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Pax-6, Eyes absent, and *Prox 1* in eye development

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ABSTRACT Eyes in different systematic groups including arthropods, molluscs and vertebrates probably have a common evolutionary origin. As a consequence of this, related genes are used for regulation of the early steps of eye development in different organisms. In this review, I briefly summarize data on three gene families which might be essential for eye development across species: *Pax-6/eyeless, Eya/eyes* absent and *Prox/prospero* with emphasis on our contribution here. Mechanisms of eye formation and the generation of different types of eyes in the course of evolution are discussed.

KEY WORDS: *eye, evolution, Pax-6, Prox 1, Eya 2, prospero, eyeless, eyes absent*

Introduction

Our understanding of development in general and the formation of organs and tissues in particular has changed dramatically during the last twenty years. It is now clear that similar developmental pathways are often involved in the organization of general body plan and the formation of analogous organs in evolutionarily distant organisms. The eye represents an excellent example of an organ whose early development is probably under the control of related regulatory cascades in different metazoan species (see Zuker, 1994; Halder *et al.*, 1995a; Callaerts *et al.*, 1997 for review).

It is well known that eyes of a very diverse type and structure can be found in the animal kingdom (Salvini-Plawen and Mayr, 1977; Land and Fernald, 1992). The morphological differences between the various eyes have been considered as evidence that they did not share a common ancestor and thus are polyphyletic in origin (Salvini-Plawen and Mayr, 1977). An alternative point of view suggested that these divergent eyes share a common evolutionary origin based on the similarity of their photoreceptors (Eakin, 1979). This hypothesis was supported by recent data from several laboratories demonstrating that similar genes may be essential for early eye development in organisms as distant as *Drosophila*, squid and human (Ton *et al.*, 1991; Quiring *et al.*, 1994; Tomarev *et al.*, 1997). Here, I will briefly summarize the available data on the involvement of the three gene families with which our laboratory is currently working, *Pax-6*, *Eya* and *Prox*, in eye development across phyla.

Eye development in vertebrates, *Drosophila* and cephalopods

Vertebrate eye development has been described in great detail and it is known to require a hierarchy of inductive interactions by which the retina, optic stalk and lens progressively become specified (see Saha *et al.*, 1992 for review). By the end of gastrulation, specification of the neural plate results in the localization of the presumptive retinal fields and the formation of the optic sulcus in the neuroepithelium. Enlargement of the sulcus generates the optic pit in the region of the future forebrain and the deepening of this pit leads to the formation of the optic vesicle which is connected to the brain through the developing optic stalk. While a lens-forming bias appears to be created through a large region of head ectoderm before the formation of the optic vesicle, the morphological manifestation of lens induction, the lens placode, is first observed after optic vesicle formation on the surface of the overlying ectodermal epithelium (Fig. 1A). The optic vesicle subsequently invaginates to form the optic cup, which gives rise to the neural retina (inner layer) and pigmented epithelium (outer layer). The lens placode first forms the lens pit and subsequently the lens vesicle which separates from the surface epithelium. After pinching off from the surface epithelium, the lens vesicle contains a single layer of cells having a columnar morphology (Fig. 1B). These cells differentiate into the anterior lens epithelial and posterior lens fiber cells. The developing lens induces overlying ectoderm to differentiate into the cornea.

In cephalopods (squid, octopus), the eye develops from a thickened ectodermal monolayer, which forms an oval mitotic placode on the dorsal surface of the head lobe and becomes multilayered (Meinertzhagen, 1991). The placode curls upwards by the outgrowth of an ectodermal fold and thus internalizes rather than invaginates (Fig. 1D). The retinal primordium is formed during this internalization and the eye vesicle is produced after enclosure by the ectodermal fold. The ectoderm at the point of closure fuses, so that the eye vesicle is sealed off by three layers of the primary eye fold: outer and inner ectodermal layers and a layer of mesoderm which separates ectodermal layers. The outer ectodermal

Abbreviations used in this paper: CNS, central nervous system; d.p.c., days post coitum; HD, homeodomain; MF, morphogenetic furrow; PRD, paired domain.

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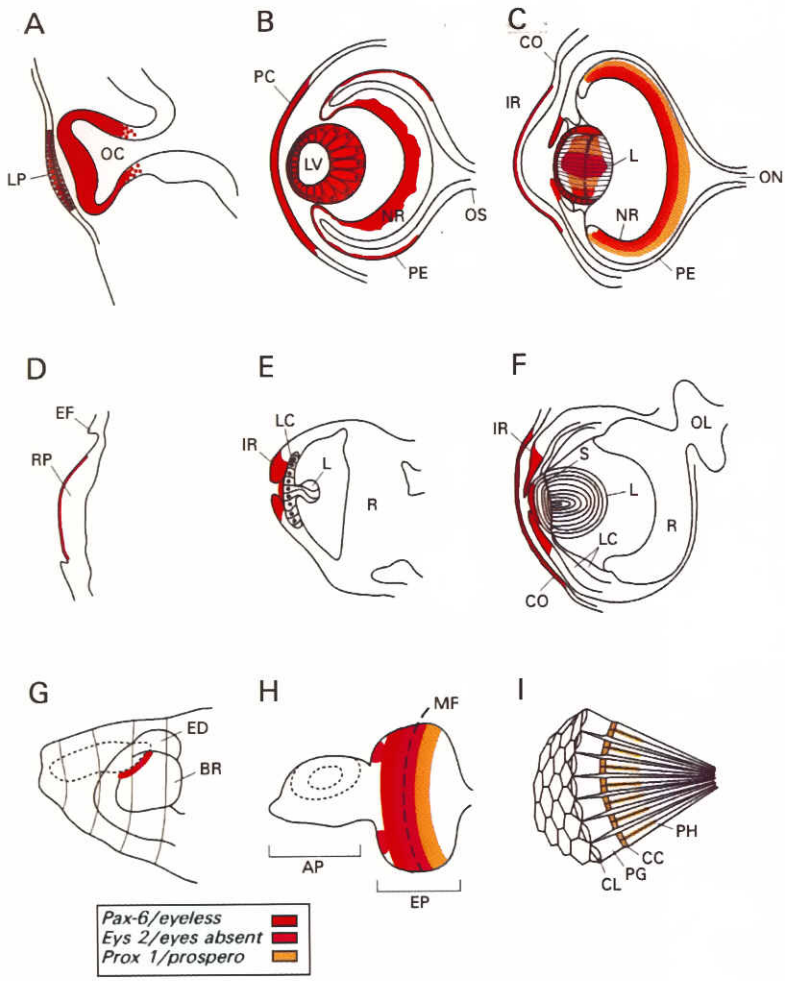

Fig. 1. Generalized schematic diagrams of eye development in vertebrates (A-C), cephalopods (D-F) and *Drosophila* (G-I). (A,D and G) *Show diagrams of eyes at early developmental stages, while* **(C,F and I)** *represent eyes of juvenile organisms. Expression patterns of Pax-6/eyeless, Eya 2/eyes absent and Prox 1/prospero are shown in red, violet and yellow, respectively. Expression patterns of the squid Eya /eyes absent is not known. Since there are some species-specific differences in the expression pattern of the Prox 1 gene in vertebrates, its expression pattern in the chicken is shown. Abbreviations: AP, antennal portion of the eye-antennal imaginal disk; BR, brain; CC, cone cells; CL, corneal lens; CO, cornea; ED, eye disc; EF, ectodermal fold; EP, eye portion of the eye-antennal imaginal disc; IR, iris; L, lens; LC, lentigenic cells; LP, lens placode; LV, lens vesicle; MF, morphogenetic furrow; NR, neural retina; OC, optic cup; OL, optic lobe; ON, optic nerve; OS, optic stalk; PC, primitive cornea; PE, pigmented epithelium; PG, pigmented cells; PH, protoreceptors; R, retina; RP, retina primordium; S, septum. For references, see text.*

layer will produce the iris and outer lens segment; the inner ectodermal layer will produce inner lens segment (Fig. 1E). The lens grows by the addition of processes derived from lentigenic cells and consists of two parts separated by septum (Fig. 1F). The cornea has a very different ectodermal origin from the remainder of the eye and is formed as a new skin fold from the edge of the forward growing arms.

All parts of the *Drosophila* visual system, both the larval and adult eye as well as the optic lobe, originate from a contiguous region in the dorsolateral head ectoderm of the embryo (Green *et al.*, 1993). The adult *Drosophila* compound eye represents a highly regular array of approximately 750 identical unit eyes, called ommatidia (Fig. 1I). Each ommatidia contains 19 cells: eight unique photoreceptors which are sensory neurons that transduce visual information to the brain, and eleven accessory cells (cone cells and pigment cells). The development of the adult eye starts in a region anteriorly flanking the optic lobe primordium on the blastoderm surface which invaginates and forms an eye imaginal disc (see Ready, 1989; Fig. 1G). The disc remains proliferative and unpatterned until late in larval life. Pattern formation and differentiation of the eye disk epithelium proceeds as a wave which moves from posterior to anterior (Fig. 1H). The leading edge of this wave is marked by a depression in the surface of the epithelium which is called morphogenetic furrow (MF). Anterior to the MF, cells are undifferentiated and divide asynchronously. As cells are drawn into the MF, they enter a synchronous cell cycle arrest. Some cells become committed to differentiate and start to express neuronal markers. Additional cells are recruited and form preclusters, composed of future photoreceptors. More posteriorly, cells form the first cluster of differentiating protoreceptors to which cone cells and pigment cells are later added.

Summarizing the mechanisms of eye development in organisms belonging to three major systematic groups, vertebrates, arthropods and molluscs, we can conclude that despite numerous differences in the mechanisms of eye formation and final structure of the adult eyes, some fundamental similarities are observed: (1) eyes have ectodermal origin; (2) eye development is closely connected to brain development; (3) inductive interactions play a critical role in eye development.

Genes essential for eye development

Pax-6/eyeless/toy

Pax-6 in vertebrates and its homologs in different species play a central role in eye development. They belong to a family of transcription factors which in vertebrates consists of at least 9 members. *Pax-6*-related genes encode proteins containing a DNA-binding paired domain (PRD) located in the N-terminal part of the protein molecule and a paired-type homeodomain (HD) located C-terminal to the PRD (Fig. 2). *Pax-6* genes have been isolated from several vertebrate species, squid, flatworm, ribbonworm, ascidian, sea urchin and nematode (see Callaerts *et al.*, 1997 for review). Attempts to isolate a *Pax-6* gene from jellyfish have led to the isolation of a gene encoding a protein with a PRD more similar to Pax-2/Pax-5/Pax-8 proteins than to Pax-6 (Piatigorsky, unpublished). It is interesting to note that the *Pax-2* gene is also involved in vertebrate eye development and mutations in this gene lead to optic nerve coloboma and retinal defects (see Graw, 1996; Macdonald and Wilson, 1996 for review).

The PRD and HD of Pax-6 are extremely conserved between species. The PRD of squid Pax-6 shows 91-95% identity with its vertebrate, *Drosophila* and nemertine homologs, while their HDs are 90-98% identical. Other parts of the protein molecules are less conserved and *Drosophila* and squid proteins do not have significant similarities in the N- and C-terminal regions or in the region between PRD and HD (Tomarev *et al.*, 1997).

The expression pattern of *Pax-6* in both vertebrates and invertebrates demonstrates remarkable similarities. We limit our discussion here to the description of *Pax-6* expression in the developing eye and olfactory systems. Detailed description of its expression in CNS can be found elsewhere (Walther and Gruss, 1991). *Pax-6* expression was first detected in a broad area of head surface ectoderm covering the prosencephalon and in head neural ectoderm (Walther and Gruss, 1991; Grindley *et al.*, 1995). In the chicken, expression in the prospective head ectoderm precedes expression in the neural ectoderm (Li *et al.*, 1994). As development proceeds, expression in the surface ectoderm became restricted to the developing lens and nasal placodes and immediately adjacent tissues (Grindley *et al.*, 1995; Fig. 1A). Ablation experiments in chick indicated that *Pax-6* expression in the head ectoderm does not require contact with the optic vesicle (Li *et al.*, 1994). Experiments with *Small eye* mice and rats, which have mutations in the *Pax-6* gene, demonstrated that *Pax-6* is essential for formation of the lens placode from the surface ectoderm and suggested a role for *Pax-6* in lens determination (Fujiwara *et al.*, 1994; Grindley *et al.*, 1995). Expression of *Pax-6* in the eye persists through development (Fig 1B,C). In the adult lens and cornea, expression is mainly observed in epithelial cells (Davis and Reed, 1996). During chicken retinal development, *Pax-6* is expressed in proliferating cells throughout the neuroepithelium. As differentiation of retinal cell layers proceeds, expression of *Pax-6* is progressively restricted to the ganglion cells layer and amacrine and bipolar cells of the inner nuclear layer (Belecky-Adams *et al.*, 1997; Fig. 1C). A similar pattern of *Pax-6* expression in the retina was observed in other species (Walther and Gruss, 1991; Puschel *et al.*, 1992; Davis and Reed, 1996; Hitchcock *et al.*, 1996). During nasal development, expression of *Pax-6* continues in the placodal epithelium in the course of nasal pit formation and, subsequently, expression is detectable in the developing olfactory epithelium (Walther and Gruss, 1991; Grindley *et al.*, 1995). In olfactory epithelium, Pax-6 protein was detected exclusively in cells of non-neuronal lineage (Davis and Reed, 1996).

In cephalopods (squid), expression of *Pax-6* was first detected in the rudimentary eye primordium (Fig. 1D). During optic vesicle formation, expression of *Pax-6* is increased and clearly seen in the developing eye, optic lobe, nerve ending rich arms and the mantle. At later stages of development (Fig. 1E,F), *Pax-6* is expressed in the brain, the very anterior portion of the anterior lens segment, developing iris and cornea, as well as in the chemosensory olfactory organ. In the olfactory organ, arms and suckers, *Pax-6* was expressed mainly in the outer cell layers which are rich in nerve endings, while in the brain expression was detected mainly in the cerebral ganglion. Surprisingly, no expression of *Pax-6* was detected in the squid retina. In adult eye tissues, a low level of *Pax-6* expression was detected in the cornea, but not in the lens (Tomarev *et al.*, 1997).

In *Drosophila*, the *Pax-6* homolog, *eyeless (ey)*, is expressed in the developing CNS, eye and salivary glands (Quiring *et al.*, 1994;

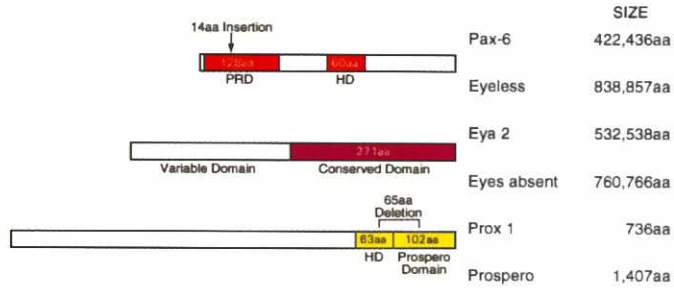

Fig. 2. Schematic representation of Pax-6/eyeless, Eab/eyes absent and Prox/prospero protein structure *(the scale is valid for the vertebrate proteins only). The sizes for vertebrate and Drosophila proteins are shown on the right.*

Callaerts *et al.*, 1997). *ey* is expressed in the eye imaginal discs throughout development (Fig. 1G,H). At the third larval stage, *ey* is expressed in the unpatterned part of the imaginal discs anterior to the morphogenetic furrow and its expression decreases after the furrow (Fig. 1H). Recently, a second *Drosophila* homolog of *Pax-6* was identified and called *twin of eyeless (toy)*. *Toy* is expressed earlier in development as compared with *ey*, but its expression in the embryonic optic anlagen and in the eye imaginal disc is very similar to that of *ey* (see Callaerts *et al.*, 1997).

Analysis of naturally occurring mutations in *Pax-6/ey* demonstrated a critical role of this gene in eye development. *Small eye* homozygous mutations in the *Pax-6* gene lead to a failure of eye, optic nerve, olfactory bulb and nose development. Mutant embryos die after birth because they can not breathe (Hogan *et al.*, 1986; Fujiwara *et al.*, 1994). Heterozygotic mutations in *Pax-6* leads to multiple anomalies, including aniridia and Peter's anomaly in human and small eyes in mouse and rat (see Hanson and van Heyningen, 1995; Callaerts *et al.*, 1997 for review). Recent data with transgenic mice carrying the human *Pax-6* locus demonstrated that not only reduced, but also increased levels of *Pax-6* can lead to severe eye abnormalities and suggests an exceptional sensitivity of cells within the eye to changes in the levels of Pax-6 (Schedl *et al.*, 1996). In *Drosophila*, inhibition of *ey* expression in the eye imaginal disc by transposon insertion in an eye-specific regulatory element leads to the complete absence of the eye (Quiring *et al.*, 1994).

Spectacular experiments on ectopic expression of *Pax-6* from different species in *Drosophila* confirmed a special role of this gene in eye development. In these experiments, *ey* and *Pax-6* genes from mouse and squid were misexpressed in different imaginal discs of *Drosophila* and were able to induce *Drosophila* eye development on antennae, legs and wings (Halder *et al.*, 1995b; Tomarev *et al.*, 1997). All *Drosophila* eye-specific structures including cornea, pigment cells, cone cells and photoreceptors with rhabdomeres were formed, but eyes were not connected to the brain. These experiments demonstrated that *Pax-6* acts at the top or near the top of the cascade of genes essential for eye development. Since *Drosophila ey* and *Pax-6* from mammals or squid possess similarity only in the PRD and HD, these domains are critical for eye development.

The pattern of *Pax-6* expression in developing and mature tissues indicates that the regulation of this gene is complex.

Different transcription factors may be involved in *Pax-6* regulation in different organs and even within the same organ during development. Some details of *Pax-6* gene regulation have begun to appear. There are some indications that Pax-6 may be involved in regulation of its own promoter (Plaza *et al.*, 1993; Grindley *et al.*, 1995). The transcription factor c-Myb was implicated in the regulation of the quail *Pax-6* gene in neuroretina (Plaza *et al.*, 1995b) and a retina-specific enhancer was identified in the quail and mouse genes (Plaza *et al.*, 1995a). It was proposed that in the developing eye and brain *Pax-6* is downregulated by sonic hedgehog (Ekker *et al.*, 1995) while in the developing spinal cord activin-like molecules may inhibit *Pax-6* transcription (Pituello *et al.*, 1995).

Analysis of *ey* expression in different *Drosophila* mutants indicates that this gene acts before several other genes *(eyes absent, sine oculis, eyes gone)*, mutations in which also lead to the absence of the eye (Halder *et al.*, 1995b). It was shown that *toy* is able to induce *ey* in the course of *Drosoplila* eye development. *Ey* in turn activates *sine oculis* which is the next target gene downstream in the cascade (Gehring, 1997). At early stages of vertebrate eye development, *Pax-6* may be involved in the formation of the lens and olfactory placodes, while at later stages, it may be required for the regulation of some tissue-specific genes. For example, some crystallin genes may be activated or inhibited by Pax-6 (for review, see Cvekl and Piatigorsky, 1996). The gene encoding the neural cell adhesion molecule is considered to be a possible target for Pax-6 (Chalepakis *et al.*, 1994) and NCAM is expressed during CNS and eye development (Thiery *et al.*, 1982; Watanabe *et al.*, 1992). *Msx-1*, or genes regulating it, were considered as possible targets for Pax-6 during nasal development (Grindley *et al.*, 1995). To add more complexity to the picture, it should be mentioned that alternatively spliced forms of Pax-6 have different DNA-binding specificities and may interact with different targets even within the same tissue (Epstein *et al.*, 1994).

Eyes absent/Eya

Eyes absent (eya) was first identified in *Drosophila* as a gene essential for differentiation and/or survival of eye progenitor cells (Bonini *et al.*, 1993). Its homologs were later isolated in vertebrates and molluscs (Duncan *et al.*, 1997; Mishima and Tomarev, 1996). In the vertebrates studied (chicken, mouse, human), there are at least three different genes homologous to *Drosophila eya*, which were called *Eya1, Eya2 and Eya3 Drosophila. Eya* and its vertebrate homologs encode proteins which are not similar to other proteins in GenBank. *Drosophila* Eya (Fig. 2) is a nuclear protein which is 760 or 766 amino acid long (due to alternative splicing). Eya2 is 532 and 538 amino acid long in mouse and human, respectively (Duncan *et al.*, 1997). Eya3 mRNA is alternatively spliced and in human encodes several proteins, the largest of which is 573 amino acid long (Tomarev and Zinovieva, unpublished).

Sequence comparison of the eya related-proteins from *Drosophila*, vertebrates and squid indicated that they consist of a conserved C-terminal domain with a length of 271 amino acids and variable N-terminal domain (Fig. 2). C-terminal domains are about 65% identical between *Drosophila* and vertebrates. Outside this area, vertebrate and *Drosophila* proteins show little similarity.

eya is expressed after *ey*, but still very early during *Drosophila* eye development in the unpatterned epithelium before formation of the MF (Bonini *et al.*, 1993; Quiring *et al.*, 1994). The expression of *eya* and eya protein first becomes detectable in cells of the eye portion on the eye-antennal disc during the second larval instar being more abundant in cells in the posterior and edges of the eye portion of the disc, than in the cells in the anterior and central region. As the MF forms during the third larval instar, the highest expression of *eya* occurs just anterior to the MF. *Eya* transcripts are also present posterior to the MF (Fig. 1H). Expression of *eya* is not eye specific. It shows a dynamic pattern of expression in embryos, beginning with the onset of zygotic gene expression the cellular blastoderm, and continuing in regions of the developing head and in segments (Bonini *et al.*, 1993).

During mouse development, expression of *Eya 2* was first detected 8.5 d.p.c. in the region of head ectoderm fated to become an olfactory placode (Duncan *et al.*, 1997). At later stages of development, *Eya 2* is expressed in a variety of neural crest derivatives including dorsal root ganglion. In the eye, expression of *Eya 2* was first detected after formation of the lens vesicle and at day 17.5 d.p.c., the highest level of *Eya 2* expression was observed in primary lens fibers undergoing denucleation (Fig. 1C). *Eya 2* was also expressed in the retina, sclera and cornea. In the 10 day postnatal mouse eye, *Eya 2* was expressed in the lens secondary fibers, retina and cornea. Preliminary data indicate that chicken *Eya 2* is also expressed in the lens and in the retina (Mishima and Tomarev, 1996).

Flies bearing an *eya* mutation in an eye disc-specific enhancer element (eya^1) do not develop adult compound eyes (Bonini *et al.*, 1993; Leiserson *et al.*, 1994). All other external structures appear normal, including adult ocelli, which develop from the edges of the eye imaginal discs. There is also loss of the first optic ganglion (lamina) and reduction in size of the second optic ganglion (medulla) and the lobula in the brain. The most severe mutations of *eya* are lethal or semilethal when homozygous. Lethality is usually embryonic. In addition, some *eya* alleles show reduced or absent ocelli, abnormal morphology of the adult brain, and female sterility (Bonini *et al.*, 1993). These data indicate that the *eya* gene, as well as the *ey* gene, is involved in the development of other tissues besides the eye. Mutations in the human *Eya1* gene lead to Branchio-Oto-Renal syndrome (Abdelhak et al., 1997). The *Eya2* gene was recently mapped (Duncan *et al.*, 1997) to human chromosome 20q13.1 and to the syntenic region of mouse chromosome 2 close to the cataract mutation *Lop4* (West and Fisher, 1986).

In summary, the existing data are consistent with the possible role of vertebrate *Eya* genes in the process of cell differentiation and/or survival. However, it is interesting to note that *Drosophila eya* is expressed very early during eye development, while *Eya2* expression was detected relatively late in eye development after the main inductive and determinative steps have already taken place. However, mouse *Eya1* gene begins to be expressed in lens placodal ectoderm at 9.5 d.p.c. and its expression there overlaps with *Pax-6* expression (Xu et al., 1997). It was proposed that the *Eya* gene family play critical roles downstream of *Pax* genes in specifying lens and nasal placode identity (Xu et al., 1997).

Prospero/Prox

The homeobox gene *prospero* was identified in *Drosophila* as a gene essential for CNS (Doe *et al.*, 1991; Vaessin *et al.*, 1991; Matsuzaki *et al.*, 1992) and eye development (Oliver *et al.*, 1993; Kauffmann *et al.*, 1996; E. Spana and C. Doe, personal communi-

cation). Its homologs were later found in *C. elegans* (Burglin, 1994; Wilson *et al.*, 1994) and vertebrates, where it was called *Prox 1* (Oliver *et al.*, 1993; Tomarev *et al.*, 1996; Zinovieva *et al.*, 1996). The second *prospero*-related gene, *Prox 2*, was recently identified in humans (S.I.T., unpublished). Prospero/Prox form a separate family of proteins since their HD is highly atypical and is divergent from a classical antennapedia or any other HD (Fig. 2). Prospero/Prox also contains a conserved C-terminal domain which was called the prospero domain (Burglin, 1994; Fig. 2). The HD of prospero/Prox are identical between chicken and human but are only 65-67% identical between these vertebrates species, *Drosophila* and *C. elegans* (Tomarev *et al.*, 1996). This is a moderate level of conservation since in general, the *Drosophila* and *C. elegans* HDs are 50-75% identical to their vertebrate homologs. The prospero domain, which is 100-102 amino acids long, is identical between chicken and human and 47-56% identical between vertebrates and *Drosophila* or *C. elegans* (Zinovieva *et al.*, 1996). Upstream of the HD, vertebrate Prox 1 and *Drosophila* prospero shows little similarity (Tomarev *et al.*, 1996). The *Prox 1* gene is at least 40 kb long in human (Zinovieva *et al.*, 1996) and its mRNA is alternatively spliced. Alternative splicing of the *Prox 1* transcript is more pronounced in lens than in other tissues studied (Tomarev *et al.*, 1996).

In *Drosophila* CNS, *prospero* is expressed in a subset of neuroblasts, sensory neuron precursors, ganglion mother cells and identified glial precursors (Doe *et al.*, 1991; Vaessin *et al.*, 1991; Matsuzaki *et al.*, 1992). Prospero was also detected in the R7 photoreceptor and the lens-secreting cone cells of the eye as well as in the midgut (Fig. 1I; Oliver *et al.*, 1993; Kauffmann *et al.*, 1996). In the eye, *prospero* expression is required for proper connectivity of R7 photoreceptor axons to their synaptic targets (Kauffmann *et al.*, 1996). Gain-of-function mutations in the *prospero* gene also led to abnormal eye development in *Drosophila* (E. Spana and C.Q. Doe, personal communication). During *Drosophila* CNS and peripheral nervous system development, prospero protein is synthesized in neuroblasts, but at mitosis, it is asymmetrically localized to the daughter ganglion mother cells and excluded from neuroblasts (Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Spana and Doe, 1995). Activity of the *inscuteable* gene is required for the asymmetric distribution of prospero (Kraut *et al.*, 1996). This asymmetric localization of prospero at mitosis potentially provides a mechanism for rapidly establishing distinct sibling cell fates in the CNS and probably other tissues (Spana and Doe, 1995).

Murine *Prox 1* is expressed in the undifferentiated neurons of the subventricular zone of CNS, the developing eye lens, and the pancreas. In the mouse eye, *Prox 1* mRNA was detected in lens fibers between days 12.5 and 18 of embryonic development but not in the proliferating lens epithelium and retina (Oliver *et al.*, 1993). Chicken *Prox 1* showed a pattern of expression similar to that of the murine *Prox 1* but with some differences. In development, chicken *Prox 1* expression was first detected at stage 14 in the early lens placode and slightly preceded the expression of the first crystallin gene, δ-crystallin (Fig. 1A). At later stages of development (Fig. 1B,C), *Prox 1* mRNA was observed throughout the lens, but it appeared more abundant around the bow region of the equator than in the anterior epithelium or the fibers (Tomarev *et al.*, 1996). In the retina, expression of the *Prox 1* gene was detected first at day 5 of embryonic development and at later stages it is present in the inner nuclear layer, mainly in the horizontal cells with a gradient toward bipolar and amacrine cells (Belecky-Adams *et al.*, 1997; Tomarev *et al.*, 1996). Distribution of Prox 1 protein in the developing chicken eye was similar to that of *Prox 1* mRNA (Belecky-Adams *et al.*, 1997; Tomarev, unpublished). It was also shown that Prox 1 is a nuclear protein which corresponds to its presumed role as a transcription factor (Belecky-Adams *et al.*, 1997).

In human, *Prox 1* was mapped to human chromosome 1q32.2-q32.3 in the region of Usher syndrome type II (Zinovieva *et al.*, 1996). In the mouse, the syntenic region is located on chromosome 1 near the retinal degeneration mutation *rd3*. Further studies are necessary to elucidate any connection between *Prox 1* and these or other diseases.

Recent data indicate that the regulation of *prospero/Prox* expression may be rather complicated. It was demonstrated that in the developing *Drosophila* eye, *prospero* transcription is regulated by *Ras1* through two different mechanisms. In the R7 equivalence group, two Ras1/MAP kinase-responsive EST transcription factors, yan and pnt are involved in activation of *prospero* transcription. High-level *prospero* expression in R7 photoreceptors includes the action of two additional nuclear factors, Phyllopod and sina (Kauffmann *et al.*, 1996). It should be pointed out that in different organs and tissues expressing Prox 1, its targets may be different. Preliminary data indicate that in the lens it may be involved in the regulation of some crystallin genes (Duncan *et al.*, 1996) and the pattern of Prox 1 expression is consistent with this possibility.

In summary, *Prox 1* represents a promising candidate gene which may be involved in eye development and function in different systematic groups. The role of *Prox 2* in eye development is still not clear but deserves further study.

Other genes involved

There are several other identified genes which are expressed in the eyes of *Drosophila* and vertebrates and may be involved in eye development in different phyla. These genes encode proteins with different structural and functional properties. *Sine oculis/Six/Optx* are HD proteins (Cheyette *et al.*, 1994; Serikaku and O'Tousa, 1994; Oliver *et al.*, 1995; Sundin *et al.*, 1996), *hedgehog/Shh* are secreted proteins essential for both long- and short-range cellular signaling (Heberlein and Moses, 1995), *seven-up/COUP* are members of the steroid receptor superfamily (Mlodzik *et al.*, 1990; Fjose *et al.*, 1993), *decapentaplegic/BMP-7* are secreted growth factors (Heberlein *et al.*, 1993; Dudley *et al.*, 1995) and *cubitus interruptus/Gli* are zinc finger proteins (Hui *et al.*, 1994; Dominguez *et al.*, 1996; see also Freund *et al.*, 1996 for other examples). Banfi and coworkers (Banfi *et al.*, 1996) recently conducted a search of identified *Drosophila* genes in the human EST database and they have found 12 human cDNA homologous of the *Drosophila* genes involved in eye development (one of them was *eyes absent*). Although connection between many of these genes and eye development in vertebrates has not been demonstrated, they can be considered as candidate genes. It should be mentioned that the finding of true homologs is often complicated by the fact that corresponding genes form families with individual members showing overlapping patterns of expression and function. The most challenging task will be to arrange these genes in regulatory cascades and determine to what extent these cascades are conserved between phyla.

Evolution of the eye

It seems reasonable to suggest that the rudimentary nervous system evolved before more sophisticated nervous, visual and olfactory systems. The development of the rudimentary nervous system should require a regulatory chain or cascade of genes. It has been proposed that the establishment of regulatory interactions between a homeodomain transcription factor and its corresponding target(s) would constitute a founder event for a type of tissue or organ (Scott, 1994). Other useful genes could come under the influence of the regulator by acquisition of corresponding regulatory elements (enhancers) due to random mutations or transposition. In all organisms studied, the development of visual and olfactory systems goes on in close association with the development of the nervous system. Moreover, the development of visual and olfactory systems has a number of common features. In vertebrates, both are ectodermally derived and depend upon determinative interactions with particular regions of the developing CNS (Grainger, 1992). In *Drosophila*, the olfactory and visual systems derive from the same eye-antennal imaginal disc and their developmental plan requires some common cellular processes (Gaines and Carlson, 1995). It is possible to imagine that some of the genes which are essential for eye development (like *Pax-6*) or even part of the regulatory cascade regionally expressed in the rudimentary nervous system could be used for the formation of visual and olfactory systems. In time, modification of gene expression together with acquisition of new eye- and olfactory-specific targets could lead to partial separation in the developmental and functional pathways of the nervous, visual and olfactory systems.

In light of the critical role of *Pax-6* in eye development in *Drosophila* and vertebrates, it was proposed that their last common ancestor at the junction of protostome-deuterostome divergence must have possessed only simple light-sensitive receptors (Valentine *et al.*, 1996). Most of the major animal groups comprise species with a simple eye spot. More elaborate optical systems capable of producing images arose later in evolution and can be found in only 6 of the more than 30 metazoan phyla; these, however, contribute about 96% of the known species (Land and Fernald, 1992). Recently, a computer-assisted model has suggested that taking a patch of pigmented light-sensitive epithelium as the starting point, camera-type eyes with refractive lens could evolve relatively quickly (Nilsson and Pelger, 1994). The diversity of eye designs and mechanisms of their formation in the course of development, as well as the different composition of their lens proteins (Tomarev and Piatigorsky, 1996) indicates that genes involved in the terminal differentiation of the eye could be different in different systematic groups. Therefore, comparative developmental biology of the eye gives an opportunity to study both universal and taxon-specific mechanisms responsible for tissue and organ formation in the course of evolution.

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