Pax-2 may play in both the developing and the mature vertebrate eye. Readers are directed to recent comprehensive reviews [14••,15••] for further discussions of invertebrate eye development.

Pax-6 is not required to initiate vertebrate eye development

Cells destined to form the eyes are located within anterior regions of the vertebrate neural plate, adjacent and probably intermixed with cells that will contribute to other regions of the dorsal diencephalon and telencephalon (see e.g. [16•]). As the neural plate closes to form the neural tube, the eye develops as an outpocketing of neuroepithelium at the boundary between rostral diencephalon and basal telencephalon. The evaginating optic vesicle subsequently contacts the surface epithelium and invaginates to form the neural and pigment epithelial layers of the retina. Proximal regions of the vesicle form the optic stalks connecting the retinae to the remainder of the forebrain (Figure 1).

The *pax-6* gene is expressed in a large area of the rostral neural plate within which all of the cells that contribute to the optic vesicles are likely to originate [17–19,20•]. At no stage, however, does *pax-6* expression specifically define which of the anterior neural plate cells will form the eyes. Indeed, we are unaware of any genes that demarcate presumptive eye cells before evagination of the optic vesicles.

The widespread expression of *pax-6* in the anterior neural plate and placode-forming epithelium (see below) is consistent with the gene defining a field of cells that are competent to form eye tissue. Pax-6 is unlikely, however, to be required for the initiation of optic vesicle formation, as this process occurs reasonably well in homozygous *Small eye* (*Sey*) mice, which probably lack all Pax-6 protein [10,21•].

Although optic vesicle evagination does occur in *Sey* mice, the resultant morphogenesis and growth are abnormal and the optic vesicle fails to form a recognizable optic cup [10,21•]. This suggests that Pax-6 may be required to maintain the proliferation of cells within the optic vesicle. Indeed, the observed reduction in eye size of heterozygous *Sey* mice [10,12] supports this possibility. Further support for a role for Pax-6 in proliferation comes from the observations that *pax-6* expression is maintained in cells at the proliferative margins of the retina (R Macdonald, J Scholes, SW Wilson, unpublished data; [22•]), is expressed in proliferative cells during retinal regeneration in goldfish [22•] and lens regeneration in urodeles [23], and is widely expressed in many other dividing cells in the CNS [17–19,24].

Thus, although all presumptive eye cells probably express *pax-6*, Pax-6 appears not to be essential for the earliest steps in the morphogenesis of the eye. Instead, at early stages of development, Pax-6 may have a more fundamental role in growth and proliferation both in the presumptive eye and in other regions of the CNS.

Pax-6 is required for retinal development

Cells at different locations within the evaginating optic primordium form different structures within the eye: distally located cells that contact the surface ectoderm form the neural layer of the retina; cells that lie adjacent to the neural retina form the pigment epithelium; and the most proximal cells of the optic vesicle give rise to the optic stalks (Figure 1). By the stage at which the optic vesicle has begun to invaginate to form the optic cup, Pax-6 is still present in all cells of both layers of the presumptive retina, but is only weakly expressed or is absent from presumptive optic stalk cells (R Macdonald, J Scholes, SW Wilson, unpublished data; [25••]) (Figure 1a).

No retinal development is evident in homozygous *Sey* mice [12,21•]. Furthermore, in zebrafish with experimentally reduced numbers of *pax-6*-expressing cells within the optic vesicles, retinal differentiation appears to be restricted to those cells that retain Pax-6 [25••,26••]. Thus, Pax-6 may be required by cells within the optic vesicle to enable them to initiate retinal development. It has yet to be conclusively demonstrated, however, that the failure to form retina is attributable to a requirement for Pax-6 within presumptive retinal cells. An alternative explanation is that Pax-6 is required within lens placodal cells that lie adjacent to the presumptive retina and that, in the absence of Pax-6, these epithelial cells fail to signal to the optic vesicle, thereby blocking retinal development.

Despite this caveat, perhaps the most favoured explanation of why retina fails to form in the absence of Pax-6 is that this protein is required to specify the retinal identity of optic vesicle cells. Indeed, it has been suggested that *pax-6* is an evolutionarily conserved master control gene that can determine the fate of expressing cells [14••]. The evidence for this comes primarily from *Drosophila*, in which ectopic expression of both mouse *pax-6* and *Drosophila eyeless* can re-specify imaginal disc cells, causing them to form extra eyes on legs, wings and antennae [27••]. This dramatic illustration of the potency of Pax-6/Eyeless in *Drosophila* raises the issue of whether Pax-6 can also specify retinal identity in cells outside the normal retinae in vertebrates.

The widespread expression of *pax-6* in regions of the vertebrate CNS that do not form eyes [17] indicates that Pax-6 is not sufficient to specify retinal development.

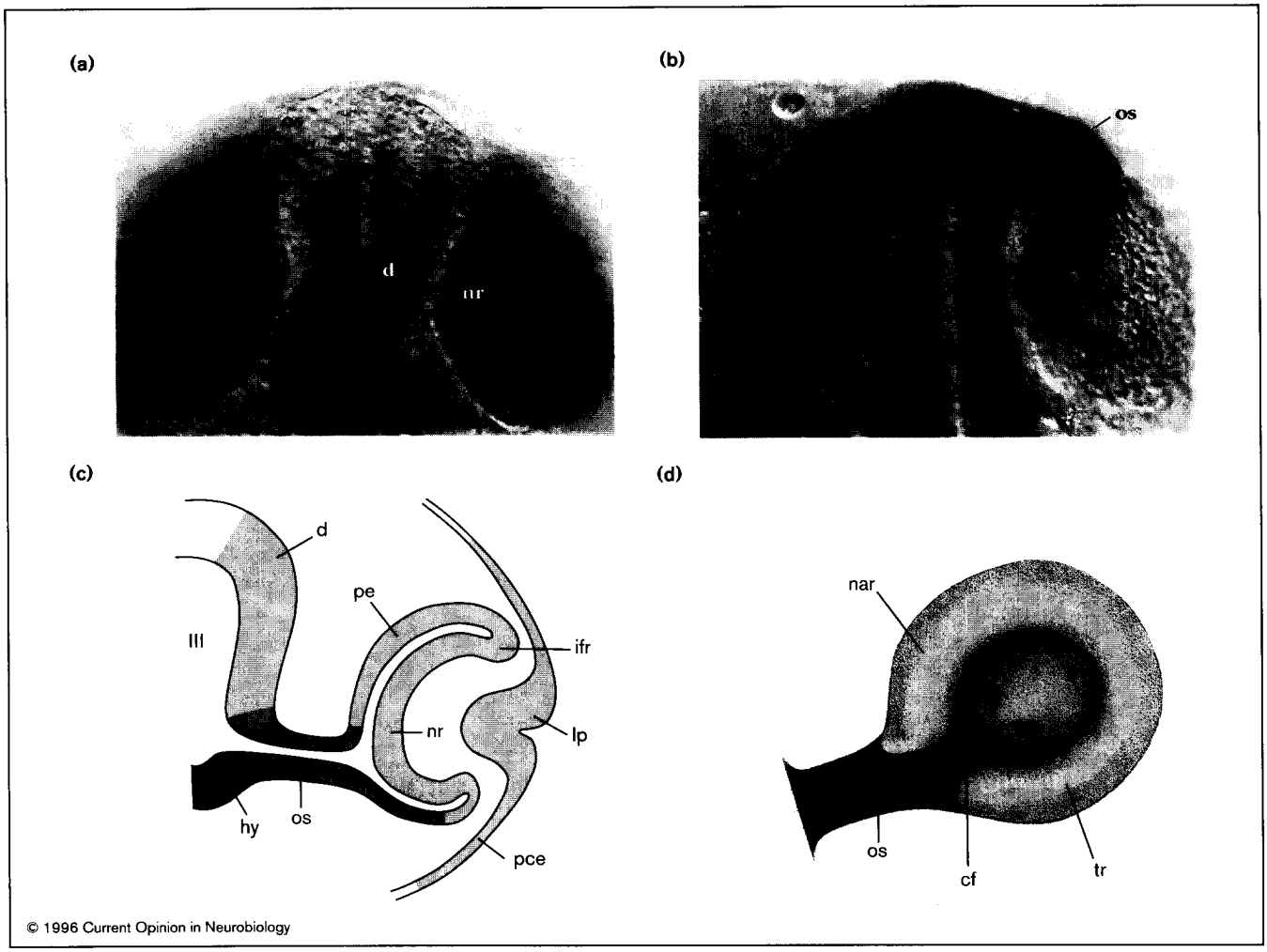
Table 1

Defects in eye development caused by mutations in Pax genes.

Gene	Species	Mutation	Phenotype	References
pax-6	*Drosophila*	*eyeless* (ey^2, ey^R)	Reduced/absent eyes	[13••]
	Mouse Rat	*Small eye* (*Sey*) *Rat Small eye* (*rSey*)	Heterozygotes: reduced eye, iris hypoplasia, cataract formation, lens vacuolization and dislocation Homozygotes: early optic vesicle forms but lens does not; at late stages, no eye or nose develops; impaired migration of midbrain neural crest to facial regions (rat)	[9•,10–12,21•,49]
	Human	Aniridia	Heterozygotes: iris hypoplasia, cataract formation, corneal vascularization, glaucoma Homozygotes/compound heterozygotes: no eyes; lethal	[43,50–54]
		Peter's anomaly	Anterior chamber and corneal defects	[55]
		Alternative splice mutation	Corneal defects, cataracts, glaucoma, poor vision, but irides intact, fovea normal	[46••]
		Autosomal dominant keratitis	Corneal opacification, and vascularization defects of iris stoma, foveal hypoplasia	[56]
pax-2[a]	Mouse	Kidney, retinal defect mouse (*Krd*)[b]	Optic nerve coloboma, retinal defects	[7]
		Null mutant	Homozygote: optic nerve coloboma	[6] (c)
	Human		Heterozygote: optic nerve coloboma	[8•]

[a]The mouse *pax-2* gene is a member of a small subfamily of Pax genes, including *pax-5* and *pax-8*. On the basis of sequence and expression analyses, it is likely that the zebrafish gene originally termed *pax-b* or *zf[pax-b]* is most closely related to *pax-2*, and this is the nomenclature that we have followed in this review. [b]The *Krd* mouse was identified in a transgenic line as a semi-dominant mutation causing kidney and retinal defects and growth retardation. Genetic and molecular analyses revealed that a deletion at the transgenic insertion site spans approximately 7 centimorgans of chromosome 19, and includes deletion of the *pax-2* locus and probably many other genes. [c]M Torres, P Gruss, personal communication.

Figure 1



Expression of *pax-6* and *pax-2* in the developing eye. Dorsal views of 18-somite zebrafish embryos labelled with antibodies that recognize (a) Pax-6 and (b) Pax-2. **(a)** Within the optic cup, Pax-6 is present in cells of the neural retina (nr) and pigment epithelium (pe), **(b)** whereas Pax-2 expression is primarily restricted to the optic stalks (os), which connect the retinae to the remainder of the forebrain. **(c)** Schematic representation of a transverse section through the diencephalon (d) and eye, illustrating the positions of cells that express *pax-6* (light grey), *pax-2* (medium grey with hatch marks), and *shh* and *twhh* (dark grey). The figure is based primarily on analysis in zebrafish, but is probably also valid for other species. hy, hypothalamus; ifr, iris-forming region; III, third ventricle; lp, lens placode; pce, presumptive corneal epithelium. **(d)** Schematic illustration of *pax-2* expression (medium grey with hatch marks) around the choroid fissure (cf). The illustration represents an oblique view of the eye looking into the optic cup after the lens has been removed. The choroid fissure is the site at which the ventral nasal and ventral temporal retina fuse to close the optic cup. The fissure provides an exit point for the axons of retinal ganglion cells (dark grey) to leave the retina en route to their central targets. Pax-2 is present both within the optic stalk and within retinal cells that line the choroid fissure. nar, nasal retina; tr, temporal retinal. Reproduced with permission from [25••].

Within the more restricted confines of the evaginating optic vesicles, however, ectopic *pax-6* expression may be sufficient to alter the fate of proximally located cells from forming optic stalks to forming retinal tissue. This possibility is suggested by analysis of the *cyclops* mutation in zebrafish, in which retina develops across the midline and optic stalks are reduced or absent [25••,28]. One interpretation of this phenotype is that proximally located cells in the optic vesicle ectopically express *pax-6* and are re-specified to form retina [25••]. This is, however, only one possible interpretation of the *cyclops* phenotype, and definitive proof that Pax-6 can specify retinal identity is still required. A key experiment will be to determine whether retinal expansion or fusion occurs in transgenic animals in which *pax-6* is ectopically expressed in presumptive optic stalk cells within the optic vesicle.

Although levels of Pax-6 protein may be important in regulating proliferation within the optic vesicle, it seems less likely that absolute levels of the protein are critical for any role in the specification of retinal identity. Thus, in *Sey* heterozygous mice with reduced levels of Pax-6, retinal specification appears to occur relatively normally even though the retina is considerably reduced in size [10].

Pax-2 is required for closure of the choroid fissure

In contrast to *pax-6* expression, *pax-2* expression is confined to cells within the optic vesicle that contribute to the optic stalk and parts of the ventral retina around the choroid fissure (R Macdonald, J Scholes, SW Wilson, unpublished data; [29–32]) (Figure 1b). The choroid fissure is the domain of the retina at which ventral nasal and ventral temporal retina fuse to create the closed optic cup (Figure 1d).

Recent evidence suggests that Pax-2 may be involved in the closure of the choroid fissure. Optic nerve colobomas, in which the choroid fissure fails to close, occur both in humans heterozygous for a mutation in the *pax-2* gene [8•] and in mice lacking *pax-2* through either targeted deletion of the gene (M Torres, P Gruss, personal communication; [6]) or a chromosomal deletion encompassing the gene [7]. Although Pax-2 appears to be required for closure of the fissure, it may not be required for its initial formation. Thus, although exogenous retinoic acid can induce the formation of ectopically located choroid fissures in the zebrafish eye [33•], these fissures can apparently form in the absence of *pax-2* expression (GA Hyatt, EA Schmitt, JE Dowling, personal communication).

Pax-6 and Pax-2 are differentially regulated by signals emanating from midline forebrain tissue

It has recently been shown that two members of the Hedgehog (HH) family of secreted signalling proteins, Sonic Hedgehog (SHH) and Tiggywinkle hedgehog (TWHH), promote the expression of *pax-2* and inhibit the expression of *pax-6* within the optic vesicles of zebrafish embryos [25••,26••]. Both *shh* and *twhh* are expressed at the base of the optic stalks adjacent to *pax-2*-expressing cells [25••,26••,34,35•], raising the possibility that these HH proteins may promote *pax-2* expression within presumptive optic stalk cells. In support of this, *pax-2* expression spreads throughout the optic vesicle following widespread overexpression of SHH or TWHH [25••,26••]. In the same embryos, massive reduction in the number of *pax-6*-expressing cells is observed and subsequent retinal development is severely impaired.

The notion that HH proteins regulate the spatial expression of *pax-6* and *pax-2* has been further tested by examining *cyclops* mutant zebrafish embryos in which neither *shh* nor *twhh* is expressed in the rostral forebrain [25••,26••,28,34]. In mutant embryos, *pax-2* expression is severely reduced and *pax-6* is abnormally expressed across the midline, thus fusing the two retinae.

The above data suggest that HH family proteins either directly or indirectly regulate the spatial localization of *pax-6* and *pax-2* expression, and the subsequent partitioning of the optic vesicle into optic stalk and retina.

Pax-6 is required for the development of other eye structures

One of the most remarkable features of *pax-6* expression is that Pax-6 is present in most cell types of the eye, regardless of their origin. Pax-6 is expressed in the neuroectodermally derived neural and pigment layers of the retina, including the iris-forming regions at the ciliary margins, and in the epithelially derived lens and cornea (R Macdonald, J Scholes, SW Wilson, unpublished data; [17,21•,36]).

Analysis of *Sey* rats and mice indicates that *pax-6* is required to initiate the formation of the lens placode [9•,21•]. Tissue-recombination experiments have demonstrated that the requirement for Pax-6 is within placodal cells. Lens formation cannot be rescued by culturing lens ectoderm from *Sey* rats adjacent to wild-type optic vesicles [9•]. Furthermore, transplantation and ablation studies in the chick have shown that induction of *pax-6* expression in the surface ectoderm occurs independently of the optic vesicle [20•], although the later development of the lens does rely upon interactions with the eye cup [37].

Mutant *pax-6* mRNA is initially detected within both the surface ectoderm and the optic vesicle of homozygous *Sey* mice, but the level of transcripts rapidly declines within lens-forming surface ectodermal cells [21•]. This decline may occur because Pax-6 is required to transactivate its own expression; *in vitro* binding studies have demonstrated that Pax-6 can recognize sites within its own promoter [38]. Thus, within the lens-forming surface ectoderm, Pax-6 appears to be required both for the initiation of lens formation and the maintenance of its own expression.

Pax-6 may have additional roles in the mature visual system

Pax-6 is downregulated in most cell types of the eye as they become postmitotic and differentiate (R Macdonald, J Scholes, SW Wilson, unpublished data; [18,36]). Expression is maintained, however, within several cell types of the mature retina, including a large population of amacrine cells (R Macdonald, J Scholes, SW Wilson, unpublished data; [18,22•,36]). Other cell types that maintain *pax-6* expression in the adult include the lens and corneal epithelia and iris (R Macdonald, J Scholes, SW Wilson, unpublished data).

Homozygous *Sey* mice fail to develop eyes and so cannot tell us anything about Pax-6 function at later stages of development. Heterozygotes do, however, exhibit eye defects that may shed light on later aspects of Pax-6 function. These defects must result from reduced levels of Pax-6 either during development or within cells that constitutively express the gene throughout life. In support of the latter possibility, defects associated with heterozygosity occur in the lens, cornea and iris (Table 1), all of which maintain *pax-6* expression in the mature eye

(R Macdonald, J Scholes, SW Wilson, unpublished data). Indeed, several studies have shown that binding sites for Pax-6 are present in the promoters of a number of different crystallin genes [39•–42•] and abnormal regulation of these genes could contribute to the lens defects in *pax-6* heterozygotes. These results imply that mutations in *pax-6* not only cause developmental defects but may also affect the function of some mature eye tissues, thus contributing to the progressive deterioration of the eye seen in aniridia patients [43].

Regulation of the *pax-6* gene is complex

The spatial and temporal distribution of Pax-6 during development and in the mature eye suggests highly complex regulation of the *pax-6* gene. Although how this occurs remains largely unknown, analyses of the quail and mouse genes have identified a neuroretina-specific enhancer [44•]. In addition, the transcription factor c-Myb binds to the *pax-6* promoter and transactivates expression *in vitro*. The c-*myb* gene is expressed in the neuroretina coincident with *pax-6*, raising the possibility that it may be an endogenous regulator of *pax-6* expression [45•]. To add further to the complexity of *pax-6* regulation, alternative splicing has been shown to generate a protein with a larger than normal paired domain. This protein has unique spatio-temporal expression and DNA-binding properties [46••]. Furthermore, humans carrying a mutation in the splicing site that alters the relative proportions of the long and short Pax-6 proteins have corneal defects and develop cataracts but have intact irises [46••].

Evolutionary aspects of Pax-6 function

It has been proposed that a role for Pax-6 in regulating eye development may have been conserved between insects and humans [14••,27••]. This suggests that some components of the genetic cascade downstream of Pax-6 and eyeless should also be conserved and will have been present in the common ancestor of both flies and vertebrates. In *Drosophila*, both eyeless and Pax-6 are sufficient to initiate a cascade of events through which competent imaginal disc cells form eyes [27••]. This finding, however, does not constitute sufficient evidence that Pax-6 initiates a similar genetic cascade during vertebrate eye development.

Given the huge differences in the differentiated eyes of insects and vertebrates, it is likely that many aspects of eye development are not conserved between these two groups. Perhaps the most likely developmental pathway to be conserved in all eye structures would be one that leads to phototransduction, as this is the defining characteristic of all eyes. Thus, the common ancestor of both insects and vertebrates may have possessed simple photoreceptive cells that expressed both Pax-6 and proteins involved in phototransduction [14••]. The simplest way in which Pax-6 function might be conserved would be direct regulation of the expression of proteins involved in phototransduction, in much the same way that Pax-6 regulates crystallin proteins in the lens. This is not the case, however, as Pax-6 is not detected within photoreceptors by the stage at which phototransduction proteins are being expressed (R Macdonald, J Scholes, SW Wilson, unpublished data). Thus, if an evolutionarily conserved genetic cascade linking Pax-6 with phototransduction exists, other components of this pathway are likely to be present in both vertebrates and invertebrates. One key avenue of research will be to determine whether the proteins that directly regulate phototransduction proteins are conserved across many species.

Current data in vertebrates are consistent with Pax-6 playing an early role in defining the regions within the forebrain and surface ectoderm from which the eyes and lenses develop. The gene may also be involved in regulating proliferation and morphogenesis in the anterior CNS. These very fundamental roles in patterning the anterior region of the embryo, as opposed to just patterning the eye, may well be conserved throughout evolution. Indeed, *vab-3*, a *pax-6* homologue in *Caenorhabditis elegans*, is also expressed in the head region [47••,48••]. *C. elegans* does not have obvious eye structures and so perhaps the original function of Pax-6 was in patterning the head region and the gene has been subsequently recruited during eye development. A secondary role for Pax-6 in eye development could nevertheless, still have evolved before evolutionary separation of insects and vertebrates.

Conclusions

Recent studies have only just begun to unravel the various roles that Pax-6 plays in the development of the vertebrate eye. Pax-6 is likely to perform discrete functions at different stages of development and in different cell types. Within the developing lens it is required to initiate the formation of the lens placode and is also implicated as a direct regulator of crystallin gene expression in mature lens cells. The roles played by Pax-6 in the optic vesicle are less clear. An early role in proliferation and morphogenesis is possible, although any such function may not be confined solely to the optic vesicle.

Work in *Drosophila* has demonstrated elegantly that eyeless can initiate a genetic cascade by which imaginal disc cells form eye tissue, indicating that this protein is both necessary and sufficient to specify eye development within responsive cells. A similar role for Pax-6 is feasible in vertebrates, but definitive evidence is still lacking. Dissection of Pax-6 function during eye development may require inactivation of *pax-6* within specific tissues at specific stages of development, as well as misexpression of the gene at times and sites when and where Pax-6 is normally absent. Determination of whether Pax-6 function has been conserved during evolution will require the identification and understanding of many more of the components of the genetic cascade between *pax-6* expression and phototransduction.

On the basis of its expression pattern and the superficial analysis of phenotypes resulting from an absence of Pax-2, it is likely that this protein will play a more restricted role in the development of the vertebrate eye. As yet, no homologue of Pax-2 has been identified in invertebrates, and it remains unknown whether Pax proteins in addition to eyeless may be required during the formation of the insect eye.

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This paper examines the role of midline signalling in regulating *pax-6* and *pax-2* expression during early eye development in the zebrafish (see also [26••]). Midline signalling is disrupted in *cyclops* mutant embryos, resulting in an expansion of the domain of *pax-6* expression to include anterior midline cells. These cells subsequently develop as retina, giving rise to a fused eye that lacks optic stalks. Overexpression of the signalling molecule SHH expands the domain of *pax-2* expression in the optic vesicle and inhibits the expression of *pax-6*, leading to hypertrophied optic stalk-like structures and reduced retinae. These findings suggest that midline signals promote the expression of *pax-2*, inhibit *pax-6* and contribute to the partitioning of the optic vesicle into retina and optic stalk.

26. •• Ekker SC, Ungar AR, Greenstein P, Von Kessler DP, Porter JA, Moon RT, Beachy PA: **Patterning activities of vertebrate hedgehog proteins in the developing eye and brain.** *Curr Biol* 1995, **5**:944–955.

Patterning of the forebrain and eye by members of the Hedgehog family of secreted proteins is investigated in zebrafish by analyzing the effects of over-expression of *shh* and *twhh* upon gene expression in the eyes and forebrain (see also [25••]). Overexpression of HH proteins promoted *pax-2* expression in the optic vesicle and inhibited *pax-6* expression. Other changes in gene expression are consistent with HH family proteins promoting ventral identity within the diencephalon. The authors suggest that HH proteins are involved in establishing the proximodistal axis in the developing eye, as well as in dorsoventral patterning of the forebrain.

27. •• Halder G, Callaerts P, Gehring WJ: **Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*.** *Science* 1995, **267**:1788–1792.

This study addresses directly the function of *eyeless* during *Drosophila* eye development. Morphologically normal and electrically active ectopic eyes develop on wings, legs and antennae following misexpression of *eyeless* or the mouse *pax-6* gene in imaginal discs. The ability to re-specify cells to another fate indicates that *eyeless* is a key regulator of eye development.

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29. Krauss S, Johansen T, Korzh V, Fjose A: **Expression pattern of zebrafish *pax* genes suggest a role in early brain regionalization.** *Nature* 1991, **353**:267–270.

30. Krauss S, Johansen T, Korzh V, Fjose A: **Expression of the zebrafish paired box gene *pax[zf-b]* during early neurogenesis.** *Development* 1991, **113**:1193–1206.

31. Püschel AW, Westerfield M, Dressler GR: **Comparative analysis of Pax-2 protein distributions during neurulation in mice and zebrafish.** *Mech Dev* 1992, **38**:197–208.

32. Nornes HO, Dressler GR, Knapik EW, Deutsch U, Gruss P: **Spatially and temporally restricted expression of *Pax2* during murine neurogenesis.** *Development* 1990, **109**:797–809.

33. • Hyatt GA, Schmitt EA, Marsh-Armstrong N, McCaffery P, Dräger UC, Dowling JE: **Retinoic acid establishes ventral retinal characteristics.** *Development* 1996, **122**:195–204.

Treatment of zebrafish embryos at the end of gastrulation with retinoic acid causes duplication of the ventral retina and an increase in the domain of *pax-2* expression. In addition, the authors showed that ectopic choroid fissure-like structures could be induced by embedding a bead soaked in retinoic acid into the dorsal retina.

34. Krauss S, Concordet J-P, Ingham PW: **A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos.** *Cell* 1993, **75**:1431–1444.

35. • Barth KA, Wilson SW: **Expression of zebrafish *nk2.2* is influenced by *sonic hedgehog/vertebrate hedgehog-1* and demarcates a zone of neuronal differentiation in the embryonic forebrain.** *Development* 1995, **121**:1755–1768.

This paper describes the isolation of a homeobox-containing gene that may be a target for regulation by SHH. Overexpression of SHH is shown to lead to severe defects in the formation of the optic cup (see also [25••,26••]).

36. Martin P, Carriere C, Dozier C, Quatannens B, Mirabel M-A, Vandenbunder B, Stehelin D, Saule S: **Characterisation of a paired box- and homeobox-containing quail gene *(Pax-QNR)* expressed in the neuroretina.** *Oncogene* 1992, **7**:1721–1728.

37. Grainger RM: **Embryonic lens induction: shedding light on vertebrate tissue determination.** *Trends Genet* 1992, **8**:349–355.

38. Plaza S, Dozier C, Saule S: **Quail *PAX-6 (PAX-QNR)* encodes a transcription factor able to bind and transactivate its own promoter.** *Cell Growth Diff* 1993, **4**:1041–1050.

39. • Cvekl A, Sax CM, Bresnick EH, Piatigorsky J: **A complex array of positive and negative elements regulates the chicken αA-crystallin gene: involvement of Pax-6, USF, CREB and/or CREM, and AP-1 proteins.** *Mol Cell Biol* 1994, **14**:7363–7376.

See annotation [42•].

40. • Cvekl A, Kashanchi F, Sax CM, Brady JN, Piatigorsky J: **Transcriptional regulation of the mouse αA-crystallin gene: activation dependent on a cyclic AMP-responsive element (DE1/CRE) and a Pax-6-binding site.** *Mol Cell Biol* 1995, **15**:653–660.

See annotation [42•].

41. • Richardson J, Cvekl A, Wistow G: ***Pax-6* is essential for lens-specific expression of ζ-crystallin.** *Proc Natl Acad Sci USA* 1995, **92**:4676–4680.

See annotation [42•].

42. • Cvekl A, Sax CM, Li X, McDermott JB, Piatigorsky J: **Pax-6 and lens-specific transcription of the chicken δ1-crystallin gene.** *Proc Natl Acad Sci USA* 1995, **92**:4681–4685.

These papers [39•–42•] demonstrate downstream target genes of Pax-6 during lens development, a process in which Pax-6 is known to be required from mutant analysis. Pax-6 binds specific promoter sites and transactivates transcription of several different lens-specific crystallin genes in several different species. This suggests that Pax-6 may have been important for the recruitment and expression of crystallin genes in the lens during evolution.

43. Glaser T, Walton DS, Cai J, Epstein JA, Jepeal L, Maas RL: ***PAX6* gene mutations in aniridia.** In *Molecular Genetics of Ocular Disease.* Edited by Wiggs J. New York: Wiley Publishers; 1995:55–81.

44. • Plaza S, Dozier C, Langlois M-C, Saule S: **Identification and characterization of a neuroretina-specific enhancer element in the quail *Pax-6 (Pax-QNR)* gene.** *Mol Cell Biol* 1995, **15**:892–903.

Promoter analysis identifies a conserved enhancer upstream of the quail and mouse *pax-6* gene that drives expression in neural retina.

45. • Plaza S, Turque N, Dozier C, Bailly M, Saule S: **c-myb acts as transcriptional activator of the quail PAX-6 (PAX-QNR) promoter through two different mechanisms.** *Oncogene* 1995, **10**:329–340.

A number of Myb-responsive elements are characterized in the quail *pax-6* promoter to which c-Myb can bind to and transactivate transcription *in vitro*. In addition, c-Myb is shown to be expressed in developing quail neural retina simultaneously with *pax-6*, suggesting that it may be an endogenous transactivator of *pax-6 in vivo*.

46. •• Epstein JA, Glaser T, Cai J, Jepeal L, Walton DS, Maas RL: **Two independent and interactive DNA-binding subdomains of the Pax6 paired domain are regulated by alternative splicing.** *Genes Dev* 1994, **8**:2022–2034.

An alternative splice product of the *pax-6* gene that results in a 14-amino-acid insertion in the paired box is described in a number of vertebrates. The authors show that distinct ocular abnormalities can be attributed to a change in the splicing acceptor site in exon 5, which results in a higher proportion of the longer form of Pax-6. Analysis of the two isoforms shows that binding specificity is altered by the insertion within the paired domain. The potential for different sets of target genes for each isoform may in part explain how Pax-6 achieves specific functions in different tissues.

47. •• Chisholm AD, Horvitz HR: **Patterning of the *Caenorhabditis elegans* head region by the *Pax-6* family member *vab-3*.** *Nature* 1995, **377**:52–55.

The authors show that the *C. elegans vab-3* gene is related to *pax-6* and is expressed in the head region. Mutants at this locus show variable head defects, including transformation of hypodermal cell fates to those of posterior homologues and abnormal specification of neurons. As nematodes do not have obvious eyes, this suggests that the earliest role for *pax-6* may be in the specification of cells in the head region, with the gene being recruited for eye development later during evolution (see also [48••]).

48. •• Zhang Y, Emmons SW: **Specification of sense-organ identity by a *Caenorhabditis elegans Pax-6* homologue.** *Nature* 1995, **377**:55–59.

In this paper, *C. elegans mab-18* is shown to be a *pax-6* homolog encoding a truncated protein consisting of the homeodomain and the carboxy-terminal regions only. The *mab-18* gene is expressed in a peripheral sense organ in the male tail and is necessary for the specification of this organ (see also [47••]).

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

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53. Glaser T, Walton DS, Maas RL: **Genomic structure, evolutionary conservation and aniridia mutations in the human *PAX6* gene.** *Nature Genet* 1992, **2**:232–239.

54. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL: ***PAX6* gene dosage effects in a family with congenital cataracts, Aniridia, anophthalmia and central nervous system defects.** *Nature Genet* 1994, **7**:463–471.

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56. Mirzayans F, Pearce WG, MacDonald IM, Walter MA: **Mutation of the PAX gene in patients with autosomal dominant keratitis.** *Am J Hum Genet* 1995, **57**:539–548.