New perspectives on eye evolution

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The highly complex eyes of vertebrates, insects and molluscs have long been considered to be of independent evolutionary origin. Recently, however, Pax-6, a highly conserved transcription factor, has been identified as a key regulator of eye development in both mammals and flies. Homologues of Pax-6 have also been identified in species from other phyla, including molluscs. The wide variety of eyes in the animal kingdom may, therefore, have evolved from a single ancestral photosensitive organ.

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Introduction

An enormous diversity in eye types and structures is found in the animal kingdom. Phylogenetic studies have revealed that almost all animal phyla have evolved some form of light-sensitive organ [1,2], ranging from simple eye-spots, comprising a small number of photoreceptor cells, to eyes with highly developed optics. About 10 distinct optical systems are distinguishable, including the pinhole eye of nautilus, eyes built like a reflecting telescope, two kinds of camera lens eyes and several kinds of compound eyes (reviewed in [2–4]). Because an almost complete gradation exists from the single light-sensitive cells to the more complex organs, it has actually become difficult to define what an eye is. In this review, we would like to apply the term 'eye' to a visual system in which single or multiple photoreceptive cells are linked by synapses to effector systems.

The three largest animal phyla have evolved different solutions to the problem of obtaining an optical image. The eyes of vertebrates and molluscs contain a single lens, whereas arthropods have a multifaceted compound eye (Fig. 1). These have been considered to be independent evolutionary innovations for a number of reasons. On the one hand, the morphologies of the compound eye of a fly and the single-lens eyes of vertebrates and molluscs are so strikingly different (Fig. 1) that no structural homology, already present in a common ancestor, can be claimed. On the other hand, clear differences between the mode of development of the single-lens eye of vertebrates and that of molluscs imply that these systems have also evolved independently, although the eyes are strikingly similar in their basic design (Fig. 1). The fundamental differences in morphology, development and photoreceptor ultrastructure of the diverse eye types found in the animal kingdom have led to the proposal that eyes evolved independently as many as 40 times [1].

In contrast to the large differences revealed by morphological studies, recent molecular genetic evidence points to a common genetic program for eye morphogenesis in organisms as diverse as humans and flies. Strikingly, these data suggest homologies not only between structural components involved in light absorption and phototransduction [5], but also between regulatory genes of the genetic cascades that ultimately control the development of the diverse eye types. The main body of evidence for such homologies between the genetic cascades comes from mutant analyses of the *Pax-6* genes in mammals and flies, showing that in both cases *Pax-6* plays a fundamental role during eye development [6–8,9••,10••]. Moreover, recent experiments have provided compelling evidence that the fly *Pax-6* gene, *eyeless*, acts as the master control gene of eye development [11••]. In this review, we will focus on these parallels in eye morphogenesis between mammals and flies. We will also discuss *Pax-6* genes from other species and the recent characterization of other conserved genes probably involved in eye development. Finally, we shall discuss how these new findings fit into current models of eye evolution and which new evolutionary perspectives they might open.

Photoreceptive cells: a common ancestor?

The basic units of the eye, the photoreceptor cells, can be divided into two main classes, a ciliary and a rhabdomeric (microvillar) type, depending on whether or not the photoreceptive membrane forms in association with a cilium. Early studies suggested that the ciliary type is common to deuterostomes and the rhabdomeric type to protostomes [12], but many exceptions to this rule have been found (reviewed in [13]). Therefore, none of these photoreceptor cell types can be correlated with major phylogenetic lineages, suggesting that various types of photoreceptor cells have originated several times independently [1]. However, the classic rhabdomeric types of cephalopods and arthropods have been found to form cilia transiently in their early development, suggesting that they shared a common ancestry with ciliary-type photoreceptors [14]. On the basis of additional evidence from species with mixed [14] or both types of photoreceptors [15], it has been argued that a primitive ciliary-type photoreceptor gave rise to the ciliary and rhabdomeric types in the course

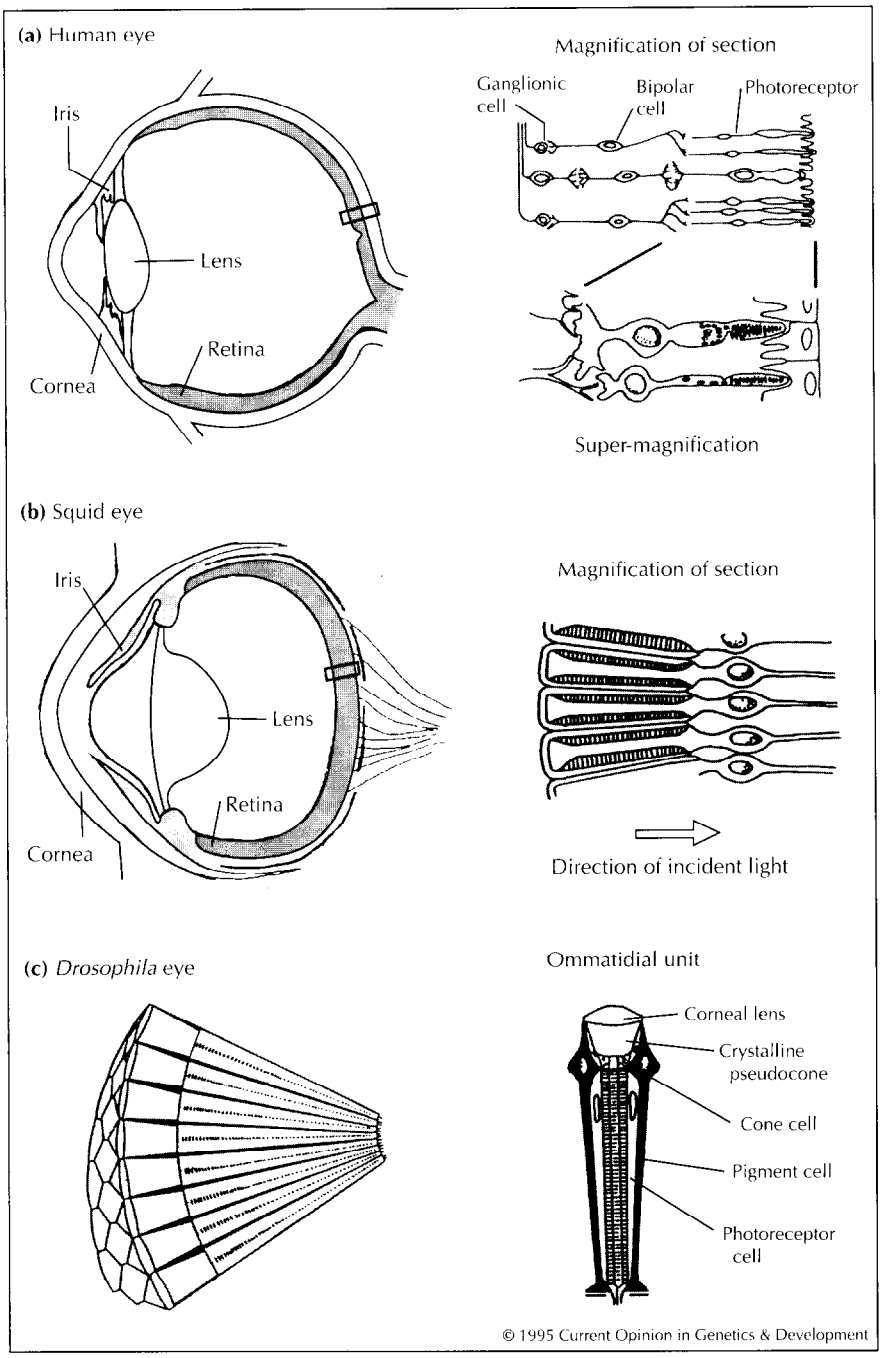

Fig. 1. Diagrammatic cross sections of the human and squid single-lens eyes and of the *Drosophila* compound eye. The **(a)** human and **(b)** squid eyes are of a strikingly similar basic design, both with a retina, a single lens, an iris and a cornea (the squid lens comprises two halves joined together). However, several fundamental differences distinguish these two eye types. Firstly, the human retina is a multilayer of photoreceptors, bipolar, ganglionic and other neuronal cell types (see the enlargement), whereas the squid retina is a monolayer of photoreceptor cells. Secondly, as shown in the super-magnification of (a), the sensory segment of the so-called 'inverted' human photoreceptor cells is directed away from the incident light, so that the latter has to penetrate the cell bodies before reaching the photoreceptive membranes. In contrast, the squid photoreceptors are directed with the sensory portion towards the light and are termed 'everse'. Thirdly, the human retina is formed by evagination from the neural ectoderm, whereas the squid retina develops from an ectodermal invagination. Fourthly, the human lens is an aggregate of transparent lens fiber cells, whereas the squid lens is formed by cellular fusions. **(c)** The morphologically very different *Drosophila* compound eye is a hexagonal array of ~800 facets or ommatidia. Each ommatidium (see enlargement) comprises a corneal lens and a crystalline pseudocone, eight photoreceptor cells and 11 accessory cells, including pigment cells, cone cells and others. (Modified after [44,65–67].)

of evolution. Many authors now suppose that various photoreceptor cell types derived independently from a ciliary precursor (reviewed in [13]).

The hypothesis of a monophyletic origin for photoreceptor cells is supported by the finding that light absorption and phototransduction in various eyes is mediated by a common underlying mechanism. All visual systems analyzed so far, including those of vertebrates, cephalopods and insects, share homologous proteins called opsins which comprise the protein component of their visual pigments. Opsins are members of the seven-transmembrane receptor family and are covalently linked to a vitamin-A-derived chromophore responsible for absorbing photons. In all cases analyzed, this complex triggers a G-protein-mediated signalling cascade when activated by light (reviewed in [16•]). Thus, the presence of opsins in divergent species and the conservation of their use in light absorption points to a common ancestry and indicates a monophyletic origin of photoreceptor cells in evolution.

Pax-6: functional conservation of a regulatory switch?

Unexpectedly, it is not only components used in light detection that are conserved between different

systems. Pax-6, a highly conserved transcription factor, has recently been shown to play a fundamental role in eye development in a number of vertebrates and invertebrates. The *Pax-6* genes are members of the *Pax* family of developmental regulatory genes encoding transcription factors containing two DNA-binding motifs: a paired-domain and a homeodomain (see [17,18,19•,20•] for reviews). *Pax-6* genes were first isolated from human [6], mouse [21,22] and zebrafish [23,24] and have subsequently been cloned from rat [8], quail [25], chicken [26], *Drosophila* [10••] and sea urchin [27]. The vertebrate genes show an unusually high conservation of both overall sequence identity and location of introns. The human and mouse proteins are identical over their entire length of 422 amino acids. The proteins encoded by the mouse and fly genes share >90% sequence identity in both DNA-binding domains. Furthermore, three splice sites affecting the two domains are conserved, indicating that these genes are orthologues [10••].

Pax-6 is required for eye development in mammals and flies

In mammals, heterozygous disruptions of the *Pax-6* gene are responsible for the human congenital disorders aniridia [28–30] and Peter's anomaly [31••] and for the mouse and rat *Small eye* phenotypes [7,8]. Aniridia and Peter's anomaly patients have variable eye malformations. Aniridia is typically characterized by a reduction or complete absence of the iris and is often accompanied by further defects in the cornea, lens, retina and the optic nerve. Peter's anomaly is most frequently associated with malformations of the anterior chamber of the eye. A similar range of eye defects as seen in humans heterozygous for *Pax-6* loss-of-function alleles is observed in mice and rats heterozygous for *Small eye*. One possible human homozygote has been described, a stillborn fetus with severe craniofacial abnormalities lacking eyes completely [32]. Homozygous *Small eye* mouse embryos are born without eyes and nose and die soon after birth [33,34]. In homozygous *Small eye* embryos, the optic vesicles start to grow out, but subsequent development leads to an abnormally shaped optic cup that does not develop further [9••,35]. Also, the lens placodes fail to form, resulting in an absence of lens and cornea. As a consequence, homozygous *Small eye* embryos completely lack all eye structures [8,9••,30,34]. Furthermore, the nasal cavities and the olfactory bulbs fail to develop and additional defects in the brain are observed [35].

Pax-6 is normally expressed in those tissues that are affected in the mutant embryos [6,9••,22]. In the neural ectoderm, *Pax-6* transcripts are detected in the developing eye (Fig. 2), first in the optic sulcus and later in the optic vesicle and differentiating retina. Furthermore, *Pax-6* is expressed in the developing olfactory bulbs and in other specific domains of the brain and spinal cord. In the overlying surface ectoderm, expression is first observed in a broad domain that becomes progressively more restricted to the lens and nasal placodes. Eventually, *Pax-6* is expressed in the developing lens and cornea (Fig. 2), as well as in the nasal epithelium.

Surprisingly, the *Drosophila Pax-6* homologue *eyeless* also plays a fundamental role in eye development and has an expression pattern similar to its vertebrate counterpart [10••]. The *eyeless* gene is first expressed in the ventral nerve cord and in discrete regions of the brain. Later in embryogenesis, it is expressed in the anlagen of the eye as early as they can be detected (Fig. 2). In subsequent larval stages, *eyeless* continues to be expressed in the primordia of the adult compound eyes, the eye imaginal discs [10••] (Fig. 2).

The existing *Drosophila eyeless* mutations arose through the insertion of transposable elements into an eye-specific enhancer [10••]. This results in a strong reduction of *eyeless* expression in the eye anlagen without dramatically affecting expression in the CNS. Flies homozygous for these hypomorphic *eyeless* alleles show a reduction or complete absence of the eyes. In these mutants, the eye imaginal discs form normally in the embryo, but during larval stages, increased cell death reduces the size of the eye discs giving rise to a head with reduced or missing eyes. Apparent null alleles are homozygous lethal, suggesting that *eyeless* has a vital function in the developing CNS in addition to its function in eye development [36].

In summary, the vertebrate and fly *Pax-6* genes are expressed at least transiently in all tissues of the developing eye (Fig. 2). Mutations in *Pax-6* genes lead to a loss of eye structures in both cases and the phenotypes suggest early requirements for *Pax-6* in eye formation.

Pax-6: a master control gene of eye development in flies and vertebrates?

In *Drosophila*, the early expression of *eyeless* in the eye primordia, and the observation that mutations in other genes involved in early eye development (including *sine oculis*, *eyes absent*, *eye gone* and *eyelish*) do not affect the expression of *eyeless*, suggested an early determinative role for this gene [10••,11••]. The potential of *eyeless* to act as a regulatory switch was tested by targeted misexpression of an *eyeless*$^+$ cDNA in imaginal discs that do not normally express *eyeless*, such as the leg, wing and antennal imaginal discs. The ectopic expression of *eyeless* resulted in the formation of supernumerary eyes on legs, wings and antennae, that is at those positions corresponding to ectopic *eyeless* expression [11••]. The finding that *eyeless* can switch on the eye developmental pathway and induce the formation of ectopic eyes in the imaginal discs of the head and thoracic segments indicates that it is a master control gene of eye development, acting high up in the regulatory cascade. The decision of a tissue to form eye structures does not solely depend upon *Pax-6* expression, however, but also on the cellular context, since in vertebrates and *Drosophila*, *Pax-6* is

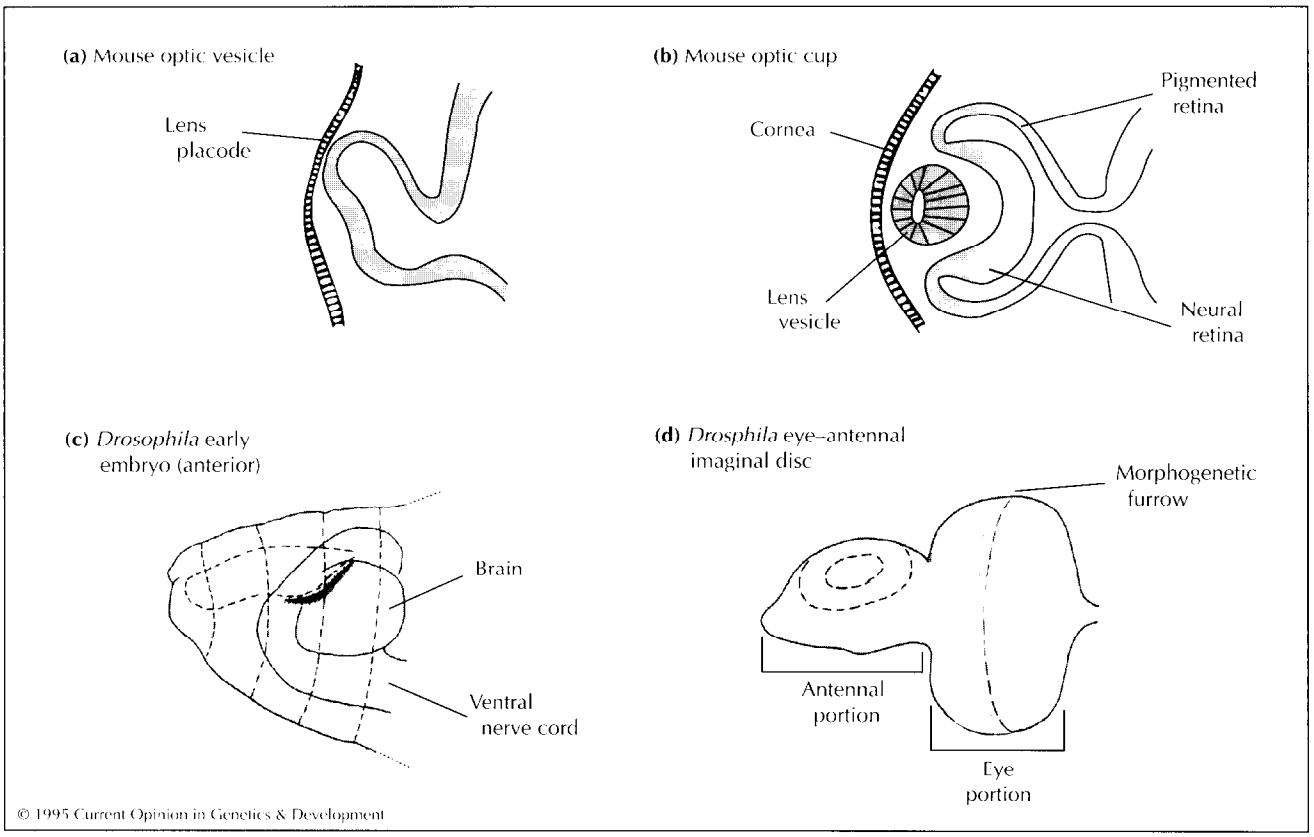

Fig. 2. Pax-6 expression during mouse and *Drosophila* eye development. The mouse eye develops from two principal components, the neural ectoderm of the optic vesicle which forms the retina, and the overlaying surface ectoderm (schematic cells indicated) which forms the lens and cornea. A protrusion from the lateral forebrain evaginates and gives rise to the optic vesicle **(a)**. The optic vesicle then contacts the overlaying ectoderm and induces it to form the lens placode, which in turn causes the optic vesicle to fold back on itself giving rise to the bilayered optic cup **(b)**. The inner layer will differentiate into the multilayered neural retina, whereas the cells of the outer layer will produce the pigmented retina. The lens placode rounds up and detaches from the surface forming the lens vesicle. The lens vesicle, in turn, induces the overlaying ectoderm to form the cornea and eventually differentiates into the lens. *Pax-6* expression is observed in all tissues of the developing eye (shaded regions) [22], i.e. in the optic vesicle and the overlaying surface ectoderm (a). Later strong expression is seen in the developing retina, lens and cornea (b). **(c,d)** The compound eye of *Drosophila*, in contrast, develops from a monolayered epithelium originating from a small domain of dorsolateral ectoderm of the early embryo. A simple epithelial sac is formed by invagination of this region and becomes internalized during the complex morphogenetic movements of the head region. This epithelial sac, the so-called eye–antennal imaginal disc, is the primordium of the adult eye, the antenna and surrounding head cuticle. (c) The anterior end of a late *Drosophila* embryo. The *Drosophila Pax-6* homologue *eyeless* is expressed in a dorsolateral domain corresponding to the eye–antennal imaginal disc anlagen (shaded area) [10••] and in the brain and ventral nerve cord (expression not indicated in the figure). During larval stages, the unpatterned imaginal disc proliferates and *eyeless* transcripts are continuously detected in the eye portion of the eye–antennal disc (d; an eye–antennal imaginal disc of the third and last larval stage is shown). The antennal portion of the disc will give rise to the antenna and head cuticle, whereas the eye portion develops into the adult eye and surrounding cuticle. During morphogenesis, a wave of pattern formation, marked by the morphogenetic furrow, moves across the eye disc in a posterior→anterior direction. In this process, cells are sequentially recruited into ommatidial clusters, which will give rise to individual facets. Ahead of the morphogenetic furrow, cells are undifferentiated and strongly express *eyeless* [10••]; behind the furrow, cells start to differentiate and *eyeless* expression drops. (Modified after [65,68].)

also expressed in the developing nervous system. The misexpression of the mouse *Pax-6* gene in *Drosophila* also leads to the formation of ectopic eyes, similar to the results obtained with the *Drosophila* gene itself [11••]. This argues that the mouse gene can activate the target genes of *eyeless*. Whether the mouse gene does so directly, or indirectly by activating *eyeless*, awaits further analysis. Nevertheless, these findings suggest that besides the sequence of the Pax-6 proteins, their biochemical activity and presumably the sequence of the DNA-binding sites is also conserved.

The place of the vertebrate *Pax-6* genes in the genetic hierarchy of eye development is not yet known. However, the early expression of *Pax-6* in the surface ectoderm and the failure of the lens placodes to form in homozygous *Small eye* embryos are consistent with a role for *Pax-6* in lens determination [9••]. Furthermore, an important function of *Pax-6* in optic vesicle development is suggested by the early expression and the defects observed in mutant embryos [9••].

Apparently, not only are the most downstream genes encoding the basic components of the phototransduction process conserved, but also regulatory genes acting high up in the genetic hierarchies. Thus, the development of the vertebrate and insect eyes seems to be controlled by conserved regulatory genes despite the huge morphological differences between the two.

Pax-6 in other phyla

The apparent conservation of *Pax-6* function in eye development in mammals and flies raises the issue of how widely distributed *Pax-6* and its function in eye development is in the animal kingdom. Recently, a *Pax-6* homologue from squid has been identified and, strikingly, it is also expressed in the developing eye (S Tomarev, WJ Gehring, unpublished data). Therefore, in vertebrates, molluscs and arthropods, the same regulatory gene seems to be essential for the development of morphologically diverged eyes. Furthermore, *Pax-6* genes have recently been cloned from nemertines (F Loosli, WJ Gehring, unpublished data), nematodes (A Chisholm, B Horvitz, personal communication; Y Zhang, S Emmons, personal communication) and ascidians (S Glardon, WJ Gehring, unpublished data) and it will be exciting to find out whether or not these genes play a role in eye development. The presence of *Pax-6* in species from several phyla suggests that this gene and its role in eye development is universal among metazoa.

More genes in common?

Early-acting genes

The genetic parallels at the top (e.g. *Pax-6*) and bottom (e.g. opsins) of the hierarchies active in the determination and differentiation of eye structures in divergent species prompt the question: are other genes acting in these cascades conserved? A first indication for further similarities comes from the isolation of *sine oculis* homologues from mice [37] and *Drosophila* [38•,39•]. In *Drosophila*, the homeobox gene *sine oculis* is required early in eye imaginal discs [38•,39•], but acts downstream of *eyeless* (P Callaerts, G Halder, WJ Gehring, unpublished data). Recently, four mouse homologues of *sine oculis* have been isolated and, remarkably, one of those is also expressed in the developing eye ([37]; G Oliver, P Gruss, personal communication). This expands the genetic parallels beyond a single transcription factor and thereby strengthens the idea that the development of these different eyes could be under the control of similar genetic cascades.

Lenses: homology or curiosity?

An unexpected homology between lens-forming cells in vertebrates and *Drosophila* may be revealed by the *Drosophila prospero* gene [40,41] and its mouse homologue *Prox-1* [42], both encoding putative transcription factors with a homeodomain [41]. The two genes are expressed in similar tissues in both species. The *Drosophila prospero* gene is transcribed in the developing CNS [40,41], in the lens-secreting cone cells of the eye and in the midgut [42]. *Prox-1* is active in the developing lens of the mouse embryo as well as in the CNS and other tissues [42]. The expression in the CNS of both organisms may reflect a homologous function for these genes [42].

Whether the expression in the lens-forming cells also reflects a homologous function is not clear. The lenses in these two species are thought to be polyphyletic in origin because of their different morphology and development [1,2]. The vertebrate lens is formed by an intracellular accumulation of lens crystallins [43], whereas the corneal lens and the pseudocone, the two lens elements of *Drosophila*, are cellular secretions (reviewed in [44]). Interestingly, the lens vesicle cells of vertebrates (reviewed in [45]) and the lens-secreting cone cells of *Drosophila* [46] both require interaction with neuronal cells for their development. Although highly speculative, the expression of *Prox-1* and *prospero* in lens-forming cells may point to a conserved molecular mechanism involved in the different developmental programs for building a lens. The characterization of *prospero* homologues from other species, in particular molluscs, in which the single lens is formed by cellular fusions [47], may yield more essential information to elucidate whether or not this is indeed the case.

The connection to the brain

Another hint at a possible conservation of molecular mechanisms involved in visual system development comes from a recently characterized family of homeobox genes. This family so far comprises the *ceh-10* gene from *Caenorhabditis elegans* [48,49•] and the vertebrate homologues *Chx-10* from mouse [50•] and *Vsx-1* from goldfish [51]. The vertebrate genes are predominantly expressed in the presumptive neural retina of the developing eye. In the mature retina, expression is restricted to cells of the inner nuclear layer, most probably to bipolar cells [50•,51]; these are interneurons that receive input from photoreceptor cells and signal to ganglionic neurons which then connect to the brain.

In *C. elegans*, a small number of neurons, including the so-called AIY interneurons, express *ceh-10* [49•]. These interneurons receive synaptic input from a thermosensory cell. Whether this sensory cell is also photosensitive is not known, but structural analysis has shown that it possesses numerous microvilli, similar to rhabdomeric photoreceptor cells [52]. Furthermore, single photoreceptor cells are located in corresponding positions in other nematodes [53]. Thus, it has been proposed that the nematode *ceh-10* expressing AIY interneurons are homologous to the *Chx-10/Vsx-1* expressing bipolar cells of the vertebrate retina [49•]. Taken together, a conserved molecular mechanism might be involved in the development of interneurons connected to photoreceptor cells in both vertebrates and nematodes. It will be interesting to characterize homologues from other species, such as *Drosophila*, and to see whether these too are expressed in interneurons coupled to photoreceptor cells.

Evolution of the eye: a model

As outlined above, *Pax-6* genes are key players in mammalian as well as fly eye development. This suggests that in addition to the sequence conservation, the function of *Pax-6* in eye development is also conserved.

Therefore, an ancestral *Pax-6* gene might have been involved in the development of a primitive eye in a common ancestor. Alternatively, the use of *Pax-6* in the development of different eye types may be explained by an independent recruitment of these conserved genes into separately evolving eye developmental pathways. However, this hypothesis would have to explain why the *Pax-6* gene in particular was adopted in both cases. The observation that the mollusc *Pax-6* gene is also expressed in the developing eye corroborates the hypothesis of a functional homology. The finding that the mouse *Pax-6* gene can function in *Drosophila* indicates that the mouse and the fly genes have retained a similar biochemical activity in the course of evolution. Taken as a whole, the evidence favours the idea of a functional homology. This implies that in a common ancestor of vertebrates, insects and molluscs, an antecedent of *Pax-6* was involved in the formation of an ancestral eye. Thus, these three very different eye types seem to have a common evolutionary origin.

The development of the hypothetical ancestral eye could have been controlled by a genetic cascade of *Pax-6* and additional regulatory genes. Whether this ancestral organ consisted of a single or multiple photoreceptive cells remains speculative, but it might have been linked to a specialized type of interneuron connecting the photoreceptive cells with effector cells such as neurons of an ancestral CNS. It seems that once a functional photoreceptive organ had evolved, its optical performance could be improved by selection in various ways and this has resulted in the enormous variety of eyes observed today.

In this evolutionary process, new genes and genes not yet functioning in eye development might have been recruited into the already existing eye developmental pathways. Acquisition of regulatory sequences, for example Pax-6 response elements, would result in additional expression of a gene in the eye allowing modifications of the eye. A glimpse at such evolutionary tinkering is offered by the lens crystallin genes (reviewed in [54–56]), encoding the structural proteins of the lens. These proteins are not specialized lens proteins, but derive from various enzymes (e.g. lactate dehydrogenase in some vertebrates) or small heat-shock proteins. During evolution, these proteins were adopted to build the lens besides their other activity, a phenomenon called gene sharing [54]. Recent reports suggest that a number of crystallin genes are direct targets of Pax-6 in the lens [57•,58,59•,60] and that their recruitment to the lens may indeed have occurred through the acquisition of Pax-6 response elements [57•]. The key regulatory genes, on the other hand, were locked into their developmental function, unable to change without disruption of the developmental pathways. Therefore, these genes were conserved and are still used today in the development of the modern eye types, although these eyes diverged almost completely in morphology and development in the course of evolution.

Conclusions

The identification of conserved regulatory and structural genes involved in the ontogeny of the eye in various species has revealed striking parallels between different eyes previously thought to have little in common. The presence of additional conserved genes involved in the morphogenesis of different types of eyes seems likely. Several genes playing a role in either vertebrate (reviewed in [61,62]) or *Drosophila* (reviewed in [63,64]) eye development are known and it will be exciting to see how many of these have homologous counterparts with a similar role. Analysis of their distribution in phylogenetically highly diverged organisms will give more essential insights into the evolutionary relationship of the diverse eye types. It will be most interesting to learn whether animals of lower phyla have homologues of the genes discussed in this review and whether or not these genes are involved in eye development in these organisms.

The discovery of conserved genes playing a role in the development of diverse eyes raises the issue of how the action of homologous regulators is interpreted differently to build the distinct eyes. It will be informative to compare the regulatory cascade required to form a *Drosophila* compound eye with that of a mouse eye, to determine how much is conserved and how many new genes have been recruited into these developmental pathways, obviously leading to the formation of different types of eyes.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Salvini-Plawen Lv, Mayr E: **On the evolution of photoreceptors and eyes.** *Evol Biol* 1977, **10**:207–263.

2. Land MF, Fernald RD: **The evolution of eyes.** *Annu Rev Neurosci* 1992, **15**:1–29.

3. Nilsson D-E: **Vision optics and evolution.** *Bioscience* 1989, **39**:298–305.

4. Nilsson D-E: **From cornea to retinal image in invertebrate eyes.** *Trends Neurosci* 1990, **13**:55–64.

5. Goldsmith TH: **Optimization, constraint, and history in the evolution of eyes.** *Quart Rev Biol* 1990, **65**:281–322.

6. Ton CCT, Hirvonen H, Miwa H, Weil MM, Monaghan P, Jordan T, Van Heyningen V, Hastie ND, Meijers-Heijboer H, Drechsler M *et al.*: **Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region.** *Cell* 1991, **67**:1059–1074.

7. Hill RE, Favor J, Hogan BLM, Ton CCT, Saunders GF, Hanson IM, Prosser J, Jordan T, Hastie ND, Van Heyningen V: **Mouse Small eye results from mutations in a paired-like homeobox-containing gene.** *Nature* 1991, **354**:522–525.

8. Matsuo T, Osumi-Yamashita N, Noji S, Ohuchi H, Koyama E, Myokai F, Matsuo N, Toniguchi 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.

9. •• Grindley JC, Davidson DR, Hill RE: **The role of Pax-6 in eye and nasal development.** *Development* 1995, **121**:1433–1442.
A detailed analysis of the ontogeny of the mouse *Small eye* phenotype in relation to *Pax-6* expression has allowed the authors to suggest a role for *Pax-6* in lens and nasal placode determination. The defects in optic vesicle development are consistent with a requirement for *Pax-6* in the neural ectoderm.

10. •• Quiring R, Walldorf U, Kloter U, Gehring WJ: **Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans.** *Science* 1994, **265**:785–789.
This paper describes the molecular characterization of *D. melanogaster eyeless* and shows that it is homologous to the mammalian *Pax-6* genes. Two spontaneous mutations of *eyeless* are caused by insertions of transposable elements and affect expression, particularly in the eye primordia.

11. •• Halder G, Callaerts P, Gehring WJ: **Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*.** *Science* 1995, **267**:1788–1792.
Targeted misexpression of an *eyeless* cDNA results in the formation of ectopic eyes on legs, wings and antennae, identifying *eyeless* as the master control gene for eye development.

12. Eakin RM: **Lines of evolution of photoreceptors.** In *General Physiology of Cell Specialisation*. Edited by Mazia D, Tyler A. New York: McGraw Hill; 1963:393–425.

13. Willmer P: *Invertebrate Relationships: Patterns in Animal Evolution*. Cambridge: Cambridge University Press; 1994.

14. Eakin RM: **Evolutionary significance of photoreceptors: in retrospect.** *Am Zool* 1979, **19**:647–653.

15. Eakin RM, Brandenberger JL: **Unique eye of probable evolutionary significance.** *Science* 1981, **211**:1189–1190.

16. • Ranganathan R, Malicki DM, Zuker CS: **Signal transduction in *Drosophila* photoreceptors.** *Annu Rev Neurosci* 1995, **18**:283–317.
A comprehensive review covering phototransduction in *Drosophila*. The similarities and differences with vertebrate phototransduction are also highlighted.

17. Noll M: **Evolution and role of *Pax* genes.** *Curr Opin Genet Dev* 1993, **3**:595–605.

18. Chalepakis G, Stoykova A, Wijnholds J, Tremblay P, Gruss P: **Pax: gene regulators in the developing nervous system.** *J Neurobiol* 1993, **24**:1367–1384.

19. • Strachan T, Read AP: **PAX genes.** *Curr Opin Genet Dev* 1994, **4**:427–438.
A valuable review with a compilation of all known mammalian *Pax* gene mutations.

20. • Mansouri A, Stoykova A, Gruss P: **Pax genes in development.** *J Cell Sci* 1994, **18(suppl)**:35–42.
This review provides considerable detail on the expression patterns of mouse *Pax* genes.

21. Walther C, Guenet J-L, Simon D, Deutsch U, Jostes B, Goulding MD, Plachov D, Balling R, Gruss P: **Pax: a murine multigene family of paired box-containing genes.** *Genomics* 1991, **11**:424–434.

22. Walther C, Gruss P: ***Pax-6*, a murine paired box gene, is expressed in the developing CNS.** *Development* 1991, **113**:1435–1449.

23. Krauss S, Johansen T, Korzh V, Moens U, Ericson JU, Fjose A: **Zebrafish pax[zf-a]: a paired box-containing gene expressed in the neural tube.** *EMBO J* 1991, **10**:3609–3619.

24. Püschel AW, Gruss P, Westerfield M: **Sequence and expression pattern of pax-6 are highly conserved between zebrafish and mice.** *Development* 1992, **114**:643–651.

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

26. Li H-S, Yang J-M, Jacobson RD, Pasko D, Sundin O: **Pax-6 is first expressed in a region of ectoderm anterior to the early neural plate: implications for stepwise determination of the lens.** *Dev Biol* 1994, **162**:181–194.

27. Czerny T, Busslinger M: **DNA-binding and transactivation properties of Pax-6: three amino acids in the paired domain are responsible for the different sequence recognition of Pax-6 and BSAP (Pax-5).** *Mol Cell Biol* 1995, **15**:2858–2871.

28. Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N, Van Heyningen V: **The human PAX6 gene is mutated in two patients with aniridia.** *Nature Genet* 1992, **1**:328–332.

29. Glaser T, Walton DS, Maas RL: **Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene.** *Nature Genet* 1992, **2**:232–238.

30. Hanson IM, Seawright A, Hardman K, Hodgson S, Zaletayev D, Fekete G, Van Heyningen V: **PAX6 mutations in aniridia.** *Hum Mol Genet* 1993, **2**:915–920.

31. •• Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ, Punnett HH, Van Heyningen V: **Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peter's anomaly.** *Nature Genet* 1994, **6**:168–173.
This paper provides evidence implicating *Pax-6* more widely in anterior segment malformations including Peter's anomaly. The authors propose that Peter's anomaly and aniridia may be alternative phenotypes resulting from loss-of-function mutations at the *Pax-6* locus.

32. Hodgson S, Saunders K: **A probable case of the homozygous condition of the Aniridia gene.** *J Med Genet* 1980, **6**:478–480.

33. Hogan BLM, Horsburgh G, Cohen J, Hetherington CM, Fisher G, Lyon MF: ***Small eyes (Sey)*: a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse.** *J Embryol Exp Morph* 1986, **97**:95–110.

34. Hogan B, Hirst EMA, Horsburgh G, Hetherington CM: ***Small eye (Sey)*: a mouse model for the genetic analysis of craniofacial abnormalities.** *Development* 1988, **103(suppl)**:115–119.

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

36. Hochman B: **Analysis of chromosome 4 in *Drosophila melanogaster*. II: Ethyl methane sulfonate induced lethals.** *Genetics* 1971, **67**:235–252.

37. Oliver G, Wehr R, Jenkins NA, Copeland NG, Cheyette BNR, Hartenstein V, Zipursky SL, Gruss P: **Homeobox genes and connective tissue patterning.** *Development* 1995, **121**:693–705.

38. • Cheyette BNR, Green PJ, Martin K, Garren H, Hartenstein V, Zipursky SL: **The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system.** *Neuron* 1994, **12**:977–996.
Molecular characterization of the *sine oculis* gene and a detailed analysis of its mutant show that it plays an essential role in controlling the initial events of pattern formation in the eye disc. The *sine oculis* gene is also expressed and required for the development of the rest of the fly visual system.

39. • Serikaku MA, O'Tousa JE: ***sine oculis* is a homeobox gene required for *Drosophila* visual system development.** *Genetics* 1994, **138**:1137–1150.
Describes isolation and characterization of the *sine oculis* gene. Mutant analysis and rescue experiments indicate that the *sine oculis* gene product is involved in the development of the larval and adult visual systems during embryogenesis.

40. Vaessin H, Grell E, Wolff E, Bier E, Jan LY, Jan YN: ***prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*.** *Cell* 1991, **67**:941–953.

41. Chu-Lagraff Q, Wright DM, McNeil LK, Doe CQ: **The *prospero* gene encodes a divergent homeodomain protein that controls neuronal identity in *Drosophila*.** *Development* 1991, **Suppl**:79–85.

42. Oliver G, Sosa-Pineda B, Geisendorf S, Spana EP, Doe CQ, Gruss P: ***Prox1*, a *prospero*-related homeobox gene expressed during mouse development.** *Mech Dev* 1993, **44**:3–16.

43. Piatigorsky J: **Lens differentiation in vertebrates. A review of cellular and molecular features.** *Differentiation* 1981, **19**:134–158.

44. Wolff T, Ready DF: **Pattern formation in *Drosophila* retina.** In *The Development of Drosophila melanogaster.* Edited by Martinez-Arias A, Bate M. New York: Cold Spring Harbor Laboratory Press; 1993:1277–1326.

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

46. Basler K, Christen B, Hafen E: **Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing *Drosophila* eye.** *Cell* 1991, **64**:1069–1981.

47. West JA, Sivak JG, Doughty MJ: **Microscopical evaluation of the crystalline lens of the squid (*Loligo opalescens*) during embryonic development.** *Exp Eye Res* 1995, **60**:19–35.

48. Hawkins NC, McGhee JD: **Homeobox containing genes in the nematode *Caenorhabditis elegans*.** *Nucleic Acids Res* 1990, **18**:6101–6106.

49. • Svendsen PC, McGhee JD: **The *C. elegans* neuronally expressed homeobox gene *ceh-10* is closely related to genes expressed in the vertebrate eye.** *Development* 1995, **121**:1253–1256.

This report describes the *ceh-10* gene and the promoter region of *ceh-10* directing the expression of a *lacZ* reporter gene to a small number of neurons. The authors draw a parallel between the bipolar cells of the vertebrate retina and an interneuron in *C. elegans*.

50. • Liu IS, Chen J-d, Ploder L, Vidgen D, Van der Kooy D, Kalnins VI, McInnes RR: **Developmental expression of a novel murine homeobox gene (*Chx10*): evidence for roles in determination of the neuroretina and inner nuclear layer.** *Neuron* 1994, **13**:377–393.

This paper describes the isolation and details of the expression pattern of *Chx-10* (a homologue of the *ceh-10* gene described in [49•]) in the developing mouse.

51. Levine EM, Hitchcock PF, Glasgow E, Schechter N: **Restricted expression of a new paired-class homeobox gene in normal and regenerating adult goldfish retina.** *J Comp Neurol* 1994, **348**:596–606.

52. Burr AJ: **The photomovement of *Caenorhabditis elegans*, a nematode which lacks ocelli. Proof that the response is to light not radiant heating.** *Photomech Photobiol* 1985, **41**:577–582.

53. Burr AH, Burr C: **The amphid of the nematode *Oncholaimus vesicarius*: ultrastructural evidence for a dual function as chemoreceptor and photoreceptor.** *J Ultrastruct Res* 1975, **51**:1–15.

54. Piatigorsky J, Wistow GJ: **Enzyme-crystallins: gene sharing as an evolutionary strategy.** *Cell* 1989, **57**:197–199.

55. Piatigorsky J, Wistow G: **The recruitment of crystallins: new functions precede gene duplication.** *Science* 1991, **252**:1078–1079.

56. Wistow G: **Lens crystallins: gene recruitment and evolutionary dynamism.** *Trends Biochem Sci* 1993, **18**:301–306.

57. • 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.

Evidence is presented that the guinea pig ζ-crystallin gene is a natural target of Pax-6. In this particular case, gene recruitment may have occurred through the acquisition of an alternative lens-specific promoter.

58. 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.

59. • 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.

The authors show by DNase I footprinting and cotransfection experiments with the δ1-crystallin enhancer/promoter that this enhancer contains functional Pax-6 binding sites. The authors suggest that Pax-6 may have been used extensively throughout evolution to recruit and express crystallin genes in the lens.

60. 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**:654–660.

61. Saha MS, Servetnick M, Grainger RM: **Vertebrate eye development.** *Curr Opin Genet Dev* 1992, **2**:582–588.

62. Kodama R, Eguchi G: **Gene regulation and differentiation in vertebrate ocular tissues.** *Curr Opin Genet Dev* 1994, **4**:703–708.

63. Dickson B, Hafen E: **Genetic dissection of eye development in *Drosophila*.** In *The Development of Drosophila melanogaster*, vol II. Edited by Bate M, Martinez Arias A. New York: Cold Spring Harbor Laboratory Press; 1993:1327–1362.

64. Zipursky SL, Rubin GM: **Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*.** *Annu Rev Neurosci* 1994, **17**:373–397.

65. Gilbert SF: *Developmental Biology*, edn 4. Sunderland, MA: Sinauer Associates Inc; 1994.

66. Wolken JJ: *Photoprocesses, Photoreceptors, and Evolution.* New York: Academic Press; 1975.

67. Remane A, Storch V, Welsch U: *Concise Textbook of Zoology* [in German]. Stuttgart: Gustav Fischer Verlag; 1985.

68. Jürgens G, Hartenstein V: **The terminal regions of the body pattern.** In *The Development of Drosophila melanogaster.* Edited by Bate M, Martinez Arias A. New York: Cold Spring Harbor Laboratory Press; 1993:687–746.

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