How to make an eye

Jessica E. Treisman

Skirball Institute of Biomolecular Medicine and Department of Cell Biology, NYU School of Medicine, 540 First Avenue, New York, NY 10016, USA

e-mail: treisman@saturn.med.nyu.edu

Development 131, 3823-3827
Published by The Company of Biologists 2004
doi:10.1242/dev.01319

Summary

The eye is an organ of such remarkable complexity and apparently flawless design that it presents a challenge to both evolutionary biologists trying to explain its phylogenetic origins, and developmental biologists hoping to understand its formation during ontogeny. Since the discovery that the transcription factor Pax6 plays a crucial role in specifying the eye throughout the animal kingdom, both groups of biologists have been converging on the conserved mechanisms behind eye formation. Their latest meeting was at the Instituto Juan March in Madrid, at a workshop organized by Walter Gehring (Biozentrum, Basel, Switzerland) and Emili Saló (Universitat de Barcelona, Spain), entitled 'The genetic control of eye development and its evolutionary implications'. The exchange of ideas provided some new insights into the construction and history of the eye.

Origin of the eye

Darwin recognized that 'organs of extreme perfection', such as the eye, presented difficulties for his theory of evolution by natural selection. The problem becomes even more daunting when one considers that the differences in eye structure between different branches of the evolutionary tree imply that complex eyes must have evolved independently at least 40 times (Salvini-Plawen and Mayr, 1977). However, despite their morphological diversity, the eyes of different organisms share many similarities, not only in function but also at the molecular level. The most striking one is the presence in almost all eye structures of the transcription factor Pax6 (Gehring and Ikeo, 1999). In *Drosophila*, as well as in vertebrates, Pax6 is both essential for eye differentiation, and sufficient to induce eye development in certain regions of the body (Chow et al., 1999; Halder et al., 1995; Hill et al., 1991; Quiring et al., 1994). This functional conservation of a specific transcription factor implies a common evolutionary origin for all eyes. How can these observations be reconciled?

A key to resolving this dispute is the definition of an eye. Walter Gehring (Biozentrum, Basel, Switzerland), who first described the central role of Pax6 in eye formation (Halder et al., 1995; Quiring et al., 1994), defines the prototypical eye, which was presumably the common ancestor of all eyes, as the combination of a photoreceptor cell and a pigment cell. This structure achieves some directional selectivity by using screening pigment to block light coming from certain directions. Based on his studies of diverse animal eyes, Michael Land (University of Sussex, Brighton, UK) prefers to define an eye as an organ that can produce an image by comparing the light intensities coming from different directions. To accomplish this, it must contain photoreceptors with more than one spatial orientation. Structures meeting this requirement range from simple pinhole eyes, like that of *Nautilus*, to the compound eyes of insects and molluscs and the complex camera eyes of cephalopods and humans, and may use either lenses or mirrors to focus light onto the photoreceptors (Land and Nilsson, 2002) (Fig. 1).

A further complication, discussed by both Land and Joachim Wittbrodt (EMBL, Heidelberg, Germany), is the existence of two types of photoreceptor cells, ciliary and rhabdomeric, in both vertebrates and invertebrates. Rhabdomeric photoreceptors fold the apical plasma membrane into microvilli to form their light-receiving surface, whereas ciliary photoreceptors fold the membrane of a modified cilium. These two photoreceptor types use divergent opsin molecules and different second messenger systems: cGMP in ciliary photoreceptors and phospholipase C in rhabdomeric photoreceptors. In vertebrates, ciliary photoreceptors are used for vision, but it was recently recognized that retinal ganglion cells express a rhabdomeric opsin, which they use for circadian clock entrainment (Berson et al., 2002). Many primitive organisms possess both photoreceptor types, sometimes within a single eye, as in the scallop *Pecten*. Both types must therefore have been present in Urbilateria, the common ancestors of all bilaterian organisms (Arendt and Wittbrodt, 2001).

The arguments for the independent evolution of complex eye structures are compelling, and include the use of spherical lenses in both vertebrates and cephalopods despite the inverted organization of their retinas, and the presence of compound eyes of differing organization in annelids, bivalve molluscs and arthropods, as described by Land and Nilsson (Land and Nilsson, 2002). However, if we accept that the prototypical eye structure is the photoreceptor/pigment cell combination, the conservation of Pax6 and rhodopsin is suggestive of a monophyletic origin (Gehring and Ikeo, 1999). The ciliary/rhabdomeric photoreceptor split could have either preceded or followed the photoreceptor/pigment cell stage; in many species, ciliary photoreceptors are not associated with pigment cells and may have a circadian rather than a visual function (Arendt, 2003; Arendt and Wittbrodt, 2001). Additional genes could have been intercalated into the eye development pathway, initially by simply providing them with transcriptional regulatory elements that could be controlled by Pax6. Different intercalations in each lineage would have allowed the evolution of diverse eye structures with a variety of refractive or reflective surfaces.

An even more primitive structure is the eye organelle or eyespot, an assembly within a single cell that contains both rhodopsin and screening pigment, and sometimes even lens material. These subcellular organelles probably first evolved in cyanobacteria (Gartner and Losi, 2003), and have been maintained either within or associated with chloroplasts (the endosymbiotic descendants of these bacteria), in green algae such as *Chlamydomonas* and *Volvox* (Ebnet et al., 1999; Dieckmann, 2003; Dyall et al., 2004). Eye organelles containing rhodopsin are also present in dinoflagellates (Greuet, 1965; Francis, 1967; Okamoto and Hastings, 2003; Ruiz-Gonzalez and Marin, 2004), single-celled eukaryotes that have now lost the chloroplasts in which these eyespots

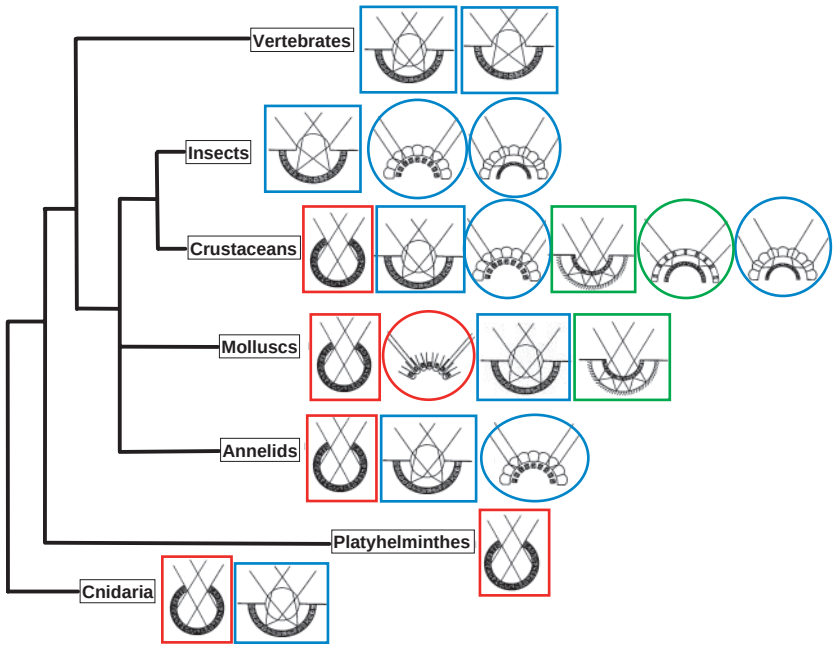

Fig. 1. Each branch of the evolutionary tree includes multiple eye types, and their distribution suggests that each type must have evolved several times independently. Single-chambered eyes are outlined in rectangles and compound eyes in ovals. The color of the outline is red for eyes that form images using only shadow, blue for eyes that use refracting devices such as lenses or corneas, and green for eyes that use mirrors. Adapted, with permission, from Land and Nilsson (Land and Nilsson, 2002).

presumably originated. Gehring made the intriguing suggestion that dinoflagellates might themselves have been engulfed by larger creatures, such as Cnidarians, and may thus be the source of the opsins and eye pigments of higher organisms.

If this scenario holds, at what point was Pax6 added to the mix? The answer, as discussed by Zbynek Kozmik (Institute of Molecular Genetics, Prague, Czech Republic), may lie in the jellyfish *Tripedalia*. This organism has a *PaxB* gene that appears to be a hybrid between *Pax6* and *Pax2/5/8*, and that can both rescue a *Pax2* mutant and induce ectopic eyes when transferred into *Drosophila* (Kozmik et al., 2003). The *Tripedalia* eye doubles as a balance organ, suggesting that duplication of the *PaxB* gene in Bilateria may have resulted in *Pax6* becoming specialized to regulate eye development, while *Pax2/5/8* took control of the ear. Interestingly, although *eyes absent* (*eya*) is downstream of *Pax6* in the eye development pathway in *Drosophila* (Bonini et al., 1993; Halder et al., 1998), mouse *Eya1* is not required in the eye but is crucial for ear development, where it probably acts downstream of *Pax2* (Xu et al., 1999). The link between eye and ear development was further confirmed by Francis Munier (Hôpital Opthalmique Jules Gonin, Lausanne, Switzerland), who described a new recessive human syndrome in which microphthalmia (small eyes) and other eye abnormalities are combined with consistent defects of external ear morphology.

Is Pax6 the master regulator?

There are some challenges to the primacy of Pax6 in eye development. For instance, planarians are able to regenerate their eyes even when *Pax6* is knocked down by RNA interference (Pineda et al., 2002). However, Emili Saló (Universitat de Barcelona, Spain) reported that *Pax6* is expressed in both the photoreceptors and the pigment cells of planarians, and is likely to be functional there, as a GFP reporter driven by three binding sites for the Pax6 homeodomain is specifically activated in the eye in these animals, as well as many others (Berghammer et al., 1999; Gonzalez-Estevez et al., 2003; Sheng et al., 1997). Regeneration may involve mechanisms distinct from those used in normal development; Panagiotis Tsonis (University of Dayton, OH, USA) showed that the secreted protein Sonic hedgehog plays a crucial role in lens regeneration in the newt, although it is never expressed in the lens during development (Tsonis et al., 2004).

Another difficulty is the relatively late phenotype of mouse *Pax6* mutants, in which the optic vesicle evaginates normally but fails to differentiate further (Grindley et al., 1995). Milan Jamrich (Baylor College of Medicine, Houston, TX, USA) described another transcription factor, Rx (Rax – Mouse Genome Informatics), with an earlier role than Pax6. *Rx* is expressed in the very early eye field, where its expression is independent of Pax6 (Zhang et al., 2000). In its absence, the optic vesicle fails to form (Mathers et al., 1997) and *Pax6* is not upregulated in the optic primordium (Zhang et al., 2000). However, Rx acts only in the retinal part of the eye and not in the lens, and its misexpression enlarges the retina but does not produce complete ectopic eyes (Mathers et al., 1997). In addition, it is not required in eyes that use the rhabdomeric type of photoreceptors. In *Drosophila*, Uwe Walldorf (Universität des Saarlandes, Homburg/Saar, Germany) reported that *Rx* is required for the development of the clypeus, a structure that pumps food into the digestive system, and of central brain regions, but that its absence has no effect on the eye (Davis et al., 2003). Using medaka and zebrafish models (Loosli et al., 2003; Loosli et al., 2001), Joachim Wittbrodt (EMBL, Heidelberg, Germany) has traced the role of Rx in optic vesicle evagination to its ability to block the epithelialization of neural tube cells. This produces a destabilized region of the neural tube that can be pushed outwards by the forces of convergent extension. It seems likely that the original function of Rx was to specify a region of the anterior neural ectoderm from which eyes later developed in some lineages (Fig. 2). Wittbrodt suggests that ciliary photoreceptors originated in *Rx*-

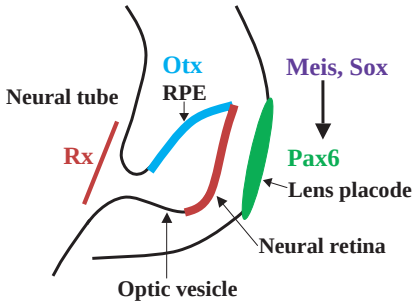

Fig. 2. Transcription factors controlling eye development, shown in a mouse embryo at the optic vesicle stage. Rx (red) is expressed in an anterior region of the neural tube and is necessary for optic vesicle evagination, proliferation of cells in the optic vesicle and retinal differentiation. Pax6 (green) is important in both the optic vesicle and the lens placode, where its expression might be regulated by Meis and Sox transcription factors. Otx proteins (blue) promote the development of the retinal pigment epithelium (RPE).

expressing regions of the brain, and that Rx-induced morphogenetic movements brought them into the periphery in vertebrates.

If not Rx, what is upstream of *Pax6*? Richard Maas (Brigham and Women's Hospital, Boston, MA, USA) is investigating this question by searching for factors that bind to *Pax6* enhancer regions. There is good evidence that Meis1 and Meis2 are activators of an enhancer that drives *Pax6* expression in the lens and cornea (Zhang et al., 2002). This is intriguing in light of the work that Fernando Casares (Universidad Pablo de Olavide, Sevilla, Spain) described, showing that expression of the fly Meis homolog Homothorax (Hth) precedes that of Pax6, and that Pax6 can only induce ectopic eyes in regions expressing Hth (Bessa et al., 2002). Maas reported that the *Pax6* lens enhancer also contains binding sites for Sox proteins, and that these sites are required for its normal expression in vivo. As mice lacking *Sox2* die before lens induction (Avilion et al., 2003), the possibility that Sox2 may activate *Pax6* expression in the lens has not yet been tested. However, a crucial role for *Sox2* in eye specification is supported by human studies described by Veronica van Heyningen (MRC Human Genetics Unit, Edinburgh, UK). Haploinsufficiency for *Sox2* is frequently associated with anophthalmia (absence of one or both eyes) or microphthalmia, a more severe phenotype than the aniridia (absence of the iris) caused by the loss of one copy of *Pax6* (Fantes et al., 2003; Hanson et al., 1993). Other regulators of *Pax6* may remain to be identified, as multiple genes can cause microphthalmia or anophthalmia, and the penetrance of this phenotype can be increased by environmental stress in humans or by blocking the function of the chaperone protein Hsp90 in zebrafish. Negative regulators of *Pax6* or other factors also play a role in restricting eye formation to the appropriate region. Work from the Casares (Universidad Pablo de Olavide, Sevilla, Spain) and Treisman (NYU School of Medicine, NY, USA) laboratories has implicated both Hth (Pichaud and Casares, 2000) and the transcriptional cofactor Chip (Milan and Cohen, 1999; van Meyel et al., 1999), which probably acts in conjunction with LIM domain proteins, in preventing eye development in the ventral head region of *Drosophila*.

Constructing the eye from its building blocks

In order to produce an eye, the specification genes discussed above must assemble all the necessary components. At the most basic level, the correct number of cells must be generated in the appropriate region of the body. Matthew Freeman (MRC Laboratory of Molecular Biology, Cambridge, UK) described the control of cell proliferation during the second mitotic wave in the *Drosophila* eye disc. Both phases of the cell cycle require specific signals: Notch promotes the G1-S transition by increasing the transcription of E2F-responsive genes and the expression of *cyclin A*, and the Epidermal growth factor receptor (EGFR) pathway promotes mitosis by inducing the transcription of *string* (Baonza et al., 2002). Activation of both pathways by signals from the developing ommatidia allows the number of cells to be adjusted to match the requirement.

In the *Drosophila* eye, photoreceptors, cone cells and pigment cells are all induced by the EGFR pathway (Freeman, 1996), but in vertebrates, the corresponding cell types arise from different tissues that are separately regulated. Several talks addressed the question of lens differentiation. Richard Lang (Children's Hospital Research Foundation, Cincinnati, OH, USA) presented evidence that Wnt signaling may negatively regulate lens formation. A conditional knockout of β-catenin in regions expressing a surface ectoderm/lens enhancer from the *Pax6* gene (Ashery-Padan et al., 2000; Williams et al., 1998) leads to the appearance of ectopic lentoid bodies anterior to the eye, whereas expressing activated β-catenin with the same enhancer blocks lens invagination. These results invite comparison to the ectopic photoreceptor differentiation induced by loss of Wingless (Wg) signaling in the anterior eye disc of *Drosophila* (Ma and Moses, 1995; Treisman and Rubin, 1995). Markus Friedrich (Wayne State University, Detroit, MI, USA) showed that Wg expression in this domain is also conserved in the grasshopper (Friedrich and Benzer, 2000). Although it seems unlikely that this was a feature of the ancestral Urbilaterian eye, it is possible that eyes have frequently formed just posterior to a Wnt-expressing region that has come to set their anterior limit.

Within the lens, crystallin expression must be activated to very high levels. Ales Cvekl (Albert Einstein College of Medicine, Bronx, NY, USA) has found that each crystallin gene is activated by a different combination of transcription factors, including Maf, Sox, Six and Retinoic acid receptor proteins, as well as two splice variants of Pax6 (Chauhan et al., 2004). These regulatory pathways may be rapidly evolving. Joram Piatigorsky (National Institutes of Health, Bethesda, MD, USA) has shown that the αB-crystallin promoter of the blind mole rat drives expression in muscle, rather than lens (Hough et al., 2002). A potential Pax3-binding site in the promoter may be responsible for this, as the introduction of a comparable site into the mouse αB-crystallin promoter decreases its activity in the lens and enhances it in muscle. The disparate nature of crystallin proteins themselves raises questions about their evolutionary origins. Many crystallins are heat-shock proteins or enzymes, which may have acquired their refractive function simply by becoming expressed at high levels in lens fiber cells. Zbynek Kozmik (Institute of Molecular Genetics, Prague, Czech Republic) raised the possibility that jellyfish may have acquired their crystallin genes by horizontal gene transfer, as they are highly homologous to fish genes but are not present in other animals.

Piatigorsky reported that enzymes or other ubiquitous proteins are also abundantly expressed in corneal cells in a species-specific manner, suggesting that they have a structural or optical function there (Piatigorsky, 2001). An interesting example is gelsolin, an actin filament-severing protein, which constitutes 50% of the water-soluble protein in the zebrafish corneal epithelium (Xu et al., 2000).

The problem of photoreceptor differentiation was represented at the meeting by Claude Desplan (New York University, NY, USA). Research in his laboratory concerns the mechanisms by which *Drosophila* acquire color vision (Cook and Desplan, 2001). For example, the inner photoreceptors R7 and R8 express different rhodopsins because of the presence of the transcription factors Prospero in R7 (Cook et al., 2003) and Senseless in R8. The exception to this rule is the dorsal rim area of the fly eye, which is specialized to receive polarized light. The multifunctional transcription factor Hth acts in this region to produce R7 and R8 cells that express the same rhodopsin, and that extend their rhabdomeres one below the other at right angles to form a polarizing filter (Wernet et al., 2003). In the remainder of the eye, R7 cells are separated into two subsets that express different rhodopsins by the apparently random activation of the bHLH-PAS transcription factor Spineless.

Finally, Paola Bovolenta (Instituto Cajal, Madrid, Spain) focused on the specification of the retinal pigment epithelium (RPE). She showed that the transcription factors Otx1 and Otx2 both contribute to differentiating the RPE from the neural retina (Martinez-Morales et al., 2001). Otx proteins can act synergistically with Microphthalmia-associated transcription factor (Mitf) to activate melanosome-specific genes such as *tyrosinase* (Martinez-Morales et al., 2003). As rhodopsin genes are also regulated by Otx proteins (Chen et al., 1997; Tahayato et al., 2003), the use of Otx in the eye may date from the first cells that expressed both opsin and pigment genes to produce an eye organelle. The complex developmental mechanisms that have appeared since that time should inspire respect for what evolution can achieve by, as Gehring put it, simply tinkering with existing components.

I thank Claude Desplan and Richard Lang for helpful comments on the manuscript.

References

Arendt, **D.** (2003). Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563-571.

Arendt, D. and Wittbrodt, J. (2001). Reconstructing the eyes of Urbilateria. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 1545-1563.

Ashery-Padan, R., Marquardt, T., Zhou, X. and Gruss, P. (2000). Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. *Genes Dev.* **14**, 2701-2711.

Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. and Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17**, 126-140.

Baonza, A., Murawsky, C. M., Travers, A. A. and Freeman, M. (2002). Pointed and Tramtrack69 establish an EGFR-dependent transcriptional switch to regulate mitosis. *Nat. Cell Biol.* **4**, 976-980.

Berghammer, A. J., Klingler, M. and Wimmer, E. A. (1999). A universal marker for transgenic insects. *Nature* **402**, 370-371.

Berson, D. M., Dunn, F. A. and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* **295**, 1070-1073.

Bessa, J., Gebelein, B., Pichaud, F., Casares, F. and Mann, R. S. (2002). Combinatorial control of *Drosophila* eye development by *eyeless*, *homothorax*, and *teashirt*. *Genes Dev.* **16**, 2415-2427.

Bonini, N. M., Leiserson, W. M. and Benzer, S. (1993). The *eyes absent* gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. *Cell* **72**, 379-395.

Chauhan, B. K., Yang, Y., Cveklova, K. and Cvekl, A. (2004). Functional interactions between alternatively spliced forms of Pax6 in crystallin gene regulation and in haploinsufficiency. *Nucl. Acids Res.* **32**, 1696-1709.

Chen, S., Wang, Q. L., Nie, Z., Sun, H., Lennon, G., Copeland, N. G., Gilbert, D. J., Jenkins, N. A. and Zack, D. J. (1997). Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* **19**, 1017-1030.

Chow, R. L., Altmann, C. R., Lang, R. A. and Hemmati-Brivanlou, A. (1999). Pax6 induces ectopic eyes in a vertebrate. *Development* **126**, 4213-4222.

Cook, T. and Desplan, C. (2001). Photoreceptor subtype specification: from flies to humans. *Semin. Cell Dev. Biol.* **12**, 509-518.

Cook, T., Pichaud, F., Sonneville, R., Papatsenko, D. and Desplan, C. (2003). Distinction between color photoreceptor cell fates is controlled by Prospero in *Drosophila*. *Dev. Cell* **4**, 853-864.

Davis, R. J., Tavsanli, B. C., Dittrich, C., Walldorf, U. and Mardon, G. (2003). *Drosophila retinal homeobox* (*drx*) is not required for establishment of the visual system, but is required for brain and clypeus development. *Dev. Biol.* **259**, 272-287.

Dieckmann, C. L. (2003). Eyespot placement and assembly in the green alga Chlamydomonas. *BioEssays* **25**, 410-416.

Dyall, S. D., Brown, M. T. and Johnson, P. J. (2004). Ancient invasions: from endosymbionts to organelles. *Science* **304**, 253-257.

Ebnet, E., Fischer, M., Deininger, W. and Hegemann, P. (1999). Volvoxrhodopsin, a light-regulated sensory photoreceptor of the spheroidal green alga *Volvox carteri*. *Plant Cell* **11**, 1473-1484.

Fantes, J., Ragge, N. K., Lynch, S. A., McGill, N. I., Collin, J. R., Howard-Peebles, P. N., Hayward, C., Vivian, A. J., Williamson, K., van Heyningen, V. et al. (2003). Mutations in SOX2 cause anophthalmia. *Nat. Genet.* **33**, 461-463.

Francis, D. (1967). On the eyespot of the dinoflagellate, Nematodinium. *J. Exp. Biol.* **47**, 495-501.

Freeman, M. (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. *Cell* **87**, 651-660.

Friedrich, M. and Benzer, S. (2000). Divergent *decapentaplegic* expression patterns in compound eye development and the evolution of insect metamorphosis. *J. Exp. Zool.* **288**, 39-55.

Gartner, W. and Losi, A. (2003). Crossing the borders: archaeal rhodopsins go bacterial. *Trends Microbiol.* **11**, 405-407.

Gehring, W. J. and Ikeo, K. (1999). Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet.* **15**, 371-377.

Gonzalez-Estevez, C., Momose, T., Gehring, W. J. and Salo, E. (2003). Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker. *Proc. Natl. Acad. Sci. USA* **100**, 14046-14051.

Greuet, C. (1965). Structure fine de l'ocelle d'Erythropsis pavillardi Hertwig, pteridinien Warnowiidae Lindemann. *C. R. Acad. Sci. (Paris)* **261**, 1904-1907.

Grindley, J. C., Davidson, D. R. and Hill, R. E. (1995). The role of *Pax-6* in eye and nasal development. *Development* **121**, 1433-1442.

Halder, G., Callaerts, P. and Gehring, W. J. (1995). Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila. Science* **267**, 1788-1792.

Halder, G., Callaerts, P., Flister, S., Walldorf, U., Kloter, U. and Gehring, W. J. (1998). Eyeless initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. *Development* **125**, 2181-2191.

Hanson, I. M., Seawright, A., Hardman, K., Hodgson, S., Zaletayev, D., Fekete, G. and van Heyningen, V. (1993). PAX6 mutations in aniridia. *Hum. Mol. Genet.* **2**, 915-920.

Hill, R. E., Favor, J., Hogan, B. L., Ton, C. C., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D. and van Heyningen, V. (1991). Mouse *small eye* results from mutations in a paired-like homeobox-containing gene. *Nature* **354**, 522-525.

Hough, R. B., Avivi, A., Davis, J., Joel, A., Nevo, E. and Piatigorsky, J. (2002). Adaptive evolution of small heat shock protein/alpha B-crystallin promoter activity of the blind subterranean mole rat, *Spalax ehrenbergi*. *Proc. Natl. Acad. Sci. USA* **99**, 8145-8150.

Kozmik, Z., Daube, M., Frei, E., Norman, B., Kos, L., Dishaw, L. J., Noll, M. and Piatigorsky, J. (2003). Role of Pax genes in eye evolution: a cnidarian PaxB gene uniting Pax2 and Pax6 functions. *Dev. Cell* **5**, 773-785.

Land, M. F. and Nilsson, D.-E. (2002). *Animal eyes*. Oxford, UK: Oxford University Press.

Loosli, F., Winkler, S., Burgtorf, C., Wurmbach, E., Ansorge, W., Henrich, T., Grabher, C., Arendt, D., Carl, M., Krone, A. et al. (2001). Medaka *eyeless* is the key factor linking retinal determination and eye growth. *Development* **128**, 4035-4044.

Loosli, F., Staub, W., Finger-Baier, K. C., Ober, E. A., Verkade, H., Wittbrodt, J. and Baier, H. (2003). Loss of eyes in zebrafish caused by mutation of *chokh/rx3*. *EMBO Rep.* **4**, 894-899.

Ma, C. and Moses, K. (1995). *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. *Development* **121**, 2279-2289.

Martinez-Morales, J. R., Signore, M., Acampora, D., Simeone, A. and Bovolenta, P. (2001). Otx genes are required for tissue specification in the developing eye. *Development* **128**, 2019-2030.

Martinez-Morales, J. R., Dolez, V., Rodrigo, I., Zaccarini, R., Leconte, L., Bovolenta, P. and Saule, S. (2003). OTX2 activates the molecular network underlying retina pigment epithelium differentiation. *J. Biol. Chem.* **278**, 21721-21731.

Mathers, P. H., Grinberg, A., Mahon, K. A. and Jamrich, M. (1997). The *Rx* homeobox gene is essential for vertebrate eye development. *Nature* **387**, 603-607.

Milan, M. and Cohen, S. M. (1999). Regulation of LIM homeodomain activity in vivo: a tetramer of dLDB and apterous confers activity and capacity for regulation by dLMO. *Mol. Cell* **4**, 267-273.

Okamoto, O. K. and Hastings, J. W. (2003). Novel dinoflagellate clock-related genes identified through microarray analysis. *J. Phycol.* **39**, 519-526.

Piatigorsky, J. (2001). Enigma of the abundant water-soluble cytoplasmic proteins of the cornea: the “refracton” hypothesis. *Cornea* **20**, 853-858.

Pichaud, F. and Casares, F. (2000). *homothorax* and *iroquois-C* genes are required for the establishment of territories within the developing eye disc. *Mech. Dev.* **96**, 15-25.

Pineda, D., Rossi, L., Batistoni, R., Salvetti, A., Marsal, M., Gremigni, V., Falleni, A., Gonzalez-Linares, J., Deri, P. and Salo, E. (2002). The genetic network of prototypic planarian eye regeneration is Pax6 independent. *Development* **129**, 1423-1434.

Quiring, R., Walldorf, U., Kloter, U. and Gehring, W. J. (1994). Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. *Science* **265**, 785-789.

Ruiz-Gonzalez, M. X. and Marin, I. (2004). New insights into the evolutionary history of type 1 rhodopsins. *J. Mol. Evol.* **58**, 348-358.

Salvini-Plawen, L. V. and Mayr, E. (1977). Evolution of photoreceptors and eyes. *Evol. Biol.* **10**, 207-263.

Sheng, G., Thouvenot, E., Schmucker, D., Wilson, D. S. and Desplan, C. (1997). Direct regulation of *rhodopsin 1* by *Pax-6/eyeless* in *Drosophila*: evidence for a conserved function in photoreceptors. *Genes Dev.* **11**, 1122-1131.

Tahayato, A., Sonneville, R., Pichaud, F., Wernet, M. F., Papatsenko, D., Beaufils, P., Cook, T. and Desplan, C. (2003). Otd/Crx, a dual regulator for the specification of ommatidia subtypes in the Drosophila retina. *Dev. Cell* **5**, 391-402.

Treisman, J. E. and Rubin, G. M. (1995). *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. *Development* **121**, 3519-3527.

Tsonis, P. A., Vergara, M. N., Spence, J. R., Madhavan, M., Kramer, E. L., Call, M. K., Santiago, W. G., Vallance, J. E., Robbins, D. J. and Del Rio-Tsonis, K. (2004). A novel role of the hedgehog pathway in lens regeneration. *Dev. Biol.* **267**, 450-461.

van Meyel, D. J., O’Keefe, D. D., Jurata, L. W., Thor, S., Gill, G. N. and Thomas, J. B. (1999). Chip and apterous physically interact to form a functional complex during *Drosophila* development. *Mol. Cell* **4**, 259-265.

Wernet, M. F., Labhart, T., Baumann, F., Mazzoni, E. O., Pichaud, F. and Desplan, C. (2003). Homothorax switches function of *Drosophila* photoreceptors from color to polarized light sensors. *Cell* **115**, 267-279.

Williams, S. C., Altmann, C. R., Chow, R. L., Hemmati-Brivanlou, A. and Lang, R. A. (1998). A highly conserved lens transcriptional control element from the *Pax-6* gene. *Mech. Dev.* **73**, 225-229.

Xu, P. X., Adams, J., Peters, H., Brown, M. C., Heaney, S. and Maas, R. (1999). *Eya1*-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. *Nat. Genet.* **23**, 113-117.

Xu, Y. S., Kantorow, M., Davis, J. and Piatigorsky, J. (2000). Evidence for gelsolin as a corneal crystallin in zebrafish. *J. Biol. Chem.* **275**, 24645-24652.

Zhang, L., Mathers, P. H. and Jamrich, M. (2000). Function of Rx, but not Pax6, is essential for the formation of retinal progenitor cells in mice. *Genesis* **28**, 135-142.

Zhang, X., Friedman, A., Heaney, S., Purcell, P. and Maas, R. L. (2002). Meis homeoproteins directly regulate *Pax6* during vertebrate lens morphogenesis. *Genes Dev.* **16**, 2097-2107.